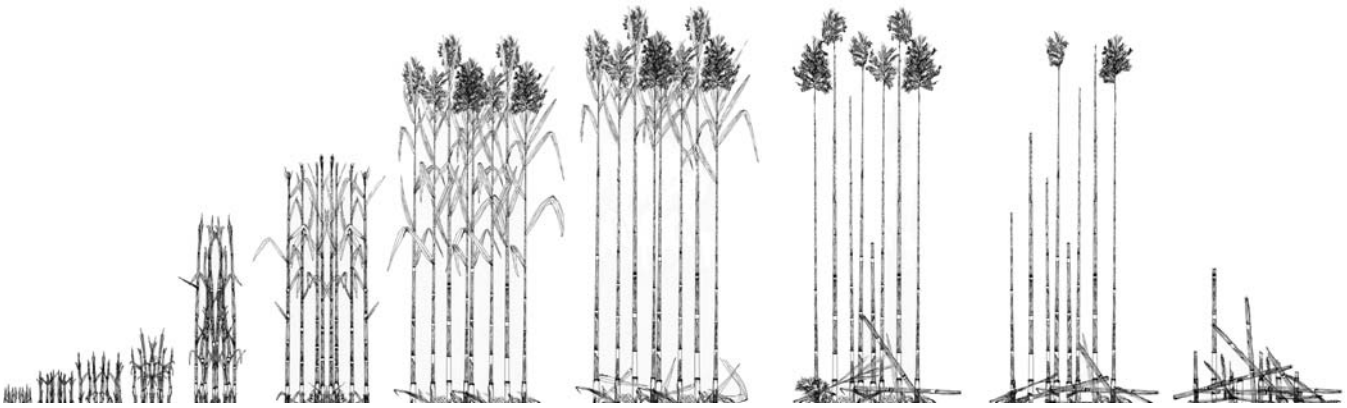
Fungi on common reed

(*Phragmites australis*)

Fungal diversity, community structure and decompositions processes

Gunther Van Ryckegem



Proefschrift voorgelegd tot het behalen van de
graad van Doctor in de Wetenschappen:
Biologie. Academiejaar 2004-2005

Promotor: Prof. Dr. Annemieke Verbeken

UNIVERSITEIT GENT
Faculty of Sciences
Department Biology
Research Group Mycology

Fungi on common reed (*Phragmites australis*)

Fungal diversity, community structure and decomposition processes

Gunther Van Ryckegem

Promotor: Prof. Dr. Annemieke Verbeken
Proefschrift voorgelegd tot het behalen van de graad van Doctor in de Wetenschappen: Biologie. Academiejaar 2004-2005

Promotor:

- Prof. Dr. A. Verbeken (Ghent University)

Overige leden leescommissie:

- Prof. Dr. M. Hoffmann (Ghent University)
- Dr. M.O. Gessner (EAWAG, Switzerland)
- Dr. H.T.S. Boschker (NIOO, The Netherlands)

Overige leden examencommissie:

- Prof. Dr. K. Sabbe (Ghent University)
- Prof. Dr. L. Lens (Ghent University)
- Prof. Dr. W. Vyverman (Ghent University)

Front page: sequence drawing of growth and decay of *Phragmites australis*; watermark symbolizing diversity of fungi on common reed with spores-conidia of a coelomycete (*Pseudorobillarda* sp.; top-left), a hyphomycete (*Arthrinium phaeospermum*; top-right), an ascomycete (*Halosphaeria hamata*; bottom-left) and a basidiomycete (*Coprinus kubickae*; bottom-right), – drawings G. Van Ryckegem

"Rather than being on the margins (of an ecosystem), fungi are… central to it, interconnecting and influencing the lives and deaths of plants and animals in countless, diverse and often surprising ways. To disregard them is to misunderstand the system"
(Rayner, 1993)

Voor Heidi, Lander en onze kleine kadee

Dankwoord – Acknowledgements

De geboorte van dit proefschrift, na een vijfvoudige olifantendracht, is niet enkel de verdienste van de verwekker maar is vooral de vrucht gegroeid door de hulp van anderen.

Mieke, bedankt voor het promotorschap met raad en daad, het lezen van mijn lange zinnen en het herlezen van alweer een andere versie. Je enthousiasme is aanstekelijk en heeft me ook gedreven. Bedankt voor de jarenlange goede sfeer op het labo, de verstandhouding en de mogelijkheid om als vrienden samen te werken. Ik apprecieerde de vrijheid in het oriënteren van mijn onderzoek en het vertrouwen. Je bezorgde me een unieke kans, merci!

Het instituut voor de aanmoediging van Innovatie door Wetenschap en Technologie in Vlaanderen (IWT) is bedankt voor de financiële steun!

Mijn supercollega's mycologen, Jorinde en Stefanie wil ik graag een kushandje toegooien. Dank voor alle hulp, de relativerende gesprekken en het delen in de mycologische miserie. Andere collega plantkundingen, waren steeds bereid voor een luchtige babbel over de geneugten des levens (o.a. eten…). Dat doet deugt, bedankt! Peter, merci voor de tips, voor die keren dat je mijn computer(-programma's) terug op gang wist te trekken maar vooral omwille van uw fysieke en verbale activiteit op de gang. Rosette, je ziet een leeftijdsverschil van 40 jaar ;-) staat géén grap en grol in de weg. Met jou kon ik het beste zeveren, ik zou je hier niet kunnen missen! Adelino, Dirk en Marleen steeds bereid om me te helpen: bedankt! Christelle, ik heb de laaste jaren jouw schaterlach gemist (al denk ik hem af en toe te horen tot op de Ledeganck). Tom, maestro, bedankt voor de hulp en initiatie in multivariate technieken. Een dikke merci aan Tim Deprez, je was een onmisbare schakel in de fabricage van mijn website! Dank aan Henry Hengledow voor het nalezen van hoofdstuk 3 op taalfouten.

De leden van mijn lees- en examencommissie wil ik bedanken voor de constructieve opmerkingen!

Also special thanks to Mark Gessner for the hospitality (and Martina!) during my (our) visit, the possibility to learn about ergosterol analysis (also thanks Daniel Steiner!) and the multiple tips and answers to my emails. It would have been great to sit in a room next to your office! Thanks to come over for my Ph.D. defence! Gratitude to Steven Newell, for answering my emails and reviewing a draft manuscript.

Dank aan Prof. Dr. J. Van Beeumen (Ghent University, Laboratory of Protein Biochemistry and Protein Engineering), Prof. Dr. Mark Stevens (Ghent University, Laboratory of Wood Technology) en Prof. Dr. Magda Vincx (Ghent University, Laboratory for Marine Biology) om mijn de mogelijkheid te bieden mijn onderzoek in hun labo uit te voeren. Specifiek zou ik Gonzalez Van Driessche (HPLC) en Bernhard De Meyer (acid detergent lignin determination) willen bedanken voor hun praktische assistentie. Dirk Van Gansbeke voerde voor mij de meeste stikstof en fosfor analyses uit. Igracias! Zonder jullie was ik misschien nog bezig…

Many mycologist helped me somehow, or sent collections of phragmiticolous fungi: André Aptroot, Hans-Otto Baral, Kevin Hyde, Thomas Læssøe, Adrian Leuchtmann, Bernard De Clercq, Conny Decock, Piere-Arthur Moreau, Peter Roberts, Carol Shearer, Brian Spooner, Karel Van de Put, Paul Van der Veken, Jan Vesterholt, Ruben Walleyn, thanks to all of you!

Ouders, schoonouders, familie en vrienden zijn een ongelofelijk kostbaar goed, iets wat je moet koesteren en onderhouden. Het spijt me dat ik jullie de laatste tijd zo verwaarloosde! Bij jullie kon ik de laatste tijd toch nog genieten van de weinige ontspanning. Het heeft me steeds opnieuw opgeladen. Vanaf nu mogen de feestjes, bezoekjes weer meer …

Deze lange lijst aan dankbetuigingen wil ik graag afsluiten met diegenen die mijn dank het meest van al verdienen. Heidi, jij bent de held van dit verhaal, zonder jouw begrip en eindeloze liefde was dit nooit geworden wat het nu is. Het spijt me voor het voorbije jaar, voor mijn egoïsme, mijn afwezigheid, je moest me veel missen (en als ik er dan was zat ik soms met mijn gedachten elders) op momenten dat ik er eigenlijk had moeten zijn. Lander, je kunt het al flink zeggen maar ik ben er niet fier op. Je vragende "papa e(k)en?" "papa e(k)en?" als ik weer eens vertrok en dan je triestig hoor vragen "spelen?". Vanaf nu beloof ik je weer tijd te maken om te ravotten! En dan is er nog onze kleine kadee, nog veilig in de buik maar wat heb ik je toch al verwaarloosd, vanaf morgen begin ik met de babbels (en ik kan ook "zingen"!) tegen mama's buik. Wacht maar…*

Gunther

---

* = werken

Contents

PART 3: FUNGAL DIVERSITY: PERSONAL OBSERVATIONS AND WORLD-WIDE DIVERSITY OF REED FUNGI

Framework of the thesis – literature study, aims and outline

Index framework

1. General introduction

Recent advances in microbial ecology highlight unknown features of fungal interactions in ecosystems and more than ever stress the general importance of fungi in ecosystem functioning not only as decomposers but as possible driving forces influencing plants growth, fitness and succession as mycorrhiza, pathogens and endophytes (e.g. Van der Putten et al., 1993; Dix & Webster, 1995; Clay & Van der Putten, 1999; Ernst et al., 2003). Despite this general acceptance, fungal research is only sparsely implemented in ecosystem studies and their diversity and the exact impact of fungi remains undocumented in most habitats. This lack of knowledge is due to several point of which we consider the following to be the most important: 1) their inconspicuous nature and an inadequate understanding of the behaviour of mycelial thalli, 2) their huge diversity combined with difficult identification and isolation and 3) the only recent development of methods to quantify fungal biomass and activity and molecular methods to fingerprint fungal communities.

Global fungal diversity is poorly known (May, 1991) and estimates of their worldwide diversity are mainly based on a presumed ratio of host specific fungi associated with single plant species (Hawksworth, 1991, 2001). However this ratio is not widely investigated and received some criticism in being not well supported by detailed monitoring. Therefore, in order to improve our understanding of fungal diversity patterns and to evaluate current estimates, there is urgent need for detailed studies focusing on single host plants (Hawksworth, 2001; Hyde, 2001). Moreover, knowledge on fungal diversity and the spatial and temporal community organisation forms the basis in understanding the functional ecology of the heterotrophic* organisms in wetlands and other ecosystems. Furthermore, the comparison of fungal species composition among wetlands could give basic information in understanding the differences in their functioning. However, beside sound qualitative-taxonomic background on the fungal species involved, quantitative information on species abundance, biomass and productivity is required to unravel functional roles of fungi in (wetland) ecosystems (e.g. Newell, 1992).

Wetlands are typically colonized by emergent macrophytes (e.g. *Spartina*, *Juncus*, *Phragmites*, *Typha*, *Carex*) of which most biomass is generally not consumed during the growing season (Mitch & Gosselink, 2000), but eventually enters the detrital pool, where it is transformed and mineralized by microbial assemblages and detritus-feeding organisms. Recent research in several wetland-ecosystems (*Spartina*-, *Juncus*-, *Phragmites*-, *Typha*- dominated sites) often revealed high fungal biomass and productivity on plant remains aboveground (Komínková et al., 2000; Newell, 2001a,b; Findlay et al., 2002). In these sites, initial aboveground decay is found to be dominated by fungi contributing significantly to microbial

* organisms only able to utilize organic sources of carbon, nitrogen etc. as starting materials for biosynthesis. Specifically fungi could be characterized as chemo-organoosmotrophs. Their primary energy source originates from chemical processes (mainly respiration under aerated conditions) after initial enzymatic extracellular, hydrolytical processes and osmotic incorporation of low molecular weight molecules (sugars, amino acids etc.).

mineralization. Bacteria are more dominant in latter stages of decay and in the soil (Benner et al., 1988; Sinsabaugh & Findlay, 1995). This makes fungi, in addition to bacteria, indispensable in sustaining the often high plant productivity and in balancing retention or removal of inorganic nutrients in wetland systems.

Although wetlands are presently highly valued (among others) for their filter capacity and their high biological importance, they are some of the most endangered habitats in the world heavily impacted by humans by land reclamation and heavy pollution. This general interest resulted in a fast body of literature on wetland biodiversity and ecosystem functioning and the effect of the former on the latter (e.g. Weinstein & Kreeger, 2000; Gessner et al., 2004). However, despite this scientific focus, current knowledge on the involvement of fungi in carbon and nutrient cycles, their impact on the food chain and influence on plants life history is very limited and clearly a vacuum in wetland research. Of all wetlands, *Phragmites* dominated wetlands are among the most geographically widespread and productive systems renowned for they filter capacity, both in natural and as constructed wetlands for wastewater treatment. More specific in the chosen study area, the Scheldt estuary, common reed is the dominant macrophyte (see §6.2.2.4) and specifically no studies have focused on fungal involvement during reed decomposition processes (Meire et al., 1997).

There are major indications that fungi are important during the life cycle of the reed plants and all aspects are discussed in depth below. In the decomposition process: (1) the high *species richness* of fungi recorded on reed (e.g. Saccardo, 1889; Taligoola 1969, Poon & Hyde 1998a; Wirsel et al., 2001); (2) a considerable fungal *biomass* develops during decay (Tanaka 1991; Komínková et al., 2000; Gessner 2001; Findlay et al., 2002; Kuehn et al., 2004), and (3) high fungal *productivity* measured during decay (Komínková et al., 2000; Findlay et al., 2002). In addition to their role as decomposers in reed stands, fungi are associated with common reed as endophytes (Peláez et al., 1998; Wirsel et al., 2001; Ernst et al., 2003), pathogens (Durska, 1969, 1970; Bán et al., 1996, 2000) and arbuscular mycorrhiza (Oliveira et al., 2001; Wirsel, 2004), having a substantial impact on the growth and fitness of the host plant. Knowledge about fungal interactions with this plant could thus prove to be important in order to understand wetland ecosystems and the plant's ecology.

2. The host plant: common reed (*Phragmites australis*)

In order to evaluate all literature data of fungal records on *P. australis* a background of systematic, synonyms and distribution of our studied substrate is relevant information. We briefly discuss host's characteristics which are important factors influencing the fungal community (see §3.1.6.1).

2.1 Systematic position & nomenclature

Class Monocotyledones
Order Poales
Family Poaceae
Subfamily Arundinoideae
Genus with 3-4 species difficult to separate from each other (Clayton, 1967; Clevering & Lissner, 1999).

- *Phragmites australis* (Cav.) Trin. ex Steud.
  Widespread in temperate and subtropical regions of both hemispheres; less common in tropical areas.
  Most important synonymes: *Arundo isiaceae* Del., *P. communis* Trin., *Arundo phragmites* L.
- *Phragmites japonicus* Steud.
  Japan, China
  By some authors also regarded as syn. of *P. australis*
- *Phragmites karka* (Retz.) Trin. ex Steud.
  Polynesia, northern Australia and tropical Asia, extending through Ethiopia and Sudan into West Africa
  Synonym: *P. vallatoria* (L.) Veldkamp
- *Phragmites mauritianus* Kunth
  Tropical Africa, and the Mascarene Islands, northern limit passing through Ethiopia, Sudan and Congo

The list of synonyms for common reed is impressive; for example Rodewald & Rudescu (1974) mention 44 synonyms. The fact that many authors described different *Phragmites* species, and still do (!) e.g. Greuter & Scholz (1996) described recently *P. fructescens* Scholz from Crete, has to do with the (sub)cosmopolitical distribution and large phenotypic plasticity of common reed grown in different ecological circumstances. A full discussion on the correct name of common reed is presented by Clayton (1968) and summarized below. Common reed was named by Linnaeus (1753) *Arundo phragmites* L. Trinius noticed that this species is not congeneric with giant reed (*Arundo donax* L.) and provided common reed with a new name: *Phragmites communis* Trin. (1820), a name still used in recent literature. However Trinius overlooked the existence of

Arundo australis Cavanilles (1799), described from Australia but also a synonym of common reed. The correct name should be a combination of the oldest ligitime genus name, *Phragmites*, with the oldest ligitime epitheton, *australis*. Steudel published the correct name in 1841: *Phragmites australis* (Cav.) Trin. ex Steud.

2.2 Distribution, die-back and expansion

Common reed is a cosmopolitan grass species (Haslam, 1972) (Fig. 1: distribution of reed). It is not found in Antarctica, the high north, certain tropical areas (such as Amazon and Indonesia) and some isles such as Iceland (Rodewald & Rudescu, 1974). The main point of its distribution is the northern hemisphere with related species (see §2.1) replacing *P. australis* in several tropical and subtropical areas. Because of the overlapping distribution of the different *Phragmites* species and the fact that they could be misidentified, it is justified to have some scepticism towards certain tropical records of fungi occurring on *P. australis*.

In contrast to a rapid expansion in North America, several European sites suffered from a severe die-back (Ostendorp, 1989; van der Putten, 1997). Results of a European project, Eureed, show that die-back of *P. australis* is due to a combination of eutrophication (by means of excessive litter accumulation) and the artificial regulation of water tables (van der Putten, 1997). Ironically, habitat destruction and manipulation of water regimes, eutrophication, pollution, and increased disturbance are often believed responsible for the population explosion of *P. australis* in North America with the replacement of several emergent macrophytes (e.g. *Typha angustiflora* L.; *Spartina patens* (Ait) Muhl.) (Marks et al., 1994; Meyerson et al., 2000). It has been suggested that the rapid spread of common reed is due to the introduction of more aggressive clones from Europe (Chamber et al., 1999). In the European context, water reed (reed along the water side of reed stands) deteriorated most from the unnatural water regimes which should normally fluctuate sufficiently with high winter levels (preventing damage at rhizomes by frost and killing some dormant pathogens in the litter layer) and low summer levels (with enhanced mineralization on larger surface of humid marsh environment and penetration of oxygen in the soil) (Graveland & Coops, 1997). Many of the reed habitats in decline are characterized by an excessive production (e.g. by eutrophication) and an accumulation of litter which stays stationary because of unnaturally low water levels during winter storms and has slower decomposition through high summer levels keeping organic litter in oxygen poor conditions at the bottom (Čižková-Končalová et al., 1992; Čižková et al., 1996; Clevering, 1998).

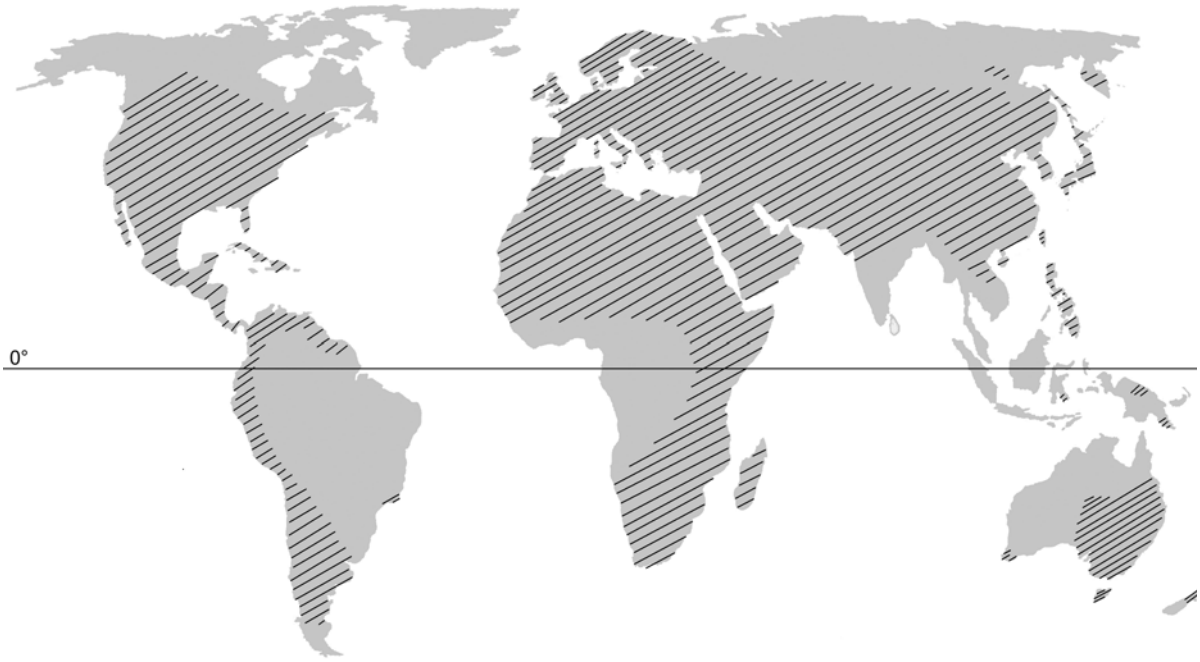


Figure 1. World-wide distribution of *Phragmites australis*. Map based data from Van der Toorn, 1972; Rodewald & Rudescu, 1974 and Clevering & Lissner, 1999.

2.3 Genotypic- phenotypic variability and ecological amplitude

Common reed has a high phenotypic variation in morphological and life-history characteristics. The very wide ecological range of *P. australis*, occurring in tidal, all sorts of non-tidal and even upland habitats exposed to fluctuating water levels (from dry sites till more than 4 m water depth), different salinities, a wide range of nutrients, other forms of pollution and a wide range of growth temperature (related to wide geographic distribution), results in several reed ecotypes (Haslam, 1972; Rodewald & Rudescu, 1974). This high phenotypic variation can be related to variance in chromosome numbers, clonal diversity, plasticity of clones or a combination of these (Clevering & Lissner, 1999). But evidence exists that responses to climate, hydrology and salt have a genetic basis (Clevering & Lissner, 1999). Furthermore, there is increasing evidence that fungi, both as mycorrhiza or as an endophyte, could play a major role in the tolerance and ecological adaptation of *P. australis* (see §3.1.3).

Although there are multiple gradients (see §6.2.2.1) within estuaries, the two important factors correlated with phenotypic difference of *P. australis* in the Scheldt estuary are salinity and secondarily flooding regime (Muylaert, 1996). Shoots in brackish conditions and in less flooded stands are smaller (e.g. in our study area about 2 m versus up to 4 m high in the freshwater areas), form denser vegetation, have narrower stems (e.g. in our study area lowest node about 6 mm diam. versus about 1 cm in the freshwater part) and smaller leaves compared

to freshwater clones (Rodewald & Rudescu 1974; Hellings & Gallagher 1992; Muylaert, 1996). Specifically, the genetic variation of *P. australis* patches is well studied in the brackish tidal marshes of the Scheldt estuary (Lamote, 2003). These genetic and phenotypic variations result in differences in substrate quality and influence micro-climate conditions in a reed stand (see §3.1.6.2).

3. Our study object: the fungi

Fungi are among the most diverse organisms in the world with presently about 80.000 taxa described (Kirk et al., 2001) but with an estimated diversity of 1.5 million (Hawksworth, 2001), leaving a huge diversity to be discovered.

Known diversity is classified in a taxonomic framework which is not stabilized so far and of which we followed a practical division of the encountered taxa with form-classes more than a natural classification (Table 1). Basidiomycota are taxa generally recognized as mushrooms with macroscopic fruit bodies characterized by the presence of microscopic basidia bearing the basidiospores. Ascomycota form asci with ascospores as a diagnostic character and were divided in two artificial form groups: discomycetes and pyrenomycetes. Discomycetes are recognizable by the free exposure of a hymenium (apothecia). Typically these taxa comprise disc-shaped fungi superficially on the host. Pyrenomycetes are characterized by their flask-shaped fruit bodies (perithecia – pseudothecia) exposing the spores (or asci) through a small channel (ostiole), often these taxa are immersed in the host. Deuteromycetes, or other names commonly used for this artificial group are anamorphic or mitosporic fungi, were divided into the coelomycetes and the hyphomycetes. Coelomycetes are characterized by sporulation structures where the conidia (asexual spores) are formed within a cavity formed by fungal tissue or a combination of fungal tissue and host tissue. Hyphomycetes comprise species which have freely exposed conidiogene cells. The majority of the deuteromycetes are probably the asexual stage of sexual ascomycetes while some taxa could represent truly asexual fungi.

Table 1. Used classification.

Kingdom	Division/Form-division	Form-class
Chromista	Oomycota	
Eumycota (Fungi)	Chytridiomycota	
	Glomeromycota	
	Zygomycota	
	Basidiomycota	basidiomycetes (incl. heterobasidiomycetes)
		teliomycetes
	Ascomycota	discomycetes
		pyrenomycetes
	deuteromycetes*	coelomycetes
		hyphomycetes

* Form-division

The latter taxonomical segregation is related to the complex life cycle of fungi which remains hidden for most time, and becoming readily apparent only during their reproductive phases. For most species the link between reproductive phases (i.e. pleiomorphy) is unknown and asexual and sexual stages are named separately because both phases result in different morphological forms possibly appearing with different timing and at other locations (Kendrick, 1979).

3.1 Fungal communities and their development

3.1.1 Introduction

With their wide geographic distribution, high species richness, and inconspicuous nature, fungi provide an immense challenge to ecologists who search for recognizable and consistent patterns of fungal communities. The community concept adopted in our study is based on the ability to recognize and measure differences among repeating assemblages of fungi occurring simultaneously in similar habitats (fide States, 1981; Cooke & Rayer, 1984). Per definition we do not imply any species interaction or organization within a community, because, for most of the recognized species assemblages, no demonstrable interactions are documented (but see §3.1.6.3). However, we assume that there are species interactions. This seems almost inevitable by the extra-cellular digestion for which at least some enzymatic interaction could be expected among the multiple species colonizing the resource.

Community studies that focus on a single resource (meaning the organic matter being decomposed (sensu Swift, 1976)) have been called component communities and this component resource could be categorized in sub-resources in terms of plant 'organs' (leaf blades, leaf sheaths and stems in the case of *P. australis*). Those component communities can be divided into physically (microhabitats) and temporally (successional phases) separated unit communities. Those unit communities should provide the base of replication in a study focussing on community structure and dynamics and the 'unit' should be chosen as homogeneous as possible (Swift, 1982) (Fig. 2). Those unit samples are the functionally integrated units of fungal activity (Swift, 1976). The body of the fungal community for identifiable, consistent fungal associations (~the fungal component community) may depend on the architecture, size and age of the host plant in a particular habitat and for many host plants probably corresponds to the individual plant. For parasites, characteristics of their hosts, such as size and morphological complexity are likely important for determining species richness (Strong & Levin, 1975; Andrews et al., 1987). Furthermore, Petrini et al. (1992) regarded each individual tree as a separate ecosystem, with plant organs and tissues representing distinct microhabitats – ecotope types – for endophytes.

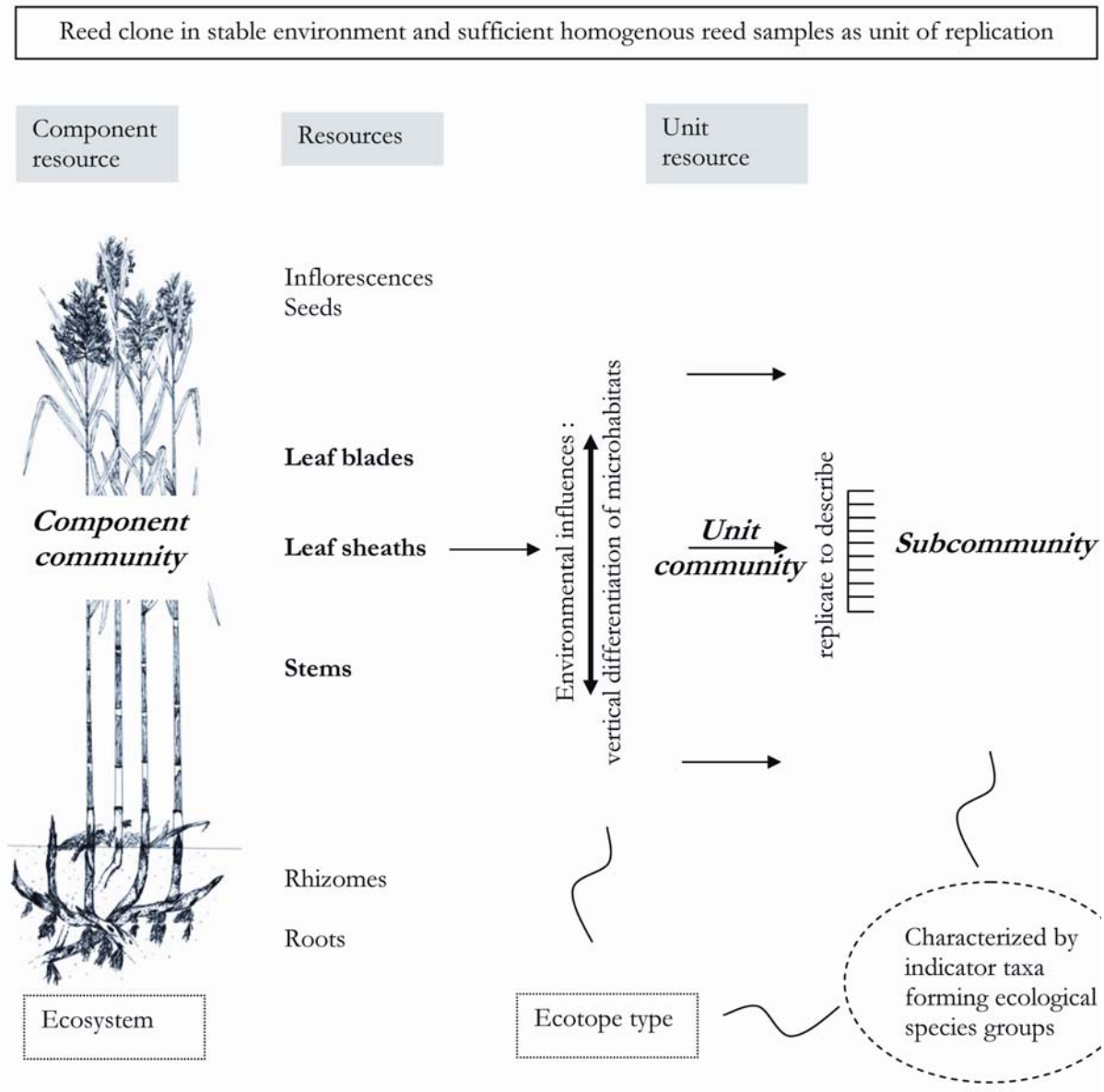


Figure 2. Schematic representation to explain some of the used terminology (see text).

For emergent macrophytes – such as reeds – with a simpler architecture compared to trees, this individual fungal-plant ecosystem entity seems at first sight too narrow. When keeping in mind that many emergent macrophytes, and in particular *P. australis,* can grow over large areas by means of vegetative, rhizomatous growth and disperses often clonally it becomes more evident that this individual scale could prove to be too large. Evidence for plant-individual, specific fungal communities is provided by the study of Ernst et al. (2003) showing systemic endophytic colonisation of the reed plant. Interestingly, three seed-dispersed fungal species could be present separately or in several combinations, so that distinct plant specific

fungal communities could already be present on the reed from the moment the seed is dispersed. That fungal (endophytical) colonization could be a very specific process is demonstrated by the fact that genotypic differences were observed in a (systemic) endophytic fungus which corresponded to a higher colonization potential on different clones of the same plant species (Ahlholm et al., 2002). Furthermore, as individual reed clones can be very large (e.g. 1736 m^2 in our study area, Lamote, 2003; or up to 3000 m^2, Koppitz et al., 1997), the colony is probably influenced by several ecological conditions which have an influence on the horizontally dispersed fungi, their colonization and growth and eventually the established community. This means that recognizable fungal component communities could exist within a reed ecosystem on one individual plant but if environmental conditions change, local spatial variability in the reed stand will alter the fungal component community. The extent to which environmental conditions may vary is unclear. However the scale could be small as gradients within reed swamps change considerably over relatively short distances (Polunin, 1984). For example, marginal sites without a specific microclimate would differ from the "buffered" central systems within large stands, or sites within a reed clone growing in different water depth, flooding frequency or locally changed edaphic factors.

For these reasons a fungal component community (ecosystem) is considered in this study to be a patch of an individual reed plant characterized by stable environmental conditions (e.g. microclimate, soil characteristics and topography,…) overruling only nearby shoot differences whereas those differences in species composition are thought to be stochastic.

3.1.2 Fungal modes of life: pathogens, endophytes and saprotrophs

When describing fungal unit communities during growth and decay of above ground parts of a plant, one finds several distinct functional groups of fungi on the host, such as pathogens, endophytes and saprobes. All of those groups are in a more or less close contact to each other. I use in my thesis a broad definition of the term endophyte referring to all fungi that spend at least a significant part of their life-cycle internally and asymptomatically. This includes plant pathogens that have extended or variable latent periods before external signs of infection appear (necrotrophic fungi) (Sinclair & Cerkauskas, 1996) to obligate mutualistic, systemic endophytes (biotrophic fungi). Our reasoning, as well as that of others (e.g. Wilson, 1995; Saikkonen et al., 1998; Latch, 1998) is that the distinction between classical plant fungal pathogens and endophytes is not clear (Sinclair & Cerkauskas, 1996). This broad concept makes it possible to place endophytic relationships on a continuum from positive (mutualistic) to negative (antagonistic) (Fig. 3) (Saikkonen et al., 1998). In this context it is clear that biotrophy, necrotrophy and saprotrophy are not necessarily mutually exclusive ways of life for a fungus (contrary!) (Parbery, 1996). They are merely different nutritional modes and there is no reason (theoretically and shown in nature) why any fungus should not be capable of all three under different circumstances. For example many pathogens prove to be endophytic

species causing disease when the plant suffers stress (e.g. Parbery, 1996; Halmschlager et al., 1997). Thus the terms pathogen, endophyte or saprotroph can be applied to any fungus, even temporarily, exhibiting one of the relevant nutritional modes (Cooke & Rayner, 1984; Wilson, 1995; Gindrat & Pezet, 1994; Sinclair & Cerkauskas, 1996; Saikkonen et al., 1998). Grass endophytes are divided in two groups: systemic and nonsystemic endophytes. Little is known about nonsystemic grass endophytes because virtually all studies have focused on systemic grass (all species within the tribe Pooidae) endophytes (clavicipitaceous species in the tribe Balansiae (Ascomycota)). This bias in research has its origins in the agricultural importance of those grass endophytes mainly because of fungal induced herbivore resistance by mycotoxin production (Saikkonen et al., 1998; Latch, 1998). Furthermore it has been shown that these fungi improve the vitality of their hosts by increasing resistance against grazing, insects, drought, and microbial pathogens (Clay, 1986; Saikkonen et al., 1998; Petrini et al., 1992) resulting in improved biomass production and nutrient status (Groppe et al., 1999; Malinowski et al., 2000; Ernst et al., 2003). In comparison little effort was done and few insights are gathered on natural plant populations and the impact of nonsystemic endophytes on the plant or their relationship with systemic endophytes in nonagricultural crops. Specifically, up till now no studies report harmful effects of endophytes towards herbivores or microbial pathogens on *Phragmites*. However one study (Ernst et al., 2003) reports on the beneficial affect of systemic (at least one is microscopically proven to be so) *Stagonospora* spp. on the growth of *Phragmites*. The mechanisms for these biomass effects by systemic grass endophytes are not settled but an obvious explanation is that endophytes act in a similar way as mycorrhizal fungi do (Marks & Clay, 1996; Malinowski et al., 2000). If phragmiticolous endophytes prove to have a beneficial impact on the life history of common reed and fungal colonization leads to biotic and/or abiotic stress resistance, it is possibly a further explanation for the widespread distribution and adaptability of *P. australis*. Furthermore, a growing awareness of the profound effects that endophytes can have on plant competitiveness, growth and persistence should help scientists to understand the ecology of natural *P. australis* vegetations in the future.

Though little studied, nonsystemic endophytes are in most cases well represented. Often a few species are dominant and those species colonize small patches on the living host (Stone, 1987; Suske & Acker 1987, 1989; Cabral et al., 1993; Carroll, 1995; Schulthess & Faeth, 1998). For those fungi Carroll (1986) introduces a second strategy of mutualism with his host, namely *inducible mutualism*, which involves a more free association between endophyte and host plant compared to the constitutive mutualism of systemic grass endophytes. For example while those small infection spots may not be a direct deterrent to herbivores or pathogens, they may serve as an inoculum source when nearby tissues are killed or stressed by insect herbivore feeding or pathogen colonization. Attacked cells are invaded by those cryptic endophytes which may kill intruders or decrease palatability for herbivores. Although this is a very interesting theory, besides observations, no real experimental evidence is presented by Carroll and to the best of our knowledge no solid prove is published so far (but see §3.1.6.3 fungal interactions).

Although our definition of endophytes excludes bacteria, it is justified to mention the existence of endophytic prokaryotes (however unknown for *Phragmites*) for which comparable mutualistic interactions with their host plant are described (e.g. Chanway, 1996).

Moreover there is an increased awareness, with important implications, that several endophytes are becoming primary saprophytes soon after plants senesce (Guo et al., 1998; Wong & Hyde, 2001; Zhou & Hyde, 2001). It is considered that most actively growing biotrophic endophytes, with their highly specialized physiological interaction with their hosts, have little or no saprotrophic ability in comparison with the necrotrophic parasites (the true pathogens considered here (Fig. 3)) and the cryptic (nonsystemic) endophytes becoming primary saprotrophs (Parbery, 1996). Although this could prove sometimes false, as for example some systemic biotrophic smuts may have yeast-like saprotrophic phases on the dead substrate (Cooke & Rayner, 1984). However, once the host dies, all fungi present enter an increasingly diverse microbial system. During the initial phases of decay, necrotrophs and biotrophs benefit from their early occupation of some tissue which can act as a base from which they can quickly invade new substrate. Strict necrotrophic parasites might depend on a system of resource management. These parasites could provide their (resting) mycelia with sufficient energy for vegetative growth or a steady state of metabolic activity on the dead host to survive till the next season and sporulate from this resource which is in many cases nearby the reappearing host (e.g. Wheeler, 1968). Nonsystemic endophytic fungi on the other hand will probably focus on life cycles with maximal reproduction at the time the host has maximal susceptibility for new inoculation and will actively grow on the dead host to obtain a maximal inoculation the next plant growth season (in temperate climates). Soon after this first phase of decay, those fungi lose the advantage against the secondary saprotrophic competitors that are able to break the active occupation of the initial decomposers and settle.

The saprotrophic fungi form a diverse community of organisms exploiting the carbon and nutrient substrates in multiple ways. The major proportion of the necromass is cell wall material, which is mainly made of lignocellulose (Boschker, 1997). This polymer is made of a variable combination of lignin, cellulose and hemicellulose. These compounds are used in different proportions depending on the species and several decay types can be characterized: white- , brown- and soft rot. White rot fungi are capable of using all cell wall components and are typically basidiomycetes (some ascomycetes, mainly Xylariales). The plant remains usually turn whitish because of bleaching by oxidation and loss of lignin, which is slightly brown. Brown rot fungi primarily use hemicellulose and cellulose components and lignin mostly remains. A wide range of fungi are capable to perform this type of decay. Soft rot fungi, mainly ascomycetes, attack all wall components but carbohydrates are preferred (Cooke & Rayner, 1984).

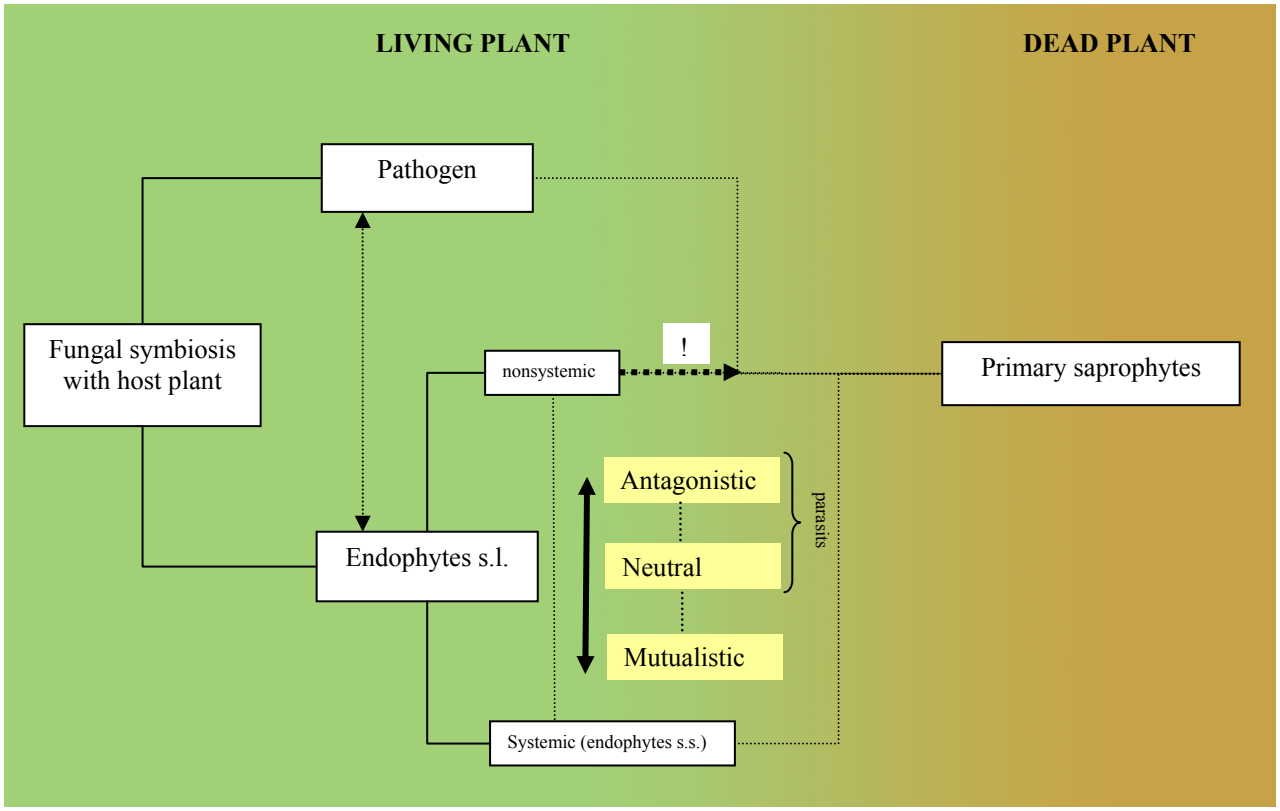


Figure 3. Different fungal symbiotic life styles during initial fungal colonization of *Phragmites australis* demonstrating the continuum and vague boundaries among them.

3.1.3 Fungi associated with Phragmites australis

High fungal biodiversity on common reed has been noticed already in the nineteenth century as Saccardo (1889) listed 146 taxa on *P. australis* on his species-host list and about 170 taxa in his whole oeuvre. More recently Taligoola (1969; Ph.D. thesis resulting in several papers: Apinis et al., 1972a, b, 1975; Taligoola et al., 1972, 1975) listed 153 taxa and Poon & Hyde (1998a) recorded 61 taxa during a smaller scaled study. In addition, several other studies rapport high fungal diversity on reed (Oliver, 1953; Rodewald & Rudescu 1974; Bán et al., 1996, 1998; Beyer 1997; Peláez et al., 1998; Wirsel et al., 2001; Wong & Hyde 2001; Luo et al., 2004). The composition of the major pseudo-systematic groups in three studies is shown in Fig. 4. Clearly the proportion of hyphomycetes is different between studies. This is related to the methodology used. The high fraction of hyphomycetes in Taligoola's study is due to incubation and agar plating of reed material, while the compilation by Saccardo is assumed to present mainly field observations.

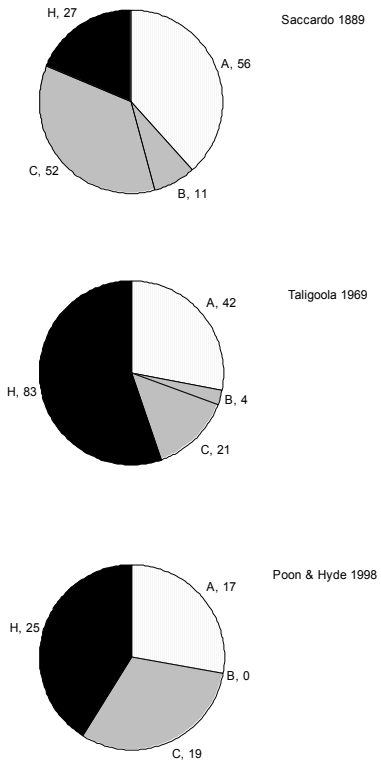


Figure 4. The proportion of the major pseudosystematic groups in three studies. A: ascomycetes; B: basidiomycetes; C: coelomycetes; H: hyphomycetes

Some studies focussed on the endophytic mycoflora present on *P. australis*. Peláez et al. (1998) found 32 taxa (their summarizing Table 1) although in Table 2 of the same paper only 28 taxa are summed for *Phragmites*, 14 genera (named) and 12 species were identified up to species level. Unfortunately the authors do not specify the plant part from which they isolated their cultures. The most common genera isolated were *Alternaria*, *Sporormiella*, *Rhizoctonia*, *Epicoccum* and *Septoriella*. It may seem odd to find representative of *Sporormiella* on *Phragmites* as this genus is known to have mainly coprophilous representatives. However, recently an increasing number of studies report presence of 'coprophilous' endophytes (Carroll, 1988; Fisher et al., 1986; Petrini, 1996) as these species are possibly adapted to sporulate after temperature rise or after fire. Probably these fungi are more common in warmer climates, which could make sense, as Peláez et al. (1998) reported phragmiticolous endophytes from Spain while none were reported by Wirsel et al. (2001) from Germany. In their more extended study Wirsel et al. (2001) found 12 (named) endophytic genera with the most common genera found: *Microdochium*, *Cladosporium*, *Trichoderma*, *Cylindrocarpon*, *Epicoccum*, *Arthrinium*, *Phoma* and *Exophiala*. In total 26 fungal taxa (26 different fungal ITS-sequences) were retrieved and

highest fungal diversity was noticed on roots (roots 19 species; stems 8 species; and leaves 6 species).

Three *Stagonospora* spp. and an *Epicoccum* strain were demonstrated to be dispersed within *Phragmites* seeds (Ernst et al., 2003). The three *Stagonospora* species were sequenced and matched, via BLAST searches, with *Stagonospora neglecta* (100% identical) being the most often isolated endophyte, *Stagonospora* sp. close to *Phaeosphaeria culmorum/ P. insignis* (99.6%) [if it corresponds to one of these it is probably *P. culmorum* which is common on *Phragmites* while the other species is not known to occur on this host (Van Ryckegem, database)] while the third *Stagonospora* matched closest to *P. pontiformis* a common species on reed. In axenic microcosm the plant growth was clearly enhanced by any of the three *Stagonospora* spp. Reed biomass was about double in presence of the endophyte compared to sterile control plants. Second, cumulated culm lengths per plant were significantly increased, which resulted from higher average culm length and a higher number of culms per plant. Furthermore inoculation in the microcosm of another *Phaeosphaeria* sp. namely *P. phragmitis* gave exactly the same results (Ernst et al., 2003) and *Stagonospora nodorum* is also known to be a seed dispersed endophyte of wheat (Sieber, 1985 in Petrini et al., 1992) indicating a high potential role in reed and in monocots vigour and ecology for this genus of endophytes.

Other common biotrophic endophytes (parasites) recorded on *Phragmites*, although not scored by previous endophytic research (wrong primers and difficult to culture) are members of the rusts (Uredinales) of the genus *Puccinia* and smuts such as *Ustilago grandis* (Ustilaginales). *U. grandis* is indeed systemic, host specific and the infected reed plants remain sterile (Vánky, 1994). However the smut should at least have some saprotrophic abilities as the teliospores are only produces on standing stems several months after the host died (January-April). This would include *U. grandis* in the group of endophytes becoming primary saprotrophs.

3.1.4 Establishment of fungal species

Some systemic endophytes are associated with the reed host already in the seed embryo and grow out together with the maturing reed plant (Ernst et al., 2003; see §3.1.3). However, for the majority of species a successful establishment within the reed plant depends upon invasive force (inoculum potential), to penetrate below the external host surface. This normally occurs by one of the following routes: (1) penetration of the cuticle and epidermal cell wall; (2) via the stomata (e.g. we observed this for *Cladosporium* sp.); (3) through lesions caused by animals, other fungi or mechanical damage. The inoculum potential is obviously related to plant characteristics such as cuticle and epidermis cell wall thickness, stomatal density and number of parasitic fungi and insects combined with the manner in which fungi arrive at the substrate surface and to their subsequent development patterns. A wide variety of dispersal mechanisms and specific spore adaptations among fungi exist (see Ingold, 1971; Jones, 1994). Dispersion of endophytes can have several pathways: vertical or horizontal transmission, while all of the real saprotrophs show a horizontal transmission on their substrate. Systemic endophytes are considered to be transmitted either clonally and vertically (by growing into the seeds) or

(a)sexually and horizontally via mito- or meiospores (Saikkonen et al., 1998). Nonsystemic endophytes and saprotrophic fungi are horizontally transmitted via mito- or meiospores by wind, water (rain or fog), insects or other vectors (see §3.1.6.3).
Furthermore the establishment of a species on above ground plant substrate can be realized via outgrow from roots and rhizomes or by mycelium in the soil or in the litter layer (Cooke & Rayner, 1984). During the life-cycle of common reed there is an ongoing colonization first by endophytes and later by saprotrophic fungi. This accumulation depends on the number of spore sources in the vicinity. Seasonal and yearly climatic factors such as humidity and rainfall may determine spread and germination success of the spores (§ 3.1.6.2).

3.1.5 Initial growth and succession

As soon as fungal colonization on the reed host starts, changes are induced. Indeed, like plant communities, fungal communities are dynamic. Directional patterns are apparent with community composition and function, changing qualitatively and quantitatively in time (Hudson, 1968; Frankland, 1981). Substratum successional sequences are degradative or heterotrophic and defined as the successive use of the dead organic resource by a number of species over a relatively short time scale (months or years) (Connell & Slatyer, 1977; Begon et al., 1996). Specifically, **fungal succession** can be defined as the sequential occupation of the same site by thalli (normally mycelia) either of different fungi or of different associations of fungi (Rayner & Todd, 1979).

3.1.6 Factors affecting the success of fungal colonization and growth

A variety of factors may influence the colonization and decomposition of plant matter (Fig. 9) (Brinson et al., 1981; Webster & Benfield, 1986). Beside the above mentioned interactions between fungal taxa the most frequently noted features are internal variables such as resource quality, being physical and chemical characteristics of the plant. The internal conditions of the solid plant resource are often influenced by a range of external environmental variables and biotic interactions. Among those external variables are temperature, water availability, water quality, aeration and biotic interactions affecting fungal colonization and decomposition.

3.1.6.1 Internal resource quality

The wide ecological amplitude where we can find growth and colonization of *Phragmites* together with the genotypic and phenotypic variation of the plant results in differences in substrate quality (see §2.3). This will have a paramount effect on the microbial decomposers regulating decomposition both in activity and diversity.

physical characteristics

Anatomical differences between different reed organs are multiple and described in general by Metcalfe (1960). Anatomy varies strongly between different ecological localities (Stant, 1953), changes during plant growth and often shows longitudinal differences which could influence fungal plant part recurrence, colonization, succession and vertical distribution pattern (cf. Webster, 1956). Anatomical features which should be considered in regard to the entry of pathogens and endophytic fungi are stomatal density, cuticle thickness and epidermal cell wall structure. During growth there are clear differences between young and mature reed leaves (Antonielli et al., 2002) with the young leaves consisting of fewer cell layers and missing sclerenchymatous bundle sheaths. Young plant parts and apical meristems would seem more susceptible to fungal invasion than mature tissues and cells with well developed cuticle and thickened cell walls. This could mean an advantage for horizontally transmitted fungi, by a higher infection rate, when they could sporulate during the period that the *Phragmites* plant grows most (April-May-June). The number of stomata increases with increasing height of insertion of the plant part on the standing shoots and generally more stomata are found on leaves [more on the abaxial site of the leaf (Antonielli et al., 2002)] compared to sheaths and stems (Willmer & Fricker, 1996). The cuticle is thicker in the upper part of the plants with only slight differences between plant parts at a certain height. The epidermal cell wall is thicker on the stem than on the leaf sheath and along the longitudinal axis of the shoot it increases in thickness to the top (personal preliminary observations). Epidermal strips reveal high amount of phytolith deposition (silica bodies, see below (chemical characteristics plant)) which could improve reed resistance against fungal infections (Samuels et al., 1991). Highest amounts of phytoliths were found in the highly photosynthetic and transpirationary upper part of shoots (Deleebeeck, 2000). Grasses (incl. *Phragmites*) posses the highest amounts of silica in the glumes of the inflorescence, followed by the leaf sheath, leaf blade, stem and rhizome (Jones & Handreck, 1967; Gráneli, 1990) no silica deposits were found in the roots of *Phragmites* (Sangster & Parry, 1981).)

Once the fungus entered the host plant, further anatomical features influence and determine the spread of the intruder. For example within the stem an internal ring of sclerenchym could slow down internal penetration by fungal hyphae or slerenchymatous bundle sheaths around a vascular tissue could slow down longitudinal spread in the reed plant. The presence of aerenchym within leaf sheaths creates a different internal environment compared to leaves where aerenchym is missing (Armstrong et al., 1996) with available physical space for fruit body formation. Air cavities within stems were only present in the two most basal internodes of reed stems (pers. obs., only brackish reed screened for this feature). Moreover, in the internal plant structure vertical differences are noticeable and could explain some of the observed difference in vertical distribution of species within one specific plant part. The reed stem is thickest at the base characterized by a continuous layer of sclerenchyma while this layer becomes thinner acropetally and even disappears in the upper parts (Bosman,

1985). The general anatomy of the nodes, as for *Dactylis* (Webster, 1956), suggests that their massive, woody appearance with extensive parenchyma, with few barriers of fungal development [however a high silica deposit is noticed in these regions (Kaufman et al., 1981)], would provide an admirable substrate for fungal development possibly also through the penetration of hyphae from the leaf sheath base.

Furthermore, in close relation to plant anatomy stands the water potential (see below *water availability*) of different reed plant tissues with generally a higher water potential and quick saturation of tissue with more aerenchym and less sclerenchym (Kuehn et al., 2004). And this is influenced by the water quality (e.g. salinity results in higher osmotic potential of the water).

chemical characteristics

Nutrient concentrations of the decomposing plant matter, in particular nitrogen and phosphorus and the proportion of refractory plant constituents are some of the main factors influencing the microbial decomposers and consequently the decay rate (Melillo et al., 1984) (see §4.4).

Some chemical differences between different plant parts are prevalent at the onset of decomposition, after resorption of the nutrients towards the rhizomes. These generally indicate higher ash, silica, nitrogen and phosphorus in the leaf compared to stem tissue (Granéli, 1990). Leaf sheaths generally fall in between for nitrogen and phosphor but have a higher amount of ash and silica compared to stems. Plant parts differ in their structural plant polymer composition (pers. obs.).

The nutrient content of standing shoots (all plant parts considered) differs along their height during their initial growth phase and senescence, with the highest amount of nitrogen measured at the apex of the growing shoots. These differences are abolished at the end of August (Kühl & Kohl, 1993) and differences of easily leaching nutrients seem to be minimal along the vertical axis of the standing dead shoots at the end of the year when the leaves are shed. Dynamics of nitrogen and phosphorus concentrations of senescing and decaying leaf blades showed about a one month delay in rhizome resorption (see Killingbeck, 1986) of the upper leaf nutrients compared to the middle leaf nutrients (Gessner, 2001) and followed the top-down senescence pattern (Granéli, 1990; 1992).

Besides nutrients, plant parts vary in other chemical (e.g. carbonhydrates, cell wall polymers) and physical properties (e.g. fiber length). And these characteristics change with the height and age of the plant (Rowell et al., 2000). However not screened for *Phragmites, Hibiscus* showed a higher lignin, glucose and xylose concentrations at the bottom of the plant compared to the top (Rowell et al., 2000).

Chemical constitution of the reed plant may differ between different habitats. This can have multiple reasons but one of the best documented factors is nitrogen supply (Čižková-Končalová et al., 1992). Excessive nitrogen supply decreases the accumulation of carbohydrate reserves and it reduces the allocation of carbon to supporting tissues (Klötzli, 1972 in Čižková-

Končalová et al., 1992); this in turn reduces the mechanical strength of shoots and increases the risk of mechanical damage, a faster decay (Haslam, 1989) and a probably increased palatability of reed tissues for herbivores (Kühl, 1989 in Čižková-Končalová et al., 1992).

3.1.6.2 External abiotic influences on the fungal community

Many environmental factors influence fungal performance to some extent and in synergy with each other and often this results in species-specific responses (Hawker, 1966). Colonization of tissues depends on availability and viability of spores, which, in turn, are influenced by abiotic climatological conditions, ground topography, microclimate and physico-chemical characteristics present in or near a plant organ (see Saikkonen et al., 1998; Petrini 1996; Cook & Rayner, 1984; see above plant characteristics). Probably the main factor in determining fungal colonization and activity on standing dead macrophytes and in the litter layer is water availability (in relation with salinity) and in addition to several abiotic factors, the outcome of microbial assemblages is influenced by biotic interactions of surrounding vegetation being a source of potential inoculum, the plant density and architecture and plant physiological conditions and other biotic interactions (see below).

The importance of climatological conditions (e.g. of temperature and precipitation) on the activity of micro-organisms and other organisms is well established and not fully discussed here. However, the subtle influence of precipitation on spatial distribution patterns of leaf endophytes illustrates the specific importance of weather conditions for plant inhabiting fungi. For example, Wilson & Caroll (1994) noticed that infections of *Discula quercina* are concentrated towards leaf petioles and on veins of *Quercus garryana*. These infection patterns seem to arise through the interaction of seasonal weather, the phenology of leaf development, and leaf topography. Infections of new leaves take place after bud burst in May and dispersal of conidia in spring rains. Leaf expansion continues into June, however, when rainfall has usually stopped. Because later leaf development occurs largely through expansion of the distal half of the leaf, *Discula* infections appear skewed towards the proximal half of the leaf. The prevalence of infections over veins results from patterns of raindrop adherence to leaves; as leaves dry, droplets occur largely over the veins, and when the droplets have evaporated completely, the conidia contained therein are stranded there on the leaf epidermis.

The microhabitat

Probably microclimatic differences in the sheltered vegetations will create microhabitats that will have an influence on the fungal species dwelling in the reed stands. The average minimum diameter of a reed stand with microclimatic conditions (altered temperature, aeration, humidity and light conditions) in the growing season compared to open air situations was stated to be 40-50 m, with a height of about 3 m and density of 25 shoots per m^2 (Rodewald & Rudescu,

1974). Within reed stands there is a vertical stratification of light, temperature and humidity as is characteristic in all kinds of more or less dense vegetation (e.g. Geiger, 1957). For example in fully outgrown reed stands on a sunny, windless day (e.g. in July for the temperate zone) only about 10% of the sunlight reaches the bottom (see Denward et al., 1999 for possible impact of this irradiation), temperature is a few degrees lower during daytime within the reed stand compared to outside while the opposite is true during night and these differences are more pronounced in the lower half of the standing reeds and in the litter layer. Furthermore humidity in lower zones in the litter is near saturation compared to about 60% in the upper zone. To what extent these microclimatic conditions have their impact on phragmiticolous fungi is poorly known. Taligoola (1969), controlling relative humidity between 75.8 and 100% in preliminary experiment (unpublished) found sporulation to depend on relative humidity of the incubation chamber and a minimum level of 96.8% was found for sporulation. No other fungi besides *Aspergillus* sp. were found to sporulate on reed below this value. Yadav & Madelin (1968a,b) showed that besides relative humidity for spore germination, daylight conditions gave a reduced sporulation of several species which were in the field confined to the lower part of standing dead plants (*Heracleum* and *Urtica*). However these observations could be due to a combined effect of heating by long wave radiation and true photoresponses (Yadav & Madelin, 1968b).

This stratification in reed stands is more marked on calm and sunny days compared to cloudy and windy days and shows clear differences during a day course, with highest relative humidity during night and lowest values on the noon. The actual relative humidity inside stands is higher than those above open water and the most important factor controlling microclimatic conditions in reed vegetations is the wind speed which is correlated with shoot density (Geiger, 1957; Rodewald & Rudescu, 1974; Taligoola, 1969).

Water availability

Both the water content of the substrate and the relative humidity of the atmosphere just above the substrate are of importance in controlling growth and reproduction of fungi (Hawker, 1966; Turian, 1974); meaning this factor could be considered as being an external or internal environmental factor influencing fungal species. However the measurements of water content in a substrate and relative humidity are only indicative as growth is actually depending on the availability of water. This accessibility is expressed as the water potential which is determined by the osmotic potential caused by solutes in the water of the substrate, matrix potential and by turgor potential. Matrix potential results from the interactions of water with interfaces and is of importance in solid resources, thus also *P. australis*. Depending on the radius of the pores where water is caught in, it becomes more or less available, for example water in the lumen of cells could be available but that in the cell wall is not. Turgor potential is the pressure exerted by the fungal wall on the content of the cell and higher turgor potential of a fungal cell increases the water potential of that cell (Carlile & Watkinson, 1994). Fungi vary greatly in their

moisture requirements as could be reflected by their vertical distribution on the substrate (Poon & Hyde, 1998b) and their salinity tolerance (e.g., Adler, 1996). General statements (one of Kleb's principals) such as those saying that conditions favourable for germination and initial colonization are also favourable for further growth and other stating that mycelial growth would continue under conditions not necessarily permitting germination are probably true for most fungi.

The water content in basal parts of standing dead shoots just above water level and in tidal wetlands is thought to be higher than in the shoot higher up (see Webster, 1956; Taligoola, 1969). Webster (1956) also noticed a reduced capacity of grass stems to retain water with age, concluding that further decomposed resource would become less suitable for fungal activity in exposed microhabitats enhancing drying. However, as decay of plant litter advances an advanced decay caused higher values of water potential at a given water content (Newell et al., 1991; Dix, 1985), possibly due to change in pore size distribution and internal surface chemistry (Boddy, 1986), making water availability less of a potential problem for saprophytes occurring on a later decay phase compared to first phase decay.

Recent research of standing dead macrophytes resulted in a better understanding and the realization of the highly specialized features of the fungal communities. Fungi growing on standing dead plants were shown to react very quickly to changes in water availability (Newell et al., 1985; Kuehn et al., 1999; Kuehn et al., 2004). For example after the substrate was initially wetted, fungal activity raised sharply within 5 min as measured by CO_2 production. This activity is able to remain high for up to 24 hours measured, meaning that the high initial burst of respiration can be maintained for prolonged periods as long as environmental conditions are favourable for inhabiting micro-organisms. When substrate was exposed to drying, CO_2 evolution declined rapidly (Kuehn et al., 1999). Under field conditions at dry days, rates of CO_2 were the highest during night and morning hours, coinciding with increased humidity and plant water potentials caused by dew wetting the plant surface (Kuehn & Suberkropp, 1998). When temperature is raised, under adequate moisture conditions, inhabitant microbial assemblages in standing litter of emergent macrophytes can stay metabolically active even when ambient temperatures become high (up till 30°C in the experiment, material from subtropics) (Kuehn & Suberkropp, 1998; Kuehn et al., 1999).

One of the most important water subsidies for fungi in the canopy of vegetations is due to formation of dew; although tides and rain perform the same function, dew is the longest-lasting wetting phenomenon (Newell et al., 1998; Kuehn & Suberkropp, 1998). The ability of dead plant tissues to let the water penetrate into the tissue as it becomes available to the fungal colonizers might be an important factor in determining potential accumulation of fungal mass and their activity among different plant parts and species (Newell, 2003).

In natural stands of *Spartina*, high densities of ascomata were produced by *Phaeosphaeria* spp. on standing-decaying blades of smooth cordgrass even during periods with little rain, suggesting a well adapted mycoflora to tidal environmental conditions. Furthermore when plants were misted during prolonged periods there was an initial raise in fungal biomass during

the first week, however after four weeks, Newell et al. (1996) noticed a pronounced negative effect upon fungal productivity, biomass and number of reproductive structures formed compared to control situation. This suggests that persistent high water content causes reduction of fungal output in the form of ascospores (cf. Newell & Wasowski, 1995) and reduced availability of concentrated fungal tissue for detritivores (cf. Newell & Bärlocher, 1993).

The effect of tidal wetting on the fungal communities is comparable with rain, increasing water availability and relative humidity near and in the substrate (Newell et al., 1985; Halupa & Howes, 1995) and showed a positive correlation with decomposition. Higher litter moisture directly related to flooding frequency and duration are important factors in determining microbial activity. However decomposition slows down if inundation frequency becomes too high (Lee, 1990). Moreover, the optimal flooding frequency and duration is probably a narrow range because a height difference of only a few centimetres on the tidal marsh showed already significant effects on both litter moisture levels and decay rates, indicating that slight changes in tidal regime may have important consequences for decay processes (Halupa & Howes, 1995). Excessive water availability in aerated conditions seems to result in higher organic matter loss. One explanation for this could be that fungi in the constantly humid conditions in combination with low nitrogen availability are induced to digest more plant material in attempting to obtain sufficient nitrogen, in the face of the higher leaching of nitrogen and organic carbon (Newell et al., 1996; Halupa & Howes, 1995).

The effect of salinity is in close association with water content of the dead tissue and in relation to the water availability for the fungus. Salinity can have a direct toxic effect or it might induce osmotic stress to the fungus (Adler, 1996). This osmotic stress makes the water less available to the fungus during drier periods. However, tissues influenced by salinity (by flooding or salt spray) become hydrophilic and water content in the tissues might increase during dew formation. Salt marsh species which are adapted to periodical high osmotic stress by salinity, could also favour periodical higher water content and longer periods of favourable water availability. This mechanism might favour fungi growing in saline circumstances (in comparison to terrestrial fungi also well adapted to osmotic stress but growing in freshwater conditions) in having longer periods of activity. This could additionally explain the higher amount of observed fungal mass in salt marsh plants compared to freshwater taxa (see Newell, 2003).

Aeration

Although some fungi are capable of growing in muddy sediments or stagnant water in nearly anaerobical conditions, (e.g. Apinis et al., 1972b), most fungi are considered to be largely aerobic organisms that fulfil their digestive tasks, growth and sporulation mainly under these aerated conditions (Hawker, 1966). Within a reed vegetation aeration is poor in the denser litter layer with locally a depletion of oxygen or excess in carbon dioxide (by respiration) and

possibly other volatile gasses all having potential deleterious effects on fungal growth and sporulation and creating specific microhabitats that could influence fungal community composition (see Griffin, 1994; Wainwright, 1992). On the tidal marshes sediment is only aerated in the top few mm, while many reed stand soils are permanently flooded. As a consequence litter entering the water mass will sink to the bottom and will be buried or decomposed in poorly aerated circumstances (but see Apinis et al., 1972b for some fungi capable of growth in mud) and most subterranean plant parts are completely surrounded by anoxic environment (Mitch & Gosselink, 2000). Not surprisingly *P. australis* is adapted to foresee his extensive belowground plant organs with oxygen (Armstrong et al., 1996) a feature not only of vital importance for plant survival but also to support the rich fungal community associated with the roots and rhizomes (see Wirsel et al., 2001; Damm et al., 2003). Furthermore, as was demonstrated by Damm et al. (2003) oxygen leaching from the root tips towards the soil triggered conidial germination and infection of reed roots by *Microdochium* sp.

Wirsel et al. (2001) demonstrated a variation in endophytic diversity at different *P. australis* sites with different oxygen conditions (caused by different flooding regimes). *Trichoderma* sp. & *Cylindrocarpon* sp. were almost exclusively recovered from roots of reed growing at dry sites, whereas *Microdochium* sp. and *Cladosporium* sp. were more frequently found at flooded sites. An influence of oxygen on the diversity of endophytic fungi on reed is illustrated for root endophytes whereas above ground parts did not show clear patterns except for *Cladosporium* which was frequently isolated from both roots and above ground plant parts and showed similar preference for the flooded sites.

3.1.6.3 Biotic interactions with the fungal community and their impact on reed decomposition

During the development of the fungal community on *P. australis*, fungal species probably interact in multiple ways with other fungi and several biological groups – bacteria, protozoa, other micro-organisms and invertebrate animals of a variety of phyla. These organisms can serve as a dispersal vector, a food source (e.g. nematode trapping fungi), or fungi serve themselves as a direct or indirect (as a part of dead plant material) food source. Hence, biotic interactions have a potential impact on the fungal community structure and subsequently on the whole detrital system.

Fungal interspecific interaction

Once a fungal species managed to establish in the reed substrate, growing mycelium can develop different strategies to exploit his host (primary resource capture) (see §3.1.4). Soon mycelia experience combat (Fig. 5) when defending their own captured resources for intruders or when they are wrestling for resources of another mycelium. All those competitive interactions (which is both primary resource capture and combat in the sense of Cooke &

Rayner, 1984) can have different outcomes: if detrimental to either or both, the interaction is described as competitive; detrimental to neither, but also not beneficial, being neutral; beneficial to both as mutualistic interation.

The high diversity and abundance of fungal endophytes and saptrotrophs in grasses increases the potential for interactions among species. For example, in natural grass populations of *Festuca arizonica*, the presence of *Acremonium* (as *Neotyphodium*, tribe Balansiae, Ascomycota) a systemic endophyte, is negatively associated with the presence of most other endophytic fungal species (Schulthess & Faeth, 1998). Furthermore antimicrobial activity of some *Phragmites* endophytes against some bacteria and fungi was demonstrated by Peláez et al. (1998), indirectly supporting the idea of inducible mutualism (see §3.1.2) for these species against pathogens (Table 2). *Periconia igniaria*, a *Phragmites* endophyte, but illustrated with a culture isolated from *Diplotaxis,* showed both strong antibacterial (*Staphyloccus, Bacillus*) and antifungal (*Saccharomyces*) activity. Fisher et al. (1986) have shown a particularly high antibiotic activity of species belonging to the genus *Microsphaeropsis*. This capability of some of the fungal endophytes to produce compounds with antibacterial and antifungal activity indicates the potential of interactions between micro-organisms, their possible role of endophytes in host resistance against certain pathogens and confirms the potential of *Phragmites* for screening programmes of bioactive natural products for among other things pharmaceutical causes. The isolation of two endophytic species (*Epicoccum purpurascens* and *Aureobasidium pullulans*), also recorded on *Phragmites,* produced auxin-like factors (indole-3-acetic acid (IAA) and indole-3-acetonitrile) which are known plant hormones involved in several plant processes (Petrini et al., 1992) is another indication for this role of fungal endophytes. Furthermore, interference competition seems to be quite common in saprotrophic fungal communities (Shearer, 1995) and was demonstrated for *Massarina aquatica* (found among others on *P. australis*, see appendix) (Fisher & Anson, 1983).

Table 2. A list of phragmiticolous endophytes with known antibacterial and antifungal activity.

	PSEUD	SERR	ENTER	MYCOB	STAPH	BACIL	CRYPT	CANDI	SACC
Phoma-like	–	–	–	–	–	–	+	–	–
Sporormiella grandispora	–	–	++	++	++	++	–	–	–
Sporormiella intermedia	–	–	–	–	–	+	–	–	–

Activities were classified according to the diameter of the inhibition zones around the point of application of the sample. ++, more than 15 mm; +, less than 15 mm; – no inhibition. PSEUD, *Pseudomonas aeruginosa*; SERR, *Serratia marcescens*; ENTER, *Enterococcus faecium*; MYCOB, *Mycobacterium smegmatis*; STAPH, *Staphylococcus aureus*; BACIL, *Bacillus subtilis*, CRYPT, *Cryptococcus neoformans*; CANDI, *Candida albicans*; SACC, *Saccharomyces cerevisiae* [Adapted from Peláez et al., (1998)].

Once a mycelial individual managed to complete a resource capture, consolidation of the conquered resources against competitive organisms may be aided by further morphogenetic

transitions. Those changes may be triggered by the presence of another individual which it can not overgrow, or on encountering physicochemical conditions which preclude growth. The reaction may end up in a dense, resistant, pseudosclerotial plate made up of proliferated hyphae. Such fungal interaction, with blackening or intensified coloration of the reaction zone, was regularly observed on *Phragmites* (Fig. 5), although considered to be more typical for wood inhabiting basidiomycetes (Rayner & Todd, 1979).

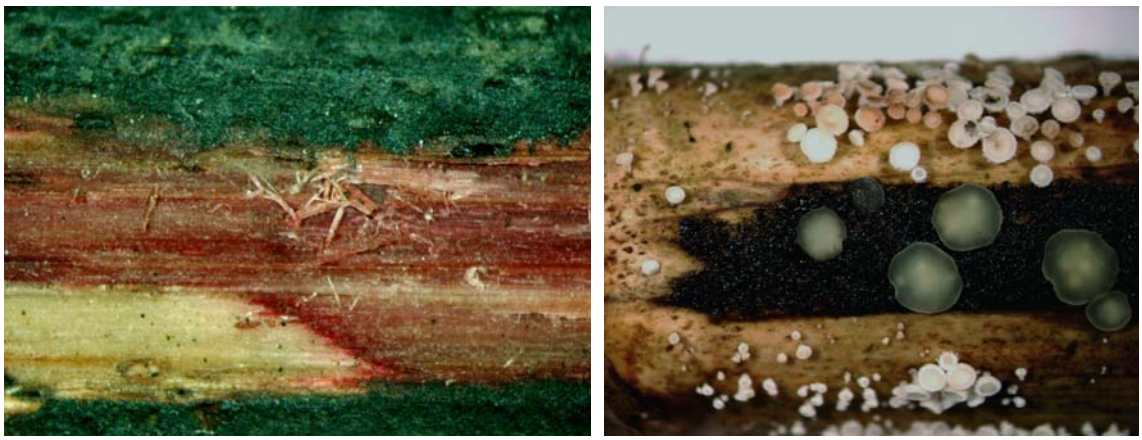


Figure 5. Fungal interaction on stems of *Phragmites australis*. Left picture shows the mycelial interaction of *Massariosphaeria typhicola*. with an unidentified species. The right picture shows the interaction of *Mollisia retincola* with *Lachum controversum*.

Bacteria

Although fungal decay is the most obvious component of the mineralization process of reed plant matter, at least during initial decomposition in aerated conditions both during standing death, on the sediment and in the water (Findlay et al., 2002; Gessner 2001; Komínková et al., 2000), it was previously postulated that bacteria fulfil a major role in the decomposition of emergent macrophytes (Mason, 1976; Benner et al., 1986; Moran et al., 1988). However, conclusions from the latter studies could have been biased because of methodological problems (e.g. inhibitors (antibiotics) used and unnatural conditions) (Newell, 1993; Newell, 1994; Gessner et al., 1997). Studies that simultaneously measure bacterial and fungal production (e.g. Komínková et al., 2000) stress the fungal importance and could further elucidate the role of organoosmotrophs.

As both eukaryotic and prokaryotic micro-organisms are present on the reed material it is possible that some species of bacteria are interacting with the fungal decomposers (e.g. Hill & Patriquin, 1992). Mille-Lindblom & Tranvik (2003) described an bilateral antagonistic relationship between bacteria and fungi in a microcosms experiment in laboratory conditions (see also Mason, 1976 and Table 2). However the extrapolation of such an experiment and the impact of these interactions in natural circumstances is difficult and doubtful because of the

unnatural starting-point: excluding fungal colonization on the stem prior to water submergence (additionally the latter is also unnatural because stems normally float in an initial phase of decay (Gessner, 2000)), the limited duration of the experiment on an unnatural sized substrate (the smaller the more attractive for bacteria (Sinsabaugh & Findlay, 1995)) and stem incubation for about 3 months, while in natural conditions fungal colonization on stems takes at least 3 months (pers. obs.). Additionally at least two of the taxa selected in Mille-Lindblom & Tranvik's experiment are common aerial fungi (*Alternaria alternata* and *Epicoccum nigrum*), perhaps not well suited to demonstrate the aquatic fungal decay of *P. australis*.

When reed remnants fall on the sediment or enter the water it is likely that bacteria get a more pronounced role in the decomposition process during initial phases when assimilating dissolved organic matter (DOM) (e.g. Oláh, 1972; Tanaka, 1991) and later once the litter gets deoxygenated (Webster & Benfield, 1986) or as the plants start to fragment (Benner et al., 1988; Sinsabaugh & Findlay, 1995).

Insects

Phytophagous insects and sap sucking insects such as aphids (e.g. *Hyalopterus pruni* is common on reed, pers. obs.) (e.g. Tscharntke, 1999) are probably some of the first organisms interacting with the fungal species present in/on the living plant. Although an increased resistance against herbivory (among others by insects) by fungal endophytic colonization is suggested (Clay, 1986; Petrini et al., 1992; Wilson & Carroll, 1997; Saikkonen et al., 1998; Gange, 2001; Gange et al., 2002) there are no reports demonstrating such a relationship for *P. australis* endophytes. However, the generally low rate of grazing on common reed by insects (e.g. van der Toorn & Mook, 1982) could perhaps be related to a species rich fungal community already present on living plants (e.g. Apinis et al., 1972a; Wirsel, 2004; pers. obs.).

A direct and close relationship between a fungus and an insect is described by Rohfritsch (1992; 1997). *Lasioptera arundinis,* a common gall midge, induces galls within stems of *P. australis*, in so far it exclusively infects side shoots induced after the plant was parasitized by *Archanara geminipunctata* (Tscharntke, 1999) and in obligate relationship with a fungal symbiont *Ramichloridium subulatum* de Hoog (Rohfritsch, C.N.R.S., Institut de Biologie Moleculaire des Plantes, Strasbourg, France, CBS identification, pers. comm.). In literature this species is mentioned as *Sporotrix* sp. or *Macrophoma* sp. (e.g. Bissett & Borkent, 1988; Rohfritsch, 1992, 1997). A closely related gall midge *L. hungarica* shows a similar close relationship with a fungus also named as *Sporotrix* sp. but close examination of this species could probably reveal its conspecificity with *R. subulatum* or a sister species. The galls of *L. arundinis* on the reed stems appear to be ambrosia galls. The gall midge collects and carries its symbiont in specialized structures: the mycangia. The highly specialized relationship is symbiotic as the biotrophic fungus is inoculated on the otherwise unavailable substrate and the fungi macerate the stem tissue for larvae to feed (Rohfritsch, 1997).

Collembolae could have a substantial impact on the decomposition of reed. These springtails are common in the freshwater part of the studied estuary. Furthermore, several species are known to be selective feeders on fungal sporulation structures and therefore they could have a potential impact on the fungal community structure (Christensen, 1989). Although to some extent their feeding activity will have its impact on the decomposition of the plant matter it was shown by controlled microcosm experiments with pine needle litter that fungal species richness, diversity, dominance, or frequency was not affected (McLean et al., 1996).

There are many other potential grazers and shredder insects of living and dead reed matter belonging to distinct insect groups (e.g. *Limnephilus* spp.,...) (e.g. Polunin, 1982).

Other micro- and macroinvertebrates

Once reed enters the detrital system several invertebrates, mainly belonging to the gastropods and amphipods, are known litter transformers (e.g. Gessner & Van Ryckegem, 2003). But in contrast to the poor assimilation efficiency of 17% for *Gammarus pulex* feeding on *P. australis* leaf blades (Mason & Bryant, 1975), the efficiency is much higher if they feed on fungi present on the detritus (for *G. pseudolimnaceus*) (Bärlocher & Kendrick, 1975). This observed adaptation results in preference pattern of many invertebrates for substrate colonized by fungi in running freshwater streams (e.g. Arsuffi & Suberkropp, 1989) and tends to be correlated with the (leaf) palatability and nutritional quality (Suberkropp, 1992).

Although their contribution to the decomposition process of *P. australis* was not assessed it is thought that periwinkles have a substantial impact on the whole process in brackish tidal marshes of the Scheldt estuary. Especially *Assiminea grayana* (≈ 2-3 mm shell length) was often observed shredding the reed substrate and the species can reach huge densities of up to 40 000 specimens per m² in brackish tidal marshes (counts near our study site) (Dumoulin, 1989); more recent counts yielded about 12 000 specimens per m² (Bruyndonck et al., 2000). The impact of gastropod grazing on the detrital system was noticed by Lee (1990) for tidal reed decomposition and is a well documented phenomenon for the *Spartina alterniflora*-saltmarsh ecosystem (summarized in Newell & Porter, 2000). In this system the snails (*Littoraria irrorata*) were able to remove living-fungal mass more rapidly compared to leaf mass, and they can efficiently digest saltmarsh-fungal mycelium. If densities are high enough (as in our brackish study site) the periwinkles have the capability of controlling fungal standing crop and are hence one of the most important mycophagous organisms controlling microbial fate by microbivory in a salt marsh (and possibly in our studied brackish tidal marsh).

Figure 6. *Assiminea grayana* dominant periwinkle in brackish tidal marsh (Saeftinghe site) grazing heavily on decomposing *P. australis*.

Within the brackish tidal marsh of the Scheldt estuary periwinkles are not the only grazers of reed litter. Amphipods (undetermined representative of the family Talitridae) are also suspects. Based on their presence in the marsh litter layer, the well-established mycovorous tendencies found for freshwater species (Suberkropp, 1992), and the demonstrated shredder activity of amphipods on common reed mainly during the summer (Polunin, 1982).

Many more invertebrates are feeding on reed litter in the Scheldt marshes and other ecosystems with reed vegetations, all could have an influence on fungal communities and fungal biomass fate. Worth mentioning from our own experience are beetles. Several times I could surprise them while they were grazing on apothecia, mites were several times observed under the microscope with their interior almost completely filled with fungal spores, the same for ciliates (Fig. 7) clearly capable of feeding on fungal spores of a certain size. Further important fauna components are nematodes: these organisms can be mycovorous (both parasites and myco-detritivores) or they are a nutrient source for nematode-destroying fungi

(e.g. Barron, 1982). Old fungal perithecia are almost without exception colonized with nematodes, whether these are fungivorous or bacterivorous is not established during our observations but for sure eggs were dropped in high numbers in the decomposing fruit bodies. Several studies observed an impact of nematode feeding on fungal populations and the ability to affect growth of fungi appears to be variable and to depend on the species of nematode and fungus (Ingham et al., 1985; Chen & Ferris, 1999).

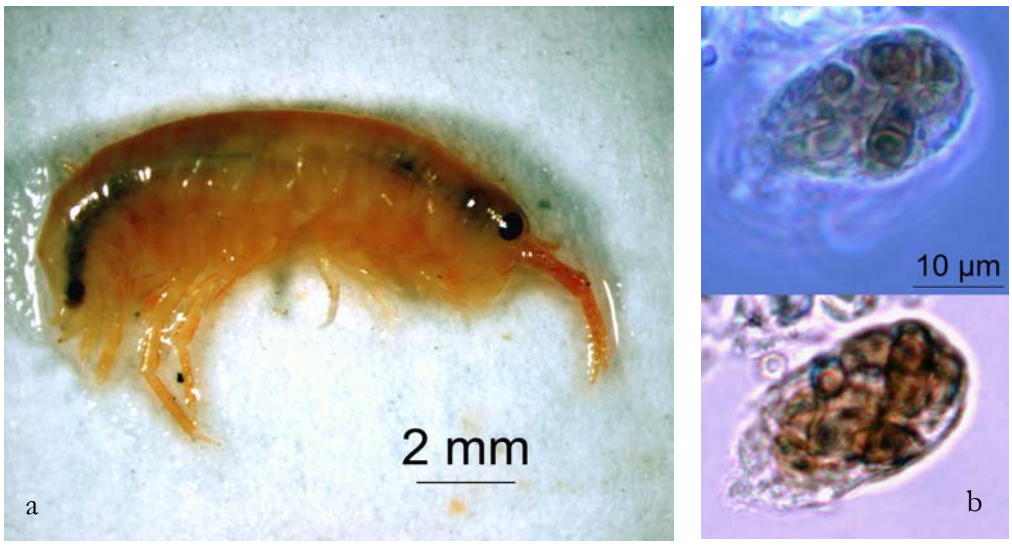


Figure 7. a) Amphipod found on the brackish tidal marsh grazing on dead organic matter. b) Ciliate feeding on *Hendersonia culmiseda* in microscopic slide.

4. Decomposition of *Phragmites australis*

4.1 Introduction

Many marsh ecosystem-functions are linked to primary plant production (e.g., Johnston et al., 1990). Within tidal and other wetland ecosystems emergent macrophytes such as *Phragmites*, *Typha*, *Carex*, *Spartina* and *Juncus* are often highly productive (Květ & Westlake, 1998; Mitsch & Gosselink, 2000), rivalling crop production in intensive farming. Generally little of this is grazed by herbivorous animals (e.g. van der Toorn & Mook, 1982; Odum, 1988; but see Haslam, 1970 reporting on substantial losses up to 80%) and after senescence, it becomes available as a habitat, carbon and energy source for microbial decomposers (Polunin, 1984; Mann, 1988; Granéli, 1989; Dvořák & Imhof, 1998).

Decomposition of all this plant litter is an important component of nutrient cycling in wetlands (Mitch & Gosselink, 2000) and changes in the processes may be one of the important actors in explaining the expansion and decline of reed vegetation all over the world (see §2.2).

Vascular plant litter first immobilizes and then releases nutrients as it decomposes. Most studies mention a first phase of leaching or releasing soluble nutrients (dissolved organic matter, DOM) immediately after death, often followed by an immobilization of exogenous nutrients. By immobilizing nutrients, litter may be a significant sink for nutrients in wetlands (Johnston et al., 1990). As decomposition proceeds further, mineralization becomes dominant and nutrients are released, furthermore the impact of fragmentation and shredder invertebrates becomes greater (Webster & Benfield, 1986; Graça et al., 2000).

The fate of above ground biomass on the tidal marsh (and in other wetlands) to the decomposers compartment has multiple pathways (Fig. 8) resulting in a variable time of standing decay before the plant material enters the litter layer. The decay time in the litter layer is also variable; finally some of this plant mass is buried by sedimentation or exported to the river as dissolved or particulate organic mater or as larger plant material. Belowground, decomposition is first aerobic in the top layers and afterwards mainly anaerobic if buried deeper in the soil. Leaves can be blown out of the reed belt in the river and tides are responsible for exchange of reed parts and other forms of organic matter between the tidal marsh and the river. In the river, the reed parts and particulate organic matter can have a floating, mainly aerobic decay phase. Once suspended, larger reed parts probably soon settle on the mainly anaerobic sediments where they slowly decompose and finally part of it is permanently buried. These various transport possibilities for above ground material are in contrast to the belowground biomass which is mineralised on the spot.

Aboveground shoots die annually, with the different plant parts showing distinct patterns. Soon after the appearance of the sharp-conical shoots from the rhizomes in March-April, the tough bud scales surrounding the first internodes senesce and form the first potential substrate for microbial decomposers (pers. obs.). Starting from the end of April, leaf blades and leaf sheaths form successively, acropetally, with the last leaf blades being formed at the end of August at the apex of the stems. As the shoots grow, the lower leaves lose their

assimilation function in the dark, sheltered environment and senesce. Falling of lower leaf blades starts in June or the beginning of July. The large bulk of leaf blades are dropped in October and November while the most upper leaf blades are dropped during winter. Senescence of stems is from top to bottom and resorption of carbohydrates and nutrients is an ongoing process during growth and senescence (Granéli et al., 1992). This pattern of successive senescence of leaves (blades + sheaths) and stems was described previously (Granéli, 1990). The leaf sheaths at a corresponding height in the canopy become senescent a few weeks (less than a month) later (pers. obs.). The lowest stem internode often remains green during the winter, possibly indicating an ongoing resorption during the winter months (pers. obs.). Some of the lower sheaths are lost by tidal wave action after the lower leaf blades were dropped. The upper part of a reed shoot is mainly formed out of tightly packed, nested leaf sheaths with only a thin stem in the centre. This part of the reed culm is very fragile and it was noticed that the majority of the upper parts were quickly (within the first year) blown off, especially when they carry a panicle. The rest of the reed culm can stay in standing aerial position for several years (Ganéli, 1990).

Decomposition processes of aboveground reed biomass has been discussed in more than 30 papers: Gorbunov (1953); Krashenninikova (1958); Olah (1972); Hargrave (1972); Pieczyńska (1972); see Rodewald & Rudescu (1974 and references herein); Mason & Bryant (1975); Mason (1976); Chang & Oh (1977); Chang et al., (1978); Andersen (1978); Úlehlová (1978); Larsen & Schierup (1981); Blake (1982); Polunin (1982); Reice & Herbst (1982); Tanaka & Tezuka (1982); Kufel & Kufel (1988); Lee (1990); Chergui & Pattee (1990); Tanaka (1991); Van der Valk et al., (1991); Hietz (1992); Akanil & Middleton (1997); Denward & Tranvik (1998); Anesio et al., (1999); Denward et al., (1999); Mun, et al., (2000); Rooth & Stevenson (2000); Komínková et al., (2000); Gessner (2000, 2001); Windham (2001); Findlay et al., (2002); Scarton et al., (2002); van Dokkum et al., (2003). It is beyond the scope of this introduction to review all those studies but some trends and relevant results of these studies are summarized below.

Probably the three most important variables determining the rate of organic matter loss among sites are oxygen, moisture and temperature differences (Brinson et al., 1981), factors evidently acting on the activity of microbial organisms (see §3.1.6). An average decomposition time of *P. australis* plant parts cannot be given (see Gessner, 2000 for a compilation of

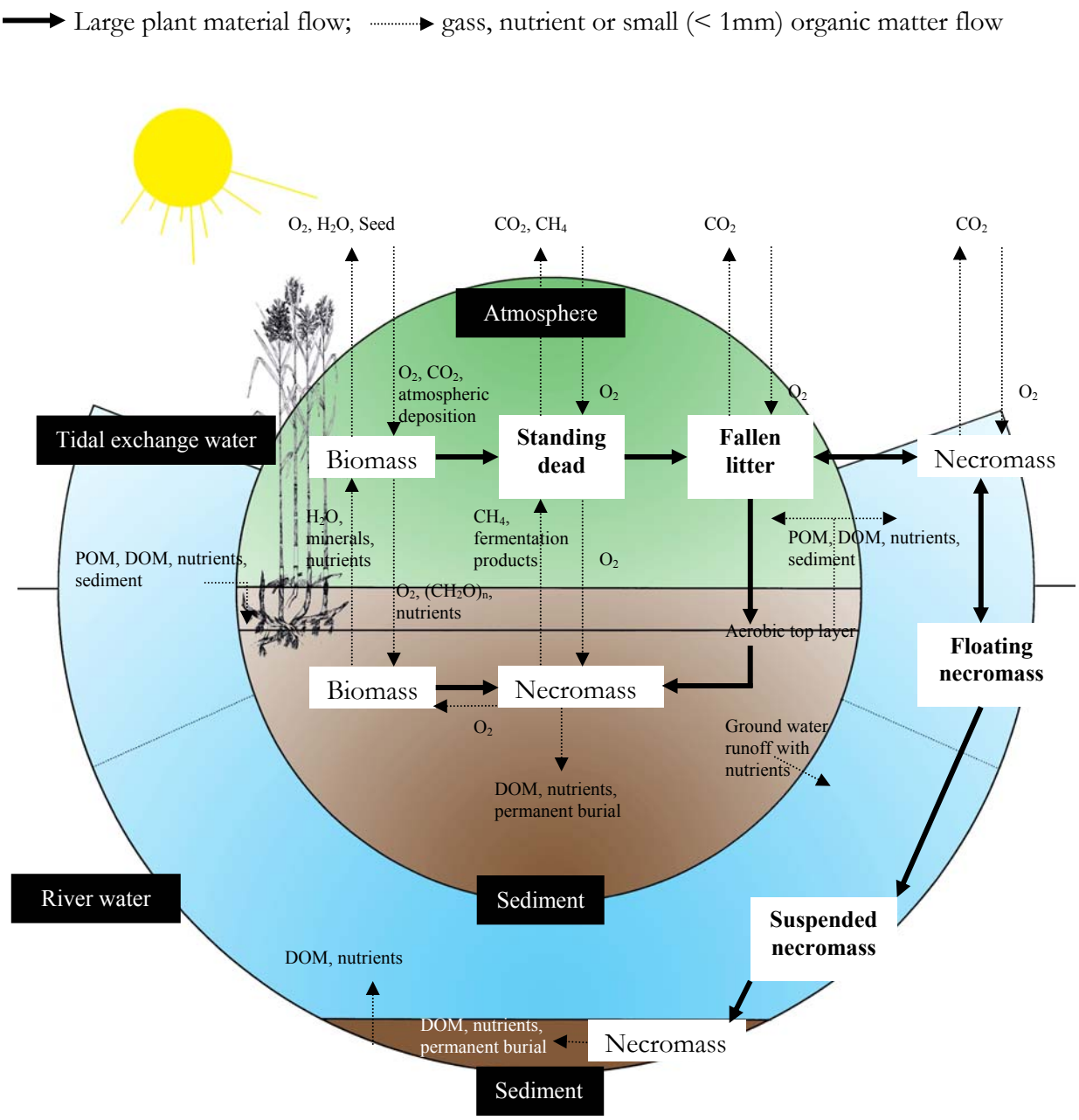


Figure 8. Schematic representation of the processes in the decomposition system of a tidal marsh ecosystem.

decomposition rates), because of the large phenotypic variation of reed resulting in obvious differences in resource quality, the incongruence of methods used to describe decomposition, and because of the large discrepancy found due to ecosystem differences. Moreover within one ecosystem, decomposition rates are often based on estimates for litter remaining on one spot in a reed stand, while litter behaves dynamically (Fig. 8) and is decomposed in a highly heterogeneous environment (this disturbance may even be more important in tidal wetlands compared to nontidal areas) making all (static) litter decay studies more or less rough approaches of natural breakdown. In conclusion this means that only approximate lengths of periods can be given for almost complete decay. Recalcitrant stems are decomposed the slowest, with studies indicating from about 3 years in ideal conditions and possibly much longer in standing aerial position if tumbling is prevented or for example in unnatural circumstances on thatched, sun exposed roofs. Rarely leaf sheaths have been studied separately (see Gessner, 2000) but their decay, seems to equal that of leaf blades, between 1 and 3 years.

4.2 Standing decay

The leaf fall and collapse of reed material to the sediment or surface waters typically does not happen immediately following carbohydrate and nutrient resorption, shoot senescence and death. As a result, large amounts of dead plant matter remain standing within reed beds (Lee, 1990; Meganck, 1998), and are colonized and decomposed in an upright aerial position (Gessner, 2001). However the proportion of culms falling is thought to vary greatly between years and sites and is influenced by extreme weather conditions (storms, heavy winds,…). Readers are referred to Asaeda et al., (2002) and Soetaert et al., (2004) [with data on our study area!] for general models simulating the collapse of standing shoots. The retention of standing dead stems is an important adaptive strategy, providing gas channels to and from rhizomes and roots via aerenchyma (Armstrong et al., 1996). Leaf sheaths stay attached to the standing dead stems and if stems stay upright long enough they can be decomposed almost completely (pers. obs.; Kuehn et al., 2004).

Despite the common occurrence of this initial standing decay phase of many emergent macrophytes (Newell, 1993; Kuehn et al., 1999; Gessner, 2001), the process is often ignored or circumvented in decomposition studies, or altered by unnatural conditions (Newell, 1993; see §6.1.2 methodology). As a consequence, studies not considering the standing decay phase to design their experiment, might result in an inaccurate description of processes and microbial dynamics during decomposition (Newell, 1993; Kuehn et al., 2004). Most decomposition studies have focused on the processes under submerged conditions, perhaps due to the assumption that decomposition occurs mainly after litter has fallen to the sediment or the water (Polunin, 1984). Hietz (1992) found that leaf litter exposed in the water lost weight much faster (45-85% of the original ash free dry mass), compared to aerial decay (losing 23-28%). For the culms differences where not that important (± 6%, Fig. 1). As discussed in §6.1.2 , the experiment altered natural conditions: the aerial decay of leaves was followed for an unnatural

long period; only very seldom leaves stay trapped in an aerial position for more than 2 years. Furthermore by hanging the stems in the air, they are not influenced by the fluctuating water level on their base, which could cause a higher microbial activity by, for example, a more humid internal atmosphere in the standing stems by gas fluxes in the aerenchyma channels of the stems. Gessner (2001) studied dead canopy leaves in a natural way on the plants. The tagged leaves lost up to 28% of their initial mass. Nitrogen and phosphorus concentrations decreased by 39-77% with time. The latter indicating that a large proportion of these nutrients are translocated to the rhizomes. Mass (mainly carbon) loss of leaf blades while hanging on the plant was found to be mediated both by plant (resorption) and by microbial activity, unlike the nutrient dynamics which appear to be largely governed by plant processes (Gessner, 2001). Kuehn et al., (2004) estimated the percentage mineralization of the annual net aboveground production to be roughly 29% for leaf sheaths and 8.5% for leaf blades (which have only a short period of standing decay in contrast to sheaths). Little carbon mineralization was recorded during the first year for culm tissue. Roughly assuming that the results of Kuehn et al., (2004) are applicable to the observations of Gessner (2001) we could estimate the mass loss observed during hanging decay of leaf blades to be for about 30% due to microbial activity and remaining (70%) mass loss is probably due to resorption and partly to leaching.

As a consequence of standing decay of leaves and culms, significantly smaller amounts of particulate organic matter reach the marsh sediments or the aquatic compartment than is inferred from estimates of net primary production alone. Therefore, budgets of carbon and nutrient flows should incorporate the organic matter dynamics in the upright position (Gessner, 2001; Kuehn et al., 2004).

4.3 Decay in the litter layer

Once the litter falls it can enter the aquatic compartment or in drier reed stands and tidal marshes it may fall onto the sediment. Within an estuary the litter can by confronted with freshwater up to salt water and ultimately it can float to the sea if not sunken to the bottom of the river or redeposited on the marsh.

Most studies focussed on fallen, submerged litter (partly summarized in Polunin, 1984; Gessner, 2000), while few have focussed on fallen litter on the sediment. Some studies have studied reed decay in tidal reed marshes, characterized by the periodical wetting of the reed substrate during decomposition (Lee, 1990; Windham, 2001; Findlay et al., 2002; Luo et al., 2002). These conditions are supposed to be optimal for decomposition if aerobic conditions are maintained and under some optimum regime of wetting and drying (Brinson et al., 1981). Lee (1990) recorded the highest decomposition rate of *P. australis* leaves on a mud plat on less flooded (47.5%) sites while decomposition was slower at the sites inundated nearly permanently (86.5 and 97.5% of the time).

4.4 Fungal performance and regulation of decomposition processes

The range of the above described physical and chemical plant characteristics and external variables will generate a variety of distinct niches within the resource. Additionally, specific niche quality changes during decomposition under influence of the microbial colonizers (mineralization and nutrient immobilisation) and by passive adsorption of external molecules. All these variables will select on the fungal component which is both diverse and each unique in life history, not surprisingly resulting in a wide spectrum of taxa and decay patterns even for a single plant resource.

Studies that attempt to establish relationships between breakdown rates, litter characteristics and fungal performance in a site generally conclude that concentrations of nutrients, lignin, phenolic compounds or a combination of these constituents are critical in determining litter decomposability (Kjøller & Struwe, 1982; Melillo et al., 1982, 1984; Enriquez et al., 1993; Gessner & Chauvet, 1994). However, general principles are difficult to formulate as regulation of decomposition in wetlands and other ecosystems seem to vary broadly according to the relative impact and interactions of a range of controlling factors, including both external ones related to specific environment and factors intrinsic to the decomposing plant material (Enriquez et al., 1993; Gessner et al., 1997).

Of the different carbon sources initial lignin concentration is generally negatively correlated with breakdown rate (but see Schaefer et al., 1985) and this is reflected by lower fungal biomass and productivity in resources with high initial lignin concentration (Melillo et al., 1982; Gessner & Chauvet, 1994; Gessner et al., 1997). Lignin is particularly recalcitrant and closely linked both physically and chemically with the other cell wall polymers (cellulose and hemicellulose), therefore it is thought that this polymer limits the decomposition rate by limiting the accessibility for micro-organisms to the more easily degraded carbon sources. Although this may be true for leaves entering streams being dominated by aquatic hyphomycetes which have limited lignin degradation capacity (Suberkropp, 1992; Gessner & Van Ryckegem, 2003), lignin in aquatic grasses might be well available for fungal colonizers (Bergbauer & Newell, 1992; Bergbauer et al., 1992b). Some genera (*Phaeosphaeria*, *Mycosphaerella*, *Buergenerula*, *Passeriniella*, *Stagonospora* and *Helicomyces)* demonstrated to degrade lignocellulose and the lignin components on *Carex* and *Spartina* resources were observed on *P. australis* during the present study (see appendix) (Bergbauer & Newell, 1992; Bergbauer et al., 1992a, b; Newell et al., 1996). Therefore, if enzymatic capacity proves to be readily available among phragmiticolous decomposer fungi, recalcitrant carbon sources might not be the most important factors determining decay rates in *P. australis*.

Initial nitrogen and phosphorus concentrations often have a positive relationship to the breakdown rate (Enriquez et al., 1993) and to the fungal biomass and activity (Newell et al., 1996) and stand in close relation to the structural plant carbon composition. Often a high correlation between initial lignin/initial nitrogen to the decay rates is observed. The positive

relationship between breakdown and initial nutrient pool among plant matter could be explained by the high requirement and incorporation of these nutrients by microbial decomposers (e.g. Anderson & Macfadyen, 1976; Beever & Burns, 1980; Dowding, 1981) and are more prevalent in sites with little exogenous nitrogen available (Melillo et al., 1982). In many decomposition studies nitrogen is stated as the limiting nutrient (e.g. Vitousek & Hobbie, 2000), however in other ecosystems, phosphorus or a combination of both nutrients is thought to be the limiting nutrient for fungal performance and efficient decay (e.g. Enriquez et al., 1993; Newell et al., 1995). Although increasing nutrient concentrations are often reported as being positively related with the rates of litter decomposition (e.g. Melillo et al., 1982) there are several studies showing that high nutrient concentrations or the addition of inorganic nitrogen suppresses litter decomposition (Fog, 1988; Carreiro et al., 2000; Hobbie & Vitousek, 2000). Carreiro et al. (2000) demonstrated the suppression of lignin phenol oxidase by additions of N in the field and their results could explain the paradoxical and contradictory effects of N on litter decay rates. In litter with high cellulose and low lignin concentrations, nitrogen stimulated the fungi capable of producing cellulolytic enzymes (brown-rot fungi) while litter with high lignin concentrations had lower decay rates with additional N. This seemed to be caused by a reduced functionality of the lignin-degrading enzymes.

Few papers investigate fungal performance in relation to the factors affecting their activity on *P. australis*. Fungi were found to be active during standing decay (Gessner, 2001; Kuehn et al., 2004), in the litter layer (Findlay et al., 2002) and in submerged conditions (Komínková et al., 2000) on all investigated plant parts (leaf blades, leaf sheaths and stems).

During decomposition, several studies report the effect of nutrient (N and sometimes P) immobilisation by litter-associated micro-organisms (Polunin, 1984; Webster & Benfield, 1986; Vitousek & Hobbie, 2000). Nutrient immobilisation refers to an increase in concentrations or even a net increase in nutrient amount compared to the original stock present at the onset of decomposition and is mediated by external nutrient incorporation and the conservation of nutrients within the resource (Gessner, 2001). Fungi colonizing solid resources seem able to access the external nutrient pool if the internal nutrients are inappropriate or inaccessible to sustain growth both in the water and on tidal marshes (Newell, 1993; Suberkropp & Chauvet, 1995; Suberkropp, 1995; Tank & Webster, 1998; Grattan & Suberkropp, 2001) and possibly, as suggested on *Spartina*, they could even obtain some of their carbon from outside the decaying plant (as dissolved organic carbon) (Newell 1993; Newell et al., 1996). Besides nutrients several other elements tend to accumulate in the decomposing litter such as phenols (tannins) (e.g. Hättenschwiler, & Vitousek, 2000) and heavy metals (e.g. Gadd, 1993), two groups of molecules known to have generally deleterious effect on fungal physiology and growth (Griffin, 1994).

With sizable fungal biomass measured during reed decay (e.g. Komínková et al., 2000) fungi could contain substantial amounts of nutrients captured within the decaying plant material. However their contribution to retention of nutrients within leaf blades prior to shedding was

thought to be low compared to the initial stock (Gessner, 2001) and probably mainly mediated by plant resorption processes and leaching.

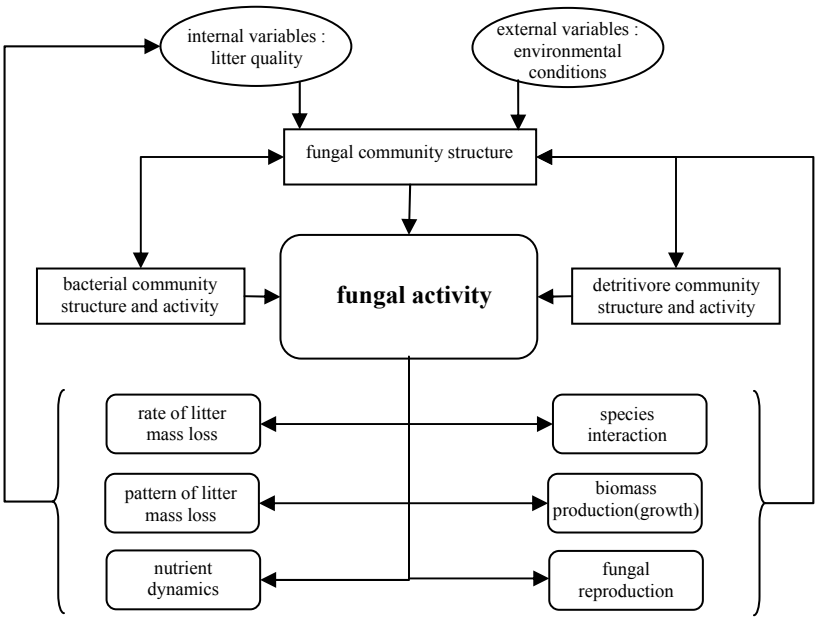


Figure 9. Conceptual model of a fungus dominated reed decomposition system (adapted from Gessner et al., 1997).

From the above literature study it is clear that fungi play an important role in the decomposition of reed and in Fig. 9 a conceptual model of a fungus dominated reed decomposition system is schematically presented viewed from a fungal and a process perspective (adapted from Gessner et al., 1997). The fungal community (discussed above) with the intrinsic fungal activity is controlled by internal (litter quality) and external (environmental conditions) variables. Biotic interactions of bacterial and detritivore activity modify fungal activity patterns and possibly may cause changes in fungal communities by direct interactions with e.g. fungal establishment by spore consumption or by changes in suitable germination conditions. The result of the fungal activity can be divided into those impacting the decomposition process and those that have an influence on fungal performance. These effects eventually have their feedback to the internal litter quality and directly to the fungal community by influencing the intra- and interspecific interactions and species fitness.

5. Aims of the study

In this thesis we investigate several aspects of fungi associated with a single host plant, common reed (*Phragmites australis*) in a wetland ecosystem. Our study area, the Scheldt estuary (Fig. 10) was chosen because *P. australis* is one of the dominant plant species in this ecosystem (§6.2.2.4) and consequently an important component in tidal marsh functioning (C, N and P cycles). Our Ph.D. contributes to the OMES* project with supplementary data within two major research areas 1) survey of biotic diversity and 2) process research in the Scheldt estuary. No information was available on the biotic diversity of eukaryotic organoosmotrophs, nor was their any information on their ecological behaviour. The presented project gives additional information on decomposition of organic material in the Scheldt estuary (Project D2.2/D2.3) (Meire et al., 1997), and provides a more detailed picture of C, N and P fluxes on a tidal marsh and the potential role of fungi in the processes.

The concrete aims of this study are:

(1) Investigating and describing the high fungal diversity on common reed suggested by previous researchers (Saccardo, 1889; Taligoola 1969, Poon & Hyde 1998a; Wirsel et al., 2001). Furthermore, we aimed to provide each taxon with critical taxonomic information based on personal observations on fresh material collected from the field. Combined with the information compiled in a database with world-wide fungal records on *P. australis* this contributes to an assessment of the world-wide diversity of reed fungi.

(2) Unravelling the fungal community structure on reed. This fungal community was suggested to be species rich and complex with several stages of fungal succession which could differ among plant parts and microhabitats (Apinis et al., 1972a). This aim is supported by our taxonomic knowledge.

(3) Investigating the functional ecology of fungi during decomposition processes by the monitoring of fungal biomass, heavy metal-, carbon- and nutrient dynamics. Fungal role during these processes is largely unknown for reed dominated wetlands but expected to be important (cf. Gessner et al., 1997; Gessner & Van Ryckegem, 2003).

(4) Studying the ecological patterns of fungal diversity along an estuarine salinity gradient. Is the fungal diversity pattern consistent with those described for other estuarine organisms (Remane, 1934; Odum, 1988)?

* OMES : Onderzoek Milieu Effecten Sigmaplan

6. Experimental design and study area

6.1 Experimental design

The monitoring of both the fungal community and the decomposition of *P. australis* were conducted in the same tidal marsh referred to as 'Saeftinghe' close to the equally named nature reserve (Fig. 10, 13). Three plots (3 x 2 m) were outlined in the marsh all adjacent to each other. Two plots (plot 1 and 2) were fenced and used for studying the fungal community. A third plot (plot 3) contained the litter bags that were used in the decomposition study.

6.1.1 The study of the fungal community (Chapter 2, 3, 4 and 5)

All aboveground plant parts (leaf blade, leaf sheath and stem; panicle excluded) were screened, if possible, in four different microhabitats (basal, middle and top canopy and the litter layer) during plant's growth and decay for fungal sporulation (Table 3).
In plot 1 we started to monitor fungi in May 2000; while in plot 2 monitoring started one year later (May 2001). Both plots were sampled monthly for a period of 24 months.
Plant parts were followed for distinct periods. Leaf blades were followed in a standing position until they were shed and further until they were nearly completely decomposed in the litter layer. Leaf sheaths were both followed in a standing position in the canopy and in the litter layer until they were nearly completely decomposed. Stems were monitored in a standing position until all tagged stems fell on the sediment. In the litter layer, stems were followed till the end of the experimental period (24 months monitoring of the reed stand) corresponding to a monitoring period of 18 months.

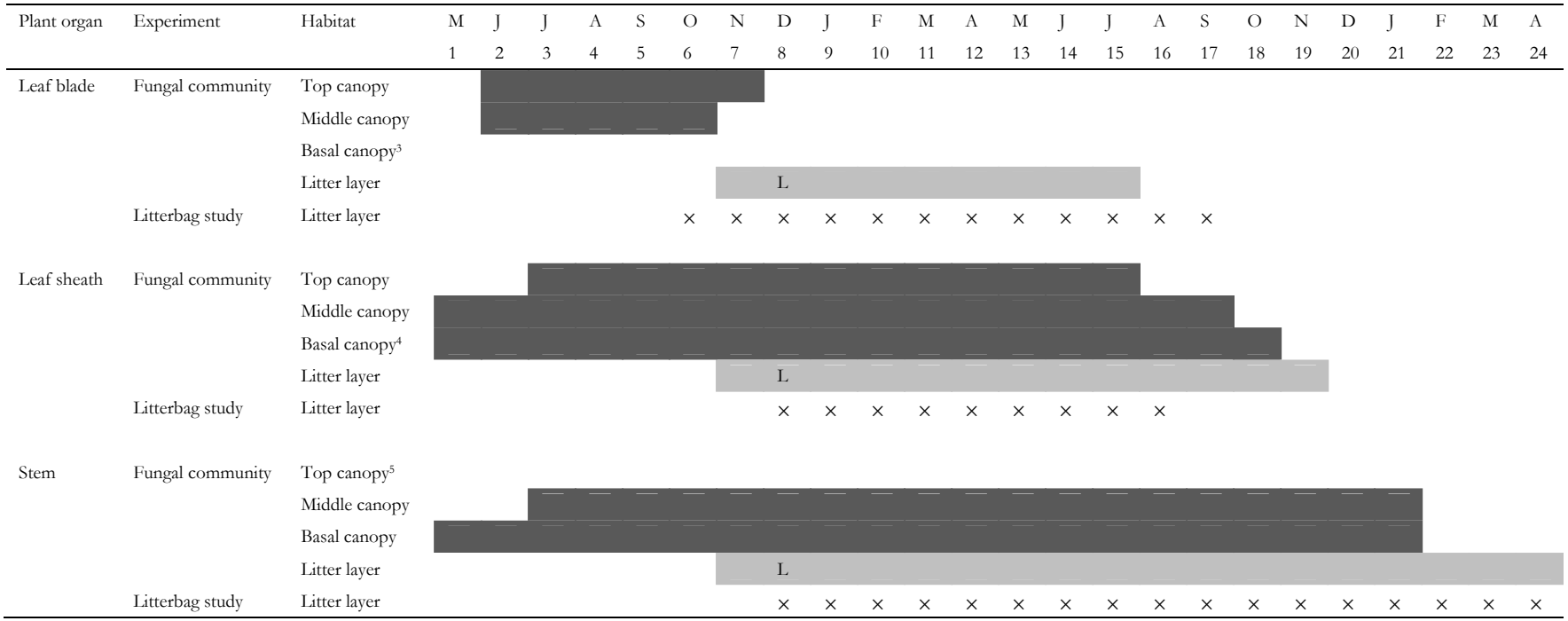
To be sure of the age and residence time of samples in the litter layer, we enclosed leaves and culm pieces from the last growing season in plastic litter bags (see §6.1.2 for comments on methods) with a 4 mm mesh, 35 × 20 cm. Leaf blades and culms (stems + leaf sheaths) were collected during the last week of November 2000 (plot 1) or 2001 (plot 2).
A cumulative species recovery experiment was done at the start of the study and set the replica for leaf blades at 10 in the canopy and in the litter layer and indicated a replica of 10 culm (leaf sheaths + stems) pieces from the canopy and 15 from the litter layer.

SAMPLE ANALYSIS:

- all plant parts were screened separately at magnification × 180 for fungal sporulation structures
- all found taxa were microscopically identified

➔ this results in an extensive database with 139 samples in each plot and for which each recorded taxon had a relative abundance score on a scale of 10 or 15 (the latter scores were transformed to a scale of 10 as a matter of conformity) on each plant organ, in each microhabitat and during each month sampled

Table 3. Sampling and dataset characteristics for the fungal community- and litterbag experiment. Months[1] for which samples were taken for each plant organ[2] in all microhabitats are indicated: fungal community monitoring in the canopy (dark grey) and fungal community monitoring in the litter layer (light grey); litterbag study in the litter layer (×).



[1] first month = May of the year X, last month (24) = April of the year X + 2, X = year 2000 or 2001

[2] Although leaf blade and leaf sheath belong morphologically to the same plant organ 'leaf' we treated them as separated plant organs

[3] leaf blades from the base of shoots were not included as they are soon dropped

[4] base samples of leaf sheaths include the bud scales tightly surrounding basal node of stems

[5] top samples of stems were not included

L: from this moment (December 2000/2001) on plant parts were sampled from the litter layer enclosed in litterbags (litterbags filled in November 2000/2001)

×: sampling of litterbags for litterbag study; start October 2001 for the leaf blades and December 2001 for the culms (leaf sheaths and stems)

6.1.2 The litter bag experiment – decomposition study (Chapter 5, 6 and 7)

Decay dynamics were followed in litter bags positioned in plot 3.

Fully brown leaf blades were collected on 4 October 2001 from standing reed shoots and included in litter bags (4 mm mesh, 35 × 20 cm) which were monthly sampled over a period of 12 months.

Culm sections (leaf sheaths + stems) were collected on 3 December 2001 one metre above the sediment. The samples were put in litter bags (see above) and monthly sampled over a period of 16 months. In those samples we could monitor leaf sheaths for only 9 months as too little material remained in later samples.

SAMPLE ANALYSIS:

- dry mass determination
- ash content
- total N and P concentrations
- structural plant polymers (acid-detergent method)
- fungal biomass determination (ergosterol analysis)

➔ this resulted in an extensive dataset describing decomposition dynamics in our study site.

Species composition on reeds in the litter bags used for the decomposition study was only superficially screened for fungal sporulation. This to ensure ourselves that reeds in the litter bags positioned in plot 3 had a comparable species composition to those followed in detail in the parallel experiment characterizing the fungal community in plot 2.

Importance of methodology

All decomposition studies (cf. §4.1) should be evaluated in terms of the methodology used. As stressed by Newell (1993) and others (Boulton & Boon, 1991; Bärlocher, 1997; Gessner et al., 1997) the methods should not fundamentally alter the natural decay conditions; otherwise false decay processes are risked to be described. A practice referred to by the above authors is the excising of green leaves and other plant parts (often not even moribund), enclosing them in litter bags and submerging them in the water or placing them on the sediment. If green plant parts are sampled for decomposition research the resorption of carbon compounds and nutrients to the rhizome (Granéli, 1990; Granéli, et al., 1992) is disrupted, thus preventing a potential pathway of mass loss resulting in higher initial concentrations of nutrients. This may affect microbial colonisation and rates of decomposition (Gessner et al., 1997). Incubation of green or prematurely sampled plant parts can give an overestimation of the initial leaching process and in subsequent increase in bacteria, assimilating those soluble compounds. Some authors manipulate reed in an unnatural way, they incubate the plant matter in a season where normally little dead reed enters the detritial pool (e.g. summer: Úlehlová, 1978; Pieczyńska,

1972) or they oven dry plant parts (Mason & Bryant, 1975; Andersen, 1978) or ground them (e.g. Tanaka & Tezuka, 1982) thus destroying the inhabitant microbial population or in the case of grounding the samples, preventing fungal colonization and stimulating bacterial production during the decomposition process (Benner et al. 1986; Sinsabaugh & Findlay, 1995).

Use of litterbags has been criticized for the artefacts it can engender (Newell & Fallon, 1989; Boulton & Boon, 1991; Bärlocher, 1997). Depending on the mesh or pore size of litterbags, large shredder invertebrates may be denied access to the plant material. However if mesh is large, possibly large fragments of plants can readily be lost (Boulton & Boon, 1991). This loss of fragments is probably limited if initial size of fragments is rather large like reed leaves or stem sections (cf. Suffling & Smith, 1974). Although internal conditions within litterbags might differ from natural litter layer conditions the circumstances probably satisfactory simulate aggregations of naturally shed plant parts, like the dense packs of litter present in tidal marshes. However natural conditions of exposure to physical and biological influences should always be carefully considered (Swift et al., 1979). For example, one should let litterbag sink naturally to the sediment surface. The direct below water incubation of litter bags ignores the initial phase of floating decay (Gessner, 2000), which is more aerobic and favourable for fungal colonization compared to the suspended or bottom decay. Suspended decay (litter bags hung up in the water) is thought to be only a short phase as most litter will, in natural conditions, sink to the bottom of the lake or river. Hietz (1992) hung up litter bags above the water surface to simulate the standing dead phase, but this alters the natural air/water connection at the base of the stems.

It is of great importance to study the different plant parts separately, as noticed by Gessner (2000) and indicate their (original) position on the whole plant. Different plant parts not only possess a specific microbial community (Apinis et al., 1972a); they show distinct decay patterns as they have a different chemical and physical composition (Granéli, 1990). Furthermore chemical and physical characteristic might differ along the vertical axis of standing shoots.

6.1.3 The fungal species composition of reeds in tidal marshes along the salinity gradient of the Scheldt estuary (Chapter 8)

A third approach was to investigate fungal ecology along a salinity gradient of the Scheldt River (Fig. 10). This was done by a survey with equal sample effort in four tidal marshes along a salinity gradient and complemented by a laboratory experiment assessing growth responses of four fungal isolates to salinity ranging 0-50 ppm.

6.2 The study area: the Scheldt estuary and the natural borders

6.2.1 Introduction

'Complex' is a term often used to describe the estuarine ecosystem. This complexity is evident from numerous studies trying to unravel the biology, geomorphology, hydrology, chemistry and all their interactions. No wonder that there are a dozen of definitions for this ecosystem. We chose to define the (Scheldt) estuary as the part of the river with tidal influence where there is a mixing of freshwater from upstream and saltwater from the sea (Fairbridge, 1980). The entire Scheldt estuary (Fig. 10) has a length of 239.2 km and includes, beside the Scheldt (160 km), which is divided into the 'Westerschelde' (Western Scheldt) on the Dutch part (56 km) and the 'Zeeschelde' (Sea Scheldt) on the Belgian (Flemish) part (104 km), six major tributaries. Those are the 'Rupel' (26.4 km), the 'Dijle' (16.7 km), the 'Durme' (13.3 km), the 'Zenne' (10.3 km), the 'Grote Nete' (8.2 km) and the 'Kleine Nete' (4.7 km) (Claessens & Belmans, 1984, Table 67).

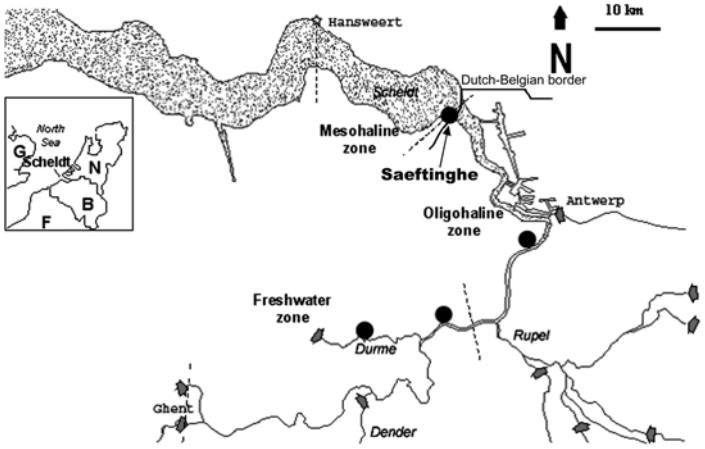


Figure 10. The Scheldt estuary with different ecological regions. The indicated dots are the main study sites with the most downstream site 'Saeftinghe' being the most intensively studied tidal marsh.

Tidal marshes form the natural borders of estuaries and are the transition zone between land and water. Therefore they combine characteristics of terrestrial and aquatic ecosystems. Aquatic since the wetlands are frequently inundated by river water and terrestrial because of the emergent macrophyte vegetation adapted to the soil conditions. The tide acts as a stress by causing submergence, salinity influence, soil anaerobiosis and sometimes excessive sedimentation; it acts as a subsidy by removing excess salts, re-establishing aerobic conditions, and providing nutrients (Mitch & Gosselink, 2000). On this interface of land and water

complex ecological interactions exist. Input and output of water and sediments with nutrients (C, N, P, Si), (an)organic ions, heavy metals, aquatic organisms and their propagules are some of the exchanges during a flooding cycle. As limnological research in the past has mainly focussed on pelagic systems, with littoral zones having received considerably less attention (Wetzel, 1990) it is only recently well accepted that river floodplains are of primary importance in the functioning of river ecosystems, also of larger rivers with proportionally less riparian wetlands (Brunet et al., 1994; Mitsch & Gosselink, 2000). Unfortunately, during history, valuable tidal habitats – also in the Scheldt estuary – were much reduced in surface mainly due to land reclamation. This makes them rather rare and vulnerable. In particular freshwater tidal marshes are an endangered habitat in Europe as in several estuaries they are absent or reduced in surface by lock construction (Meire et al., 1994). Furthermore they are generally smaller than marine tidal wetlands and because they are more inland, more prone to human impact.

Within the Scheldt estuary, taking only the river Scheldt in account (Fig. 10), we have 3011 ha of tidal marsh left. 5% (± 150 ha) are characterized as salt marshes. 83% (± 2500 ha) of all tidal marshes are defined as brackish tidal marshes (with the large nature reserve 'Verdronken Land van Saeftinghe' (2020 ha)). The remaining 12% (± 360 ha) are freshwater tidal marshes with some additional small relicts along the tributaries with tidal influence (most important: Durme with ± 60 ha) (Meire et al., 1992; Van Damme et al., 1999).

6.2.2 Tidal marshes within the estuarine ecosystem

Estuarine ecosystems (both pelagic and intertidal areas) are not only important because of the specialized biological diversity, determined by several gradients of which salinity is often the most pronounced, but also because of their filterfunction and high productivity.

6.2.2.1 Gradients in the Scheldt estuary

The key elements in determining the existing gradients in the Scheldt estuary are the combination of tide and seasonally fluctuating river discharge. The tide brings in seawater twice a day and a runoff of fresh inland water causes several gradients because of the different characteristics of these two water bodies, combined with the chemical and biological transformation processes taking place during the trip of water to the sea.
The gradient with most impact on the biological diversity in the Scheldt estuary is the salinity gradient: it determines to large extent the pelagic, bentic and intertidal communities (Van Damme et al., 1999). The salinity influence is noticeable up to Temse (100 km inland). As the characterization of the Scheldt estuary by Van Damme et al. (1999) is mainly based on pelagic features derived from the model MOSES (Soetaert & Herman, 1995b,c) and additional data from Muylaert (1999) they conclude the oligohaline zone up to Temse. This pelagic characterization, mainly based on salinity, is shifted downstream for about 20 km if we look at the tidal marsh characterization reflected by plant and arthropod species composition (Hoffmann, 1993; Desender & Malfait, 1999) (as indicated in Fig. 10). A seasonally shifting oligohaline zone, with rather variable salinity influence extends roughly from Antwerp city towards Burcht (86 km inland). Downstream this zone we find a mesohaline zone extending towards the Dutch-Belgian border. The Western Scheldt is polyhaline to euhaline near the North-sea (Van Damme et al., 1999).

Some other abiotic pelagic gradients are just mentioned below but not discussed in depth, for an overview see Van Damme et al. (1999); diversity gradients are discussed below.

Oxygen has low concentrations in the Sea Scheldt, which is anaerobe during long parts of the year, while higher O_2 concentrations are prevalent in the Western Scheldt estuary.

Nitrogen shows changing dominant oxidation states along the longitudinal axes of the estuary. If oxygen is available, the large anthropogenic input of ammonium is soon transformed to nitrate which is a major export nutrient towards the sea. In anaerobic conditions this element is denitrificated to N_2 gas, the main pathway to lose nitrogen from an ecosystem.

Another major anthropogenic input concerns phosphorus. Different chemical forms, also show differentiated patterns along the longitudinal axes of the estuary: for example high orthophosphate concentrations in the freshwater part and much lower concentrations downstream the turbidity maximum, near Temse.

6.2.2.2 Biodiversity

Gradients are best reflected by the occurring ecological communities. They form the natural coenoclines determining the distribution of species. Tidal influence, with salinity and flooding characteristics, is the most important factor modelling the tidal marsh biota besides human impact by infrastructuring and nature management.

In the Scheldt tidal marshes we find specific and some rare organisms adapted to the harsh environment. See for an overview Van den Bergh et al. (2001). However fungi are not mentioned.

Plant distributional patterns are best explained by the salinity influence they experience (Odum, 1988; Hoffmann, 1993; Criel et al., 1999). On salt marshes, species distribution is mainly determined by marsh topography resulting in a different degree of exposure to water (salinity). This makes vegetations less species rich, with few dominant species and a clear spatial distribution on the marsh reflecting succession (Odum, 1988; de Leeuw et al., 1993). Freshwater tidal marshes show more complex vegetation structure with a higher plant species richness of eurytopic taxa. This results in patchy vegetation with a diffuse zonation and less clear succession (Odum, 1988; Criel et al., 1999).

General patterns in biodiversity on tidal marshes are discussed by Odum (1988) and for the Scheldt estuary by Van den Bergh et al. (2001). Different groups of organisms show distinct patterns of species richness along the salinity gradient of the Scheldt estuary. In general the number of species found on the tidal marsh increases from salt to fresh and from higher to lower inundation frequencies. The vascular plant species diversity-curve is gradually decreasing towards salt marshes, as is the number of mosses, lichens and nesting birds. Organisms in close relationship with the water and sediments seem to correspond with the Remane curve (Remane, 1934) suggesting a species impoverishment in the brackish zone and higher species richness in saline and freshwater conditions. Few species are adapted to both harsh physiological circumstances of either saline or freshwater.

6.2.2.3 Filter function, biogeochemical pathways and the role of tidal marshes

The Scheldt estuary is exposed to large loads of carbon and nutrients. This leads to an eutrophication problem characterized by reduced oxygen concentrations and a huge pelagic efflux of CO_2 over the entire estuary (Van Damme & Meire, 2001). The filter capacity of the estuary to purify the water is important but at present it cannot cope with the vast amount of emissions of waste water. An increase of water treatment combined with restoration and development of tidal wetlands is necessary to improve the quality of the Scheldt-ecosystem (Meire et al., 1997; Van Damme & Meire, 2001).

In the Scheldt estuary natural boundaries are reduced in surface and the knowledge on the exact role of the tidal wetlands is starting to get clearer (see below). Functionally, riparian wetlands are considered as important buffers (sources or sinks) with high filter capacity for subsurface water moving toward the river and for river water entering the marshes during tides (Mitch & Gosselink, 2000). The former is probably limited in the Scheldt estuary as most of the marshes are higher then surrounding valley grounds (Van Damme et al., 1999). The impact of tidal marshes on the river water highly depends on the height of the tide in the river (estuary morphology) and determines the flooding frequency of the marshes. Sometimes tides are low and only flood the bare creeks and mudflats. Although this also has an impact on water characteristics, the largest impact is expected when vegetated zones are also flooded. This evidently also implies a proportionally larger part of a tidal marsh flooded. The most important filter functions of riparian wetlands are the bioreactor activity for nitrate and ammonium and the efficient sediment and suspended matter removal (Haycock et al., 1993; Castelle et al., 1994; Gilliam, 1994). (In)organic nitrogen removal can through direct sequestration into plant tissues (e.g. Jordan et al., 1989) or via immobilization or via direct atmospheric efflux by microbial denitrification.

Carbon and the role of the tidal marsh

Huge organic carbon inputs, more than 200.000 tons yr^{-1}, enter the Scheldt estuary. The input is realized by several pathways: 1) phytoplankton incorporating CO_2 by photosynthesis is responsible for 10-60% of the load, while 2) carbon derived from riparian vegetation only takes 2-5%, maximal 10% of the load, 3) waste water input is responsible for the overall large bulk of organic carbon input (Hellings et al., 1999).
Highly productive pelagic bacteria are mainly responsible for the mineralization of the organic carbon sources and make the estuary a heterotrophic ecosystem (Goosen et al., 1995; Frankignoulle et al., 1998). This results in an enormous efflux of CO_2 to the atmosphere. Heterotrophy is more important in the upstream freshwater area and less prevalent in the Western Scheldt (Van Damme et al., 1999).
The role in the C- cycle of tidal marshes is mainly derived from the primary plant production on the marsh, which consists of ± 50% carbon. The carbon removal from the marsh to the river, i.e. aquatic decay opposed to *in situ* tidal marsh mineralization is a relevant research topic. However, for the Scheldt estuary these exchanges are not well quantified as methodological problems make accurate measurements difficult. Tackx et al. (2000) investigated the

redeposition of plant material on the freshwater tidal marshes from the water column, but methodology from this study seems inaccurate. Their figures probably indicate intra marsh transport of necromass more than a deposition of plant material from the river. Muylaert (1996) estimated the average litter output and burial for two reed sites at 48% after comparing the maximal summer biomass with the present litter (standing and fallen) in March the following year. However a decomposition component should be added to this percentage as for leaves and leaf sheaths making 40% of total biomass, it can by estimated that about 50% (or even more) of the dry mass can decompose over 6 months (e.g. Gessner 2000, 2001; chapter 6). This would mean that at least 20% of the observed 'output' is due to decomposition, while burial by sedimentation can account for another substantial amount of litter disappearance. These observations suggest that a large part of the produced reed material is decomposed as autochtonous material on the tidal marsh, with large differences among sites, determined by exposure degree of site to the river, geomorphology of marsh and the vegetation characteristics (cf. Hemminga et al., 1993).

However, a substantial part of the sedimentation on the marshes is allochthonous riverine POC (particulate organic carbon) (Middelburg et al., 1997), while measured concentrations of recognizable plant material (1 mm - 1 cm) floating or in subsurface layers of the freshwater part of the Scheldt river is comparably low (0.002-0.03 g C m^{-3}) (Tackx et al., 1999). The mean bulk of recognizable plant species in these samples were common reed (*Phragmites australis*), willows (*Salix* spp.), and indian balsam (*Impatiens glandulifera*) (Tackx et al., 1999). However, these measured amounts of floating plant material are possibly an underestimation of the realized removal of plant mass from the marsh because plant material could sink fast or could soon fragment in small particles. Some experiments were conducted (Tackx et al., 2000) in the Scheldt estuary. Complete stems (and leaf sheaths) remained floating for long periods (> 60 days) while leaves sank after about 30 days if they were placed directly from the canopy on the water surface. However if they are first incubated under water for a sufficiently long period (7 days in the experiment) reed leaves all sank after 24 hours after being placed on the water surface. However, incubation of the leaves under water prior to putting in the water is an unnatural situation, forcing air out of the internal spaces and ignores the natural hydrophobic features of leaves. From Tackx et al. (2000), I would conclude that the floating time of a reed leaf blade will be between 7 (or shorter) and 30 days depending on factors such as decay-state, the time on the tidal marsh before entering the main river and probably also the frequency by which a leaf was flooded in the litter layer.

Because of the low river runoff compared to the total volume of the estuary, water residence time is high. Plant material entering the water of the river Scheldt is transported on average 2 km downstream in one tidal cycle, as there are two cycles, the average daily downstream transport is about 4 km. This means that an object entering the river near Ghent will reach the sea in about 40 days (Graré, 2004). This estimate is probably at the lower end because combined data from Soetaert & Herman (1995) and Muylaert et al. (2000) suggests a residence time of about 65-85 days. The latter estimate is related to the effective longer residence time in the freshwater zone of the estuary (Van Damme et al., 1999).

Nitrogen and the role of the tidal marsh

Large anthropogenic inputs of nutrients characterize the Scheldt estuary and part of those are carried to the sea and caused an intensified eutrophication the last few years. The key element in explaining this phenomenon is silicium (Van Damme & Meire, 2001). Models predicted that by water treatment a reduced C-input in the river, would improve oxygen quality causing an intensified nitrification. Furthermore less favorable conditions for denitrification are created, reducing the atmospheric efflux of N from the estuary (= paradox of the Scheldt estuary). Still, form intensive monitoring at the Dutch-Belgian border this increase of N-transport to the sea, with an improved water quality is not recorded, although algae blooms (e.g. *Phaeocystis* sp.) are recorded more frequently (Van Damme et al., 1999). Reasons for this could be 1) better water treatment, 2) the possibility that denitrification processes go on in anaerobic flocs in the water and 3) that the observed trend is caused by tidal influence as more seawater penetrates further upstream (Van Damme & Meire, 2001).
In the Scheldt estuary, with few tidal marshes, the main pathway for nitrogen removal is denitrification on the tidal marsh surface and in the anaerobic sediments of mud flats (Middelburg et al., 1995; Van Damme et al., 1998). Middelburg et al. (1995) estimated that intertidal areas were responsible for 14% of the total N-efflux of the estuary (Vlissingen to Rupelmonding), while Van Damme et al. (1998) found even a more intensive denitrification in freshwater tidal marshes. However, the impact of marshes as nutrient removers or as nutrient spiraling zones is limited (but not unimportant!) due to their small area (Van Damme et al., 1998).

Phosphorus and the role of the tidal marsh

Generally phosphorus, another important effluent of human wastewaters and agricultural runoff, is reasonable well removed by buffer zones if attached to the sediment, but little filtering happens when P is dissolved (Gilliam, 1994). For the Scheldt estuary with phosphorus concentrations 1-2 orders of magnitude higher than in natural situations a variant of this filtering mechanism was observed at 'Tielrode-schor' a freshwater marsh. Particular phosphate fraction was lower in out coming water compared to ingoing water, while orthophosphate concentrations showed, by remobilisation, an output from the marsh to the Scheldt water (Van Damme et al., 1998). Along the longitudinal axes of the estuary phosphate is controlled by different processes: physicochemical in the freshwater zone (precipitation and resuspension, often complexation with iron and other elements leading to large sinks in the sediment) and more biological processes downstream (e.g. phytoplanktonic uptake) (Van Damme et al., 1999). As there is almost no netto removal of P from the river, only retention by nutrient spiralling or soil complexation, water treatment is stated to be the key for reduced P-output toward the sea (Van Damme et al., 1999).

6.2.2.4 Scheldt tidal marsh productivity: the reed vegetation

In the Scheldt estuary detailed information on the annual above ground biomass and seasonal dynamics of common reed are available (Muylaert, 1996; Meganck, 1998; Soetaert et al., 2004). Muylaert (1996) estimated the average annual above ground biomass of common reed in the

Scheldt estuary to be 21.9 ± 7.2 ton/ha. However, this result is an overestimation as the mass of larger fertile stems was extrapolated to calculate the biomass, without taking non-fertile stems into account (Meganck, 1998). A more accurate figure, used as working number in this thesis, is presented by Meganck (1998) estimating the average annual above ground biomass to be 10.0 ± 3.8 ton/ha.

On a whole of 3011 ha of tidal marsh along the Scheldt (see §6.2.1), 219 ha (7%) is covered by common reed (about 160 ha monospecific reed vegetation and 59 ha mixed herbaceous vegetation, the latter especially in the freshwater part (± 42 ha), with *Urtica dioica*, *Calystegia sepium*, *Impatiens glandulifera*,… (Hoffmann, 1993)). We find 179 ha reed on a whole of 512 ha (i.e. 35%) tidal marsh left along the Sea Scheldt. Most of these reed marshes (70%, 125 ha) are situated in the brackish part of the Sea Scheldt (with 187 ha brackish tidal marsh), meaning that in this brackish zone 67% of vegetation cover is common reed. In the freshwater zone, more than half of the tidal marshes (53%, 192.5 ha) are covered by willow (*Salix* spp.) while *Phragmites* covers 53.5 ha (13.4%) of freshwater tidal marsh (Van Damme et al., 1999 Table 5). The presented figures indicate that *Phragmites* is mainly of importance as dominant vegetation along the Sea Scheldt, and in particular in the oligohaline and in the lower end of the mesohaline brackish part.

Combining the above data of surface covering and productivity we can estimate the biomass production of reed to be 2190 ton yr^{-1} of which 1790 ton yr^{-1} is produced along the Sea Scheldt. 1255 ton yr^{-1} in the brackish part and 535 ton yr^{-1} in the freshwater part. An additional production of about 100 ton yr^{-1} is expected from tidal freshwater tributaries (mainly the Durme).

Culm material (stems) accounts for the greatest portion of this production but other shoot components, such as leaf blades and sheaths, also make up a substantial fraction (Fig. 12). The inflorescences have less impact on the total biomass with 1-2% in the freshwater tidal marsh and 4-5% in the brackish tidal marsh (Meganck, 1998).

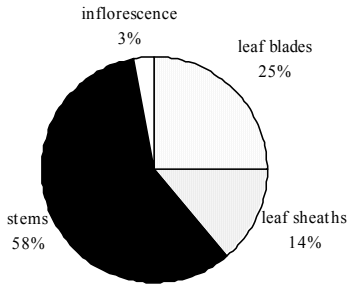


Figure 12. Average proportion of the different plant parts in above ground biomass production in a brackish tidal marsh 'Saeftinghe'. Based on Meganck (1998) and own measurements.

Outline of the thesis

The thesis is divided into 3 larger parts each containing two or more chapters. The parts discuss the obtained results at different levels. The first part focuses on the fungi in a single brackish tidal marsh (Saeftinghe, The Netherlands). This site was intensively screened for fungal occurrence. Furthermore we conducted a decomposition study associated with a fungal biomass monitoring and we followed heavy metal dynamics in the decaying reed material. In the second part of the thesis we looked at a larger scale and investigated the fungal diversity and ecological patterns along the estuarine salinity gradient and summarized the impact of fungi in the entire Scheldt estuary. In the third part we focused on fungal diversity and we extrapolated our dataset and literature database to an estimation of world-wide diversity of fungi occurring on *Phragmites australis*.

Part 1: Fungi in a brackish tidal marsh

The first six chapters explore fungal diversity, community structure and decomposition processes in a single study site. A brackish tidal marsh was chosen as case study site. This brackish tidal marsh was selected because of the homogeneous nature of the reed vegetation. Furthermore 'PQ 206' adjacent to our study site 'Saeftinghe' (Fig. 13) is (was) intensively monitored by the Institute of Nature Conservation (Brussels – E. Van den Bergh) and by the University Antwerp (P. Meire). This makes that there is much data available about our study site (e.g. Muylaert, 1996; Meganck, 1998; Soetaert et al., 2004). Tidal brackish marshes are of particular interest in the Scheldt estuary because they have the largest surface area and *P. australis* forms the dominant climax vegetation on these marshes (see above § 6.2.2.4).

In chapter 2 we investigated the fungal community pattern by multivariate analysis. It was hypothesized that fungal communities could differ among plant parts, among heights in the canopy and that we would be able to recognize different phases in the decomposition process based on fungal sporulation on the reed parts (cf. Apinis et al., 1972).

Because our dataset was very large, temporal effects of fungal succession on the different plant parts were less obvious from the analysis. Therefore in order to further explore our interesting dataset, we investigated each plant part separately respectively in chapter 3 (leaf sheaths), 4 (stems) and 5 (leaf blades).

In order to describe the role of fungi during the decomposition process we set up a litter bag experiment. In chapter 5 and 6 we superimposed the data from the fungal community (fungal succession data) on the decomposition pattern observed in the experiment. However, for practical reasons the plant parts used to study the fungal community were not the same (although incubated on the same marsh and followed in parallel) as those used in de decomposition study (see § 6.1; Table 3). During this experiment we studied the

decomposition of leaf blades, leaf sheaths and stems by monitoring there weight loss, nutrient (N, P) dynamics, cell wall polymers (only sheaths and stems) and concentrations in heavy metals. Furthermore we followed fungal biomass dynamics during decay by using ergosterol as a proxy. In chapter 5 we discuss the decomposition of leaf blades and in chapter 6 we studied leaf sheaths and stems. We concentrated on decay processes in the litter layer but in chapter 6 we illustrate and discuss the importance of standing decay. The possible impact of fungi in nutrient dynamics is illustrated in both chapters. In chapter 7 we further elaborate our scope and screen for a possible interaction between fungal biomass dynamics and changes in the metal concentrations within the different reed parts.

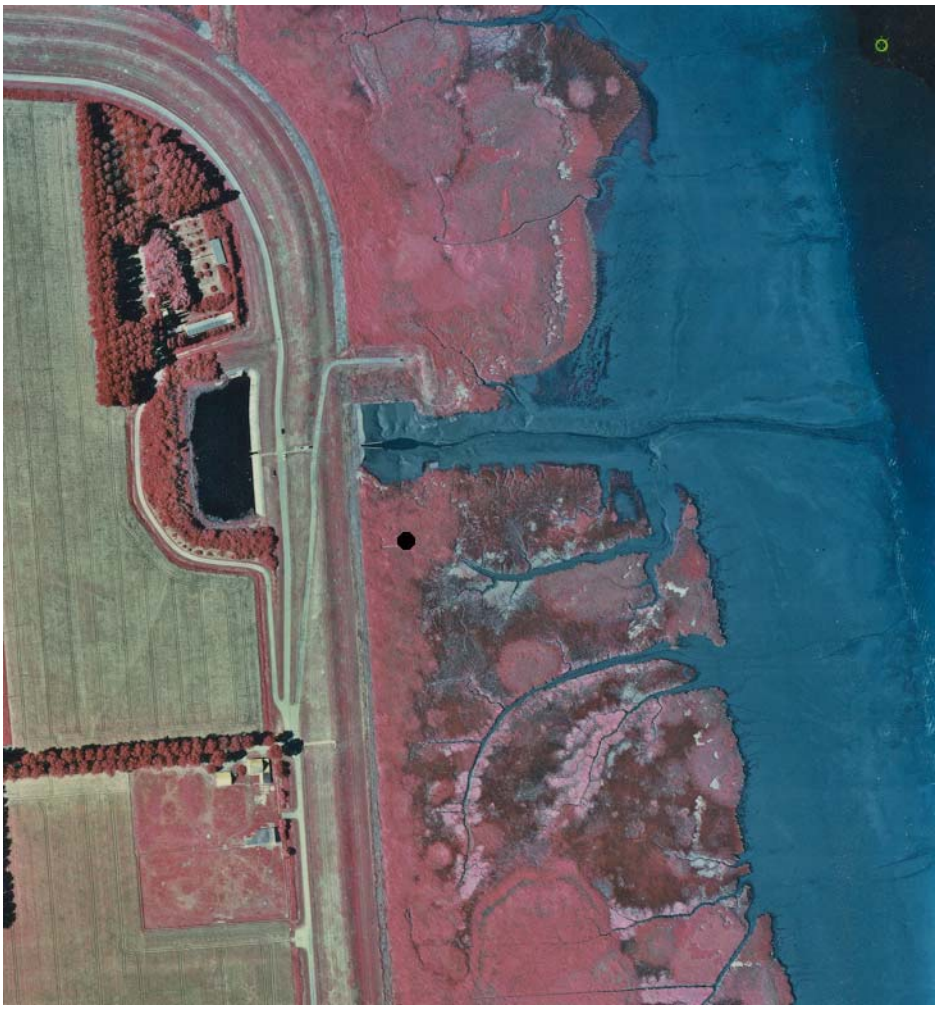


Figure 13. Aerial photo showing the location of our study site 'Saefinghe' (•) – just beside PQ 206. Photo 1:5000

Part 2: Fungi in an estuary

The next two chapters discuss fungi in an estuarine environment. Chapter 8 focuses on the natural salinity gradient and flooding characteristics present in estuaries. The main question was: do fungal communities differ among sites along the Scheldt estuary? We address the effect of salinity on fungal diversity and we discuss the effect of flooding.
Chapter 9 is a concluding chapter summarizing the established and potential impact of fungi in estuarine ecosystems and in particular for the Scheldt estuary.

Part 3: Fungal diversity: personal observations and world-wide diversity of reed fungi

The following chapters 10 and 11 report on the fungal diversity observed. Chapter 10 is a short chapter summarizing the observed diversity. This observed diversity is subsequently used in chapter 11 together with literature information to extrapolate fungal diversity on a single host plant, common reed.

In chapter 12 a synthesis of the results will be given, provided with a general discussion and with a selection of perspectives. Chapter 13 is the Dutch translation of the previous chapter.

The appendix of this thesis provides the sound basis of all ecological information abovementioned. It offers the reader the opportunity to verify species identity and provides detailed information for comparison with past and future research focussing on fungi on *P. australis*. To save paper, make accessibility to everyone and provide a dynamic framework all figures and descriptions are hosted on a website (http://biology.ugent.be/reedfungi).

Personal contribution to the thesis

Except for chapter 7 all field work, laboratory work, fungal identification, analyses, photographs, drawings and (initial) writing for the chapters were carried out by G. Van Ryckegem. The presented manuscripts were thereafter discussed, corrected and commented on by the respective coauthors (A. Verbeken, M.O. Gessner, G. Van Driessche, G., J.J. Van Beeumen, Du Laing, F. Tack and Verloo, M.). However, see acknowledgements in each chapter for the many people that helped during all processes. G. Du Laing (Ghent University) performed metal analysis and in a joint project we produced the manuscript presented in chapter 7. Nitrogen and phosphorus analysis presented in chapter 6 were done by Ir. Dirk Van Gansbeke (Ghent University). The website is a first exploration of the html-language and invaluable help by Tim Deprez (Ghent University) was much appreciated.

5. References

ADLER, L. (1996). Fungi and salt stress. *In* FRANKLAND, J.C., MAGAN, N. & GADD, G.M. (Eds.). Fungi and environmental change. Cambridge University Press, Cambridge. 217-234.

AHLHOLM, J.U., HELANDER, M., HENRIKSSON, J., METZLER, M. & SAIKKONEN, K. (2002). Environmental conditions and host genotype direct genetic diversity of *Venturia ditricha*, a fungal endophyte of birch tree. Evolution 56: 1566-1573.

AKANIL, N. & MIDDLETON, B. (1997). Leaf decomposition along the Porsuk River, Eskisehir, Turkey. Canad. J. Bot. 75: 1394-1397.

ANDERSEN, F.O. (1978). Effects of nutrient level on the decomposition of *Phragmites communis* Trin. Arch. Hydrobiol. 84: 42-54.

ANDERSON, J.M. & MACFADYEN, A. (EDS.) (1976). The role of terrestrial and aquatic organisms in decomposition processes. Blackwell Scientific, Oxford. 474 pp.

ANDREWS, J.H., KINKEL, L.L., BERBEE, F.M. & NORDHEIM, E.V. (1987). Fungi, leaves and the theory of island biogeography. Microb. Ecol. 14: 277-290.

ANTONIELLI, M., PASQUALINI, S., BATINI, P., EDERLI, L., MASSUCCI, A. & LORETO, F. (2002). Physiological and anatomical characterisation of *Phragmites australis* leaves. Aquatic Bot. 72: 55-66.

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1972a). Colonisation of *Phragmites communis* leaves by fungi. Nova Hedwigia 23: 113-124.

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1972b). Microfungi colonizing submerged standing culms of *Phragmites communis* Trin. Nova Hedwigia 23: 473-480.

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1975). Microfungi colonizing nodes and internodes of aerial standing culms of *Phragmites communis* Trin. Nova Hedwigia 26: 495-507.

ARMSTRONG, J., ARMSTRONG, W., BECKETT, P.M., HALDER, J.E., LYTHE, S., HOLT, R. & SINCLAIR, A. (1996). Pathways of aeration and the mechanisms and beneficial effects of humidity- and Venturi-induced convections in *Phragmites australis* (Cav.) Trin. ex Steud.. Aquatic Bot. 54: 177-197.

ARSUFFI, T.L. & SUBERKROPP, K. (1989). Selective feeding by shredders on leaf-colonizing stream fungi: comparison of macroinvertebrate taxa. Oecologia 79: 30-37.

ASAEDA, T. & NAM, L.H. (2002). Effects of rhizome age on the decomposition rate of *Phragmites australis* rhizomes. Hydrobiologia 485: 205-208.

ASAEDA, T., NAM, L.H., HIETZ, P., TANAKA, N. & KARUNARATNE, S. (2002). Seasonal fluctuations in live and dead biomass of *Phragmites australis* as described by a growth and decomposition model: implications of duration of aerobic conditions for litter mineralization and sedimentation. Aquatic Bot. 73: 223-239.

BÁN, R., ENGLONER, A.I. & VIRANYI, F. (2000). Occurrence of fungal pathogens in relation to reed quality. Z. Pflanzenkrankh. Pflanzenschutz 107: 258-266.

BÁN, R., FISCHL, G. & VIRANYI, F. (1996). A spatio-temporal analysis of fungal pathogens on reed in natural habitats. Acta Phytopathol. Entomycol. Hung. 31: 219-227.

BÁN, R., VIRANYI, F. & OBADOVICS C. (1998). Survey of pathogenic fungi in different reed stands. Növénytermelés 47: 407-416.

BÄRLOCHER, F. (1997). Pitfalls of traditional techniques when studying decomposition in aquatic habitats. Limnetica 13(2): 1-11.

BÄRLOCHER, F. & KENDRICK, W.B. (1975). Assimilation efficiency of *Gammarus pseudolimnaeus* (Amphipoda). Oikos 26: 55-59.

BARRON, G.L. (1982). Nematode-destroying fungi. *In* BURNS, R.G. & SLATER, J.H. (EDS.) Experimental Microbial Ecology. Blackwell, Oxford. 533-552.

BEEVER, R.E. & BURNS, D.J.W. (1980). Phosphorus uptake, storage and utilization by fungi. Adv. Bot. Res. 8: 127-219.

BEGON, M., HARPER, J.L. & TOWNSEND, C.R. (1996). Ecology: individuals, populations and communities. 3rd ed.. Blackwell Science, Oxford. 1068 pp.

BENNER, R., LAY, J., K'NEES, E. & HUDSON, R.E. (1988). Carbon conversion efficiency for bacterial growth on lignocellulose: implications for detritus-based food webs. Limnol. Oceanogr. 33: 1514-1526.

BENNER, R., MORAN, M.A. & HUDSON, R.E. (1986). Biogeochemical cycling of lignocellulosic carbon in marine and freshwater ecosystems: relative contributions of prokaryotes and eukaryotes. Limnol. Oceanogr. 31: 89-100.

BERGBAUER, M. & NEWELL, S.Y. (1992). Contribution to lignocellulose degradation and DOC formation from a salt marsh macrophyte by the ascomycete *Phaeosphaeria spartinicola*. FEMS Microb. Ecol. 86: 341-348.

BERGBAUER, M., EGGERT, C. & KALNOWSKI, G. (1992a). Biotreatment of pulp mill bleachery effluents with the coelomycetous fungus *Stagonospora gigaspora*. Biotechnol. Lett. 14: 317-322.

BERGBAUER, M., MORAN, M.A. & HODSON, R.E. (1992b). Decomposition of lignocellulose from a freshwater macrophyte by aero-aquatic fungi. Microb. Ecol. 23: 159-167.

BEYER, W. (1997). Ascomyceten und einige Basidiomyceten an *Typha latifolia* L. and *Phragmites australis* (Cav.) Trin.. Beitr. Kenntn. Pilze Mitteleuropas 11: 49-113.

BISSETT, J. & BROKENT, A. (1988). Ambrosia galls: the significance of fungal nutrition in the evolution of the Cecidomyiidae (Diptera). *In* PIROZYNSKI, K.A. & HAWKSWORTH, D.L. Coevolution of fungi with plants and animals. Academic Press, London. 203-225.

BLAKE, G. (1982). Characterization of decomposition of plant material in an alpine lake. Hydrobiol. Bull. 16: 5-9.

BODDY, L. (1986). Water and decomposition processes in terrestrial ecosystems. *In* AYRES, P.G. & BODDY, L. (EDS.) Water, Fungi and Plants. Cambridge University Press, Cambridge. 375-398.

BOSCHKER, H.T.S. (1997). Decomposition of organic matter in the littoral sediments of a lake. Thesis Wageningen Agricultural University, Wageningen, 120 pp.

BOSMAN, M.T.M. (1985). Some effects of decay and weathering on the anatomical structure of the stem of *Phragmites australis* Trin. ex Steud.. Int. Ass. Wood. Anat. Bull. 6: 165-170.

BOULTON, A.J., BOON, P.I. (1991). A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over an old leaf? Aust. J. Mar. Freshwat. Res. 42: 1-43.

BRINSON, M.M., LUGO, A.E., BROWN, S. (1981). Primary productivity, decomposition and consumer activity in freshwater wetlands. Ann. Rev. Ecol. Syst. 12: 123-161.

BRUNET, R.C., PINAY, G., GAZELLE, F. & ROQUES, L. (1994). Role of floodplain and riparian zone in suspended matter and nitrogen retention in the Adour river, South-west France. Regulated rivers: research & manag. 9: 55-63.

BRUYNDONCK, L., JORDAENS, K. & MEIRE, P. (2001). De weekdieren van de schorren van het Schelde-estuarium. De Levende Natuur 102: 70.

BRUYNDONCK, L., JORDAENS, K., DE WOLF, H., MEIRE, P. & BACKELJAU, T. (2000). New records of *Assiminea grayana* Fleming, 1828, *Myosotella myosotis* (Draparnaud, 1801) and *Pisidium subtruncatum* Malm, 1855 (Mollusca: Gastropoda, Bivalvia) in the Scheldt estuary. Bull. Inst. Roy. Sci. Nat. Belgique, Biol. 70: 103-106.

CABRAL, D., STONE, J.K. & CARROLL, G.C. (1993). The internal mycobiota of *Juncus* spp. microscopic and cultural observations of infection patterns. Mycol. Res. 97: 367-376.

CARLILE, M.J. & WATKINSON, S.C. (1994). The Fungi. Academic Press Limited, London. 482 pp.

CARREIRO, M.M., SINSABAUGH, R.L., REPERT, D.A. & PARKHURST, D.F. (2000). Microbial enzyme shift explain litter decay responses to simulated nitrogen deposition. Ecology 81: 2359-2365.

CARROLL, G.C. (1986). The biology of endophytism in plants with particular reference to woody perennials. *In* FOKKEMA, N.J. & VAN DEN HEUVEL, J. (EDS.) Microbiology of the Phyllosphere. Cambridge University Press, Cambridge, UK. 205-222.

CARROLL, G.C. (1995). Forest endophytes: pattern and process. Canad. J. Bot. 73 (Suppl. 1): 1316-1324.

CASTELLE, A.J., JOHNSON, A.W. & CONOLLY, C. (1994). Wetland and stream buffer size requirements - A review. J. Environ. Qual. 23: 878-882.

CHAMBERS, R.M., MEYERSON, L.A. & SALTONSTALL, K. (1999). Expansion of *Phragmites australis* into tidal wetlands of North America. Aquatic Bot. 64: 261-273.

CHANG, N.K. & OH, K.H. (1977). The decomposition rates of the organic constituents of the litter in *Phragmites longivalvis* grassland in a delta of the Nakdong-River. Coll. of thesis, Coll. of Eduction, S.N.U. 15: 129-142.

CHANG, N.K., OH, K.H. & AN, B.H. (1978). The turnover rates of N, P, K, Ca and Na of the litter in *Phragmites longivalvis* grassland in a delta of the Nakdong-River. Science Education, S.N.U. 3: 17-24.

CHANWAY, C.P. (1996). Endophytes: they're not just fungi!. Canad. J. Bot. 74: 321-322.

CHEN, J.C. & FERRIS, H. (1999). The effects of nematode grazing on nitrogen mineralization during fungal decomposition of organic matter. Soil Biol. Biochem. 31: 1265-1279.

CHERGUI, H. & PATTEE, E. (1990). The processing of leaves of trees and aquatic macrophytes in the network of the River Rhone. Int. Rev. ges. Hydrobiol. 75: 281-302.

CHRISTENSEN, M. (1989). A view of fungal ecology. Mycologia 81: 1-19.

ČÍŽKOVÁ, H., STRAND, J.A. & LUKAVSKÁ, J. (1996). Factors associated with reed decline in a eutrophic fishpond, Rožmberk (South Bohemia, Czech Republic). Folia Geobot. Phytotax. 31: 73-84.

ČÍŽKOVÁ-KONČALOVÁ, H., KVĚT, J. & THOMPSON, K. (1992). Carbon starvation: a key to reed decline in eutrophic lakes. Aquatic Bot. 43: 105-113.

CLAESSENS, J. & BELMANS, H. (1984). Overzicht van de tijwarrnemingen in het Zeescheldebekken gedurende het decennium 1971-1980. Tijds. Openb. Werken België 3: 1-96.

CLAY, K. (1986). Grass endophytes. *In* FOKKEMA, N.J. & VAN DEN HEUVEL, J. (EDS.) Microbiology of the Phyllosphere. Cambridge University Press, Cambridge, UK. 188-204.

CLAY, K. & VAN DER PUTTEN, W. (1999). Pathogens and plant life histories. *In* VUORISALO, T.O. & MUTIKAINEN, P.K. Life history evolution in plants. Kluwer Academic Press, London. 275-301.

CLAYTON, W.D. (1967). Studies in the Graminae. XIV. Kew Bull. 21: 113-117.

CLAYTON, W.D. (1968). The correct name of the Common reed. Taxon 17: 168-169.

CLEVERING, O. A. (1998). Effects of litter accumulation and water table on morphology and productivity of *Phragmites australis*. Wetland Ecol. Mangement 5: 275-287.

CLEVERING, O. A. & LISSNER, J. (1999). Taxonomy, chromosome numbers, clonal diversity and population dynamics of *Phragmites australis*. Aquatic Bot. 64: 185-208.

CONNELL, J.H. & SLATYER, R.O. (1977). Mechanisms of succession in natural communities and their role in community stability and organisation. Amer. Naturalist 111: 1119-1144.

COOKE, R.C. & RAYNER, A.D.M. (1984). Ecology of saprotrophic fungi. Longman Group Limited, London. 415 pp.

CRIEL, B., MUYLAERT, W., HOFFMANN, M., DE LOOSE, L. & MEIRE, P. (1999). Vegetatiemodellering van de buitendijkse gebieden langs de Zeeschelde. RUG, Instituut voor Natuurbehoud, Brussel.

DAMM, U., BRUNE, A. & MENDGEN, K. (2003). In vivo observation of conidial germination at the oxic-anoxic interface and infection of submerged reed roots by *Microdochium bolleyi*. FEMS Micorbiol. Ecol. 45: 293-299.

DELEEBEECK, N. (2000). Fytolietenstudie van Riet (*Phragmites australis* (Cav.) Steudel (Poaceae)) langsheen Schelde en Durme (België). Ongepubliceerde licentiaatsscriptie, Gent. Deel 1, 73 pp.

DENWARD, D.M.T. & TRANVIK, L.J. (1998). Effects of solar radiation on aquatic macrophyte litter decomposition. Oikos 82: 51-58.

DENWARD, D.M.T., EDLING, H. & TRANVIK, L.J. (1999). Effects of solar radiation on bacterial and fungal density on aquatic plant detritus. Freshwater Biol. 41: 575-582.

DESENDER, K. & MAELFAIT, J. (1999). Diversity and conservation of terrestrial arthropods in tidal marshes along the River Schelde: a gradient analysis. Biol. Conservation 87: 221-229.

DIX, N.J. (1985). Changes in relationship between water content and water potential after decay and its significance for fungal successions. Trans. Brit. Mycol. Soc. 85: 649-653.

DIX, N.J. & WEBSTER, J. (1995). Fungal ecology. Chapman & Hall, London. 549 pp.

DOWDING, P. (1981). Nutrient uptake and allocation during substrate exploitation by fungi. *In* WICKLOW, D.T. & CAROLL, G.C. (EDS.) The fungal community, its organization and role in the ecosystem. Marcel Dekker, New York. 621-635.

DUMOULIN, E. (1989). Overzicht van de brakwatermollusken van België.. Verhandelingen van het symposium "Invertebraten van België" Brussel: 87-94.

DURSKA, B. (1969). Distribution of some parasitic fungi on *Phragmites communis* Trin. in Poland. Acta Mycol. 5: 117-133.

DURSKA, B. (1970). Changes in the reed (*Phragmites communis* Trin.) condition caused by diseases of fungal and animal origin. Polsk. Arch. Hydrobiol. 17: 373-396.

DVOŘÁK, J. & IMHOF, G. (1998). The role of animals and animal communities in wetlands. *In* WESTLAKE, D.F., KVĚT, J. & SZCZEPAŃSKI, A. The production ecology of wetlands. Cambridge university press, Cambridge. 211-318.

ENRIQUEZ, S., DUARTE, C.M. & SAND-JENSEN, K. (1993). Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94: 457-471.

ERNST, M., MENDGEN, K.W. & WIRSEL, S.G.R. (2003). Endophytic fungal mutualists: seed-borne *Stagonospora* spp. enhance reed biomass production in axenic microcosms. Molec. Pl.-Microbe Interact. 16: 580-587.

FAIRBRIDGE, R. (1980). The estuary: its definition and geodynamic cycle. *In* OLAUSSON, E. & CATO, I. (EDS.) Chemistry and biochemistry of estuaries. Wiley, New York. 1-35.

FINDLAY, S.E.G., DYE, S. & KUEHN, K.A. (2002). Microbial growth and nitrogen retention in litter of *Phragmites australis* compared to *Typha angustifolia*. Wetlands 22: 616-625.

FISHER, P.J. & ANSON, S.E. (1983). Antifungal effects of *Massarina aquatica* growing on oak wood. Trans. Brit. Mycol. Soc. 81: 396-398.

FISHER, P.J., ANSON, A.E. & PETRINI, O. (1986). Fungal endophytes in *Ulex europaeus* and *Ulex gallii*. Trans. Brit. Mycol. Soc. 86: 153-156.

FOG, K. (1988). The effect of added nitrogen on the rate of decomposition of organic matter. Biol. Review 63: 433-462.

FRANKIGNOULLE, M., ABRIL, G., BORGES, A., BOURGE, I., CANON, C., DELILLE, B., LIBERT, E., THEATE, J.M. (1998). Carbon dioxide emission from European estuaries. Science 282: 434-436.

FRANKLAND, J.C. (1981). Mechanisms in fungal successions. *In* WICKLOW, D.T. & CARROLL, G.C. (EDS.) The Fungal community. Its organization and role in the ecosystem. Marcel Dekker, New York. 403-426.

FRANKLAND, J.C. (1992). Mechanisms in fungal succession. *In* CAROLL G.C. & WICKLOW, D.T. The fungal community. Its organisation and role in the ecosystem. 2de ed.. Marcel Dekker, New York. 383-401.

GADD, G.M. (1993). Interactions of fungi with toxic metals. New Phytol. 124: 25-60.

GANGE, A.C. (2001). Species-specific responses of a root- and shoot-feeding insect to arbuscular mycorrhizal colonization of its host plant. New Phytologist 150: 611-618.

GANGE, A.C., STAGG, P.G. & WARD, L.K. (2002). Arbuscular mycorrhizal fungi affect phytophagous insect specialism. Ecol. Letters 5: 11-15.

GEIGER, R. (1957). The climate near the ground. Harvard university press, . 494 pp.

GESSNER, M.O. (2000). Breakdown and nutrient dynamics of submerged *Phragmites* shoots in the littoral zone of a temperate hardwater lake. Aquatic Bot. 66: 9-20.

GESSNER, M.O. (2001). Mass loss, fungal colonisation and nutrient dynamics of *Phragmites australis* leaves during senescence and early decay. Aquatic Bot. 69: 325-339.

GESSNER, M.O. & CHAUVET, E. (1994). Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75: 1807-1817.

GESSNER, M.O., INCHAUSTI, P., PERSSON, L., RAFFAELLI, D.G. & GILLER, P.S. (2004). Biodiversity effects on ecosytem functioning: insights from aquatic systems. Oikos 104: 419-422.

GESSNER, M.O. & VAN RYCKEGEM, G. (2003). Water fungi as decomposers in freshwater ecosystems. *In* BITTON, G. (ED.) Encyclopedia of Environmental Microbiology. Wiley, New York. (online edition: DOI: 10.1002/0471263397.env314).

GESSNER, M.O., SUBERKROPP, K. & CHAUVET, E. (1997). Decomposition of plant litter by fungi in marine and freshwater ecosystems. *In* WICKLOW, D.C. & SÖDERSTRÖM, B. (EDS.) The mycota IV, Environmental and microbial relationships. Springer-Verlag, Berlin Heidelberg. 303-322.

GILLIAM, J.W. (1994). Riparian wetlands and river quality. J. Environm. Qual. 23: 896-900.

GINDRAT, D. & PEZET, R. (1994). Paraquat: a tool for the quick development of latent fungal infections and endophytic fungi. J. Phytopathol. 141: 86-98.

GOOSEN, N.K., VAN RIJSWIJK, P. & BROCKMANN, U. (1995). Comparison of heterotrophic bacterial production rates in early spring in the turbid estuaries of the Scheldt and the Elbe. Hydrobiologia 311: 31-42.

GORBUNOV, K.V. (1953). Raspad ostatkov vysshikh vodnykh rastenii i ego ekologicheskaia vol'v vodoemakh nizhnei zony Delty Volgi. Tr. Vses. Gidrobiol. Ova 5: 158-202.

GRAÇA, M.A., NEWELL S.Y., KNEIB, R.T. (2000). Grazing rates of organic matter and living fungal biomass of decaying *Spartina alterniflora* by three species of salt-marsh invertebrates. Mar. Biol. 136: 281-289.

GRANÉLI, W. (1989). Influence of standing litter on shoot production in reed, *Phragmites australis* (Cav.) Trin. ex Steudel. Aquatic Bot. 35: 99-109.

GRANÉLI, W. (1990). Standing crop and mineral content of reed, *Phragmites australis* (Cav.) Trin. ex Steud., in Sweden - management of reed stands to maximize harvestable biomass. Folia Geobot. Phytotax. 25: 291-302.

GRANÉLI, W., WEISNER, S.E.B. & SYTSMA, M.D. (1992). Rhizome dynamics and resource storage in *Phragmites australis*. Wetlands Ecol. Manag. 1: 239-247.

GRARÉ, W. (2004). Scheldemonitor: het getijde op de Schelde. Schelde Nieuwsbrief 38: 5.

GRATTAN II, R.M. & SUBERKROPP, K. (2001). Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. J. N. Am. Benthol. Soc. 20: 33-43.

GRAVELAND, J. & COOPS, H. (1997). Verdwijnen van rietgordels in Nederland, oorzaken, gevolgen en een strategie voor herstel. Tijdschrift voor landschapsecologie en milieukunde 14: 67-86.

GREUTER, W. & SCHOLZ, H. (1996). *Phragmites* in Crete, Cenchrus frutescens, and the nomenclature of the common reed (Gramineae). Taxon 45: 521-523.

GRIFFIN, D.H. (1994). Fungal Physiology. 2nd. Edition. Wiley-liss, New York. 458 pp.

GROPPE, K., STEINGER, T., SANDERS, I.I., SCHMID, B., WIEMKEN, A. & BOLLER, T. (1999). Interaction between the endophytic fungus *Epichloë bromicola* and the grass *Bromus erectus*: effects of endophyte infection, fungal concentration and environment on grass growth and flowering. Mol. Ecol. 8: 1827-1835.

GUO, L.D., HYDE, K.D. & LIEW, E.C.Y. (1998). A method to promote sporulation in palm endophytic fungi. Fungal Div. 1: 109-113.

HALMSCHLAGER, VON, E., BUTIN, H. & DONAUBAUER, E. (1993). Endophytische pilze in Blattern und Zweiben von *Quercus petraea*. Eur. J. Forest Pathol. 23: 51-63.

HALUPA, P.J. & HOWES, B.L. (1995). Effects of tidally mediated litter moisture content on decomposition of *Spartina alterniflora* and *S. patens*. Mar. Biol. 123: 379-391.

HARGRAVE, B.T. (1972). Aerobic decomposition of sediment and detritus as a function of particle surface area and organic content. Limnol. Oceanogr. 17: 583-596.

HASLAM, S.M. (1970). The development of the annual population in *Phragmites communis* Trin. Ann. Bot. 34: 571-591.

HASLAM, S.M. (1972). Biological flora of the British Isles no. 128, *Phragmites communis* Trin. J. Ecol. 60: 585-610.

HASLAM, S.M. (1989). Early decay of Phragmites thatch: an outline of the problem. Aquatic Bot. 35: 129-135.

HÄTTENSCHWILER, S. & VITOUSEK, P.M. (2000). The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends Ecol. Evol. 15: 238-243.

HAWKER, L.E. (1966). Environmental influences on reproduction. *In* AINSWORTH, G.C. & SUSSMAN, A.S. (EDS.) The Fungi. Vol. 2. Academic Press, New York. 435-469.

HAWKSWORTH, D.L. (1991). The fungal dimension of biodiversity: magnitude, significance and conservation. Mycol. Res. 95: 641-655.

HAWKSWORTH, D.L. (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. 105: 1422-1432.

HAYCOCK, N.E., PINAY, G. & WALKER, C. (1993). Nitrogen retention in river corridors: European perspective. Ambio 22: 340-346.

HELLINGS, L., DEHAIRS, F., TACKX, M., KEPPENS, E., BAEYENS, W. (1999). Origin and fate of organic carbon in the freshwater part of the Scheldt Estuary as traced by stable carbon isotope composition. Biogeochem. 47: 167-186.

HELLINGS, S.E. & GALLAGHER, J.L. (1992). The effects of salinity and flooding on *Phragmites australis*. J. Appl. Ecol. 29: 41-49.

HEMMINGA, M.A., KLAP, V.A., VAN SOELEN, J. & BOON, J.J. (1993). Effect of salt marsh inundation on estuarine particulate organic matter characteristics. Mar. Ecol. Prog. Ser. 99: 153-161.

HIETZ, P. (1992). Decomposition and nutrient dynamics of reed (*Phragmites australis* (Cav.) Trin. ex Steud.) litter in Lake Neusiedl, Austria. Aquatic Bot. 43: 211-230.

HILL, N.M. & PATRIQUIN, D.G. (1992). Interactions between fungi and nitrogen-fixing bacteria during decomposition. *In* CAROLL G.C. & WICKLOW, D.T. The fungal community. Its organisation and role in the ecosystem. 2de ed.. Marcel Dekker, New York. 783-796.

HOFFMANN, M. (1993). Vegetatiekundig-ecologisch onderzoek van de buitendijkse gebieden langs de Zeeschelde met vegetatiekartering. Universiteit Gent, . 223 pp.

HUDSON, H.J. (1968). The ecology of fungi on plant remains above the soil. New Phytol. 67: 837-874.

HYDE, K.D. (2001). Where are the missing fungi? Does Hong Kong have any answers? Mycol. Res. 105: 1514-1518.

INGHAM, R.E., TROFYMOW, J.A., INGHAM, E.R. & COLEMAN, D.C. (1985). Interactions of bacteria, fungi and their nematode grazers: effects on nutrient cycling and plant growth. Ecol. Monogr. 55: 119-140.

INGOLD, C.T. (1971). Fungal spores. Their liberation and dispersal. Clarendon Press, Oxford. 302 pp.

JOHNSTON, C.A., DETENBECK, N.E. & NIEMI, G.J. (1990). The cumulative effect of wetlands on stream water quality and quantity: a landscape approach. Biogeochem. 10: 105-142.

JONES, E.B.G. (1994). Fungal adhesion. Mycol. Res. 98: 961-981.

JONES, L.H.P. & HANDRECK, K.A. (1967). Silica in soils, plants and animals. Advances Agron. 19: 107-149.

JORDAN, T.E., WHIGHAM, D.F. & CORRELL, D.L. (1989). The role of litter in nutrient cycling in a brackish tidal marsh. Ecology 70: 1906-1915.

KAUFMAN, P.B., DAYANANDAN, P., TAKEOKA, Y., BIGELOW, W.G., JONES, J.D. & ILER, R. (1981). Silica in shoots of higher plants. *In* SIMPSON, T.L. & VOLCANI, B.E. (EDS.) Silican and Siliceous Structures in Biological Systems. Springer-Verlag, New York. 409-449.

KENDRICK, B. (1979). Dialogue on the ecology of anamorphs and teleomorphs. The Conference. *In* KENDRICK, B. (ed.). The Whole Fungus. The Sexual-Asexual Synthesis. Volume 2: 623-634. National Museum of Natural Sciences and National Museums of Canada, Ottawa.

KILLINGBECK, K.T. (1986). The terminological jungle revisited: making a case for use of the term resorption. Oikos 46: 263-264.

KIRK, P.M., J.C.DAVID, AND J.A. STALPERS. (2001). Ainsworth and Bisby's Dictionary of the Fungi (including the lichens). 9th ed.

KJØLLER, A. & STRUWE, S. (1982). Microfungi in ecosystems: fungal occurrence and activity in litter and soil. Oikos 39: 391-422.

KOMÍNKOVÁ, D., KUEHN, K.A., BÜSING, N., STEINER, D. & GESSNER, M.O. (2000). Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of a large lake. Aquat. Microb. Ecol. 22: 271-282.

KOPPITZ, H., KÜHL, H., HESSE, K. & KOHL, J.G. (1997). Some aspects of the importance of genetic diversity in *Phragmites australis* (Cav.) Trin. ex Steudel for the development of reed stands. Bot. Acta 110: 217-223.

KRASHENNINIKOVA, S.A. (1958). Mikrobiologicheskie protsessy raslada vodnoi rastitel nosti v litorali Rybinskogo Vodokhranilishcha. Byull. Inst. Biol. Vodokhr. 2: 3-6.

KUEHN, K.A. & SUBERKROPP, K. (1998). Diel fluctuations in rates of CO_2 evolution from standing dead leaf litter of the emergent macrophyte *Juncus effusus*. Aquatic Microb. Ecol. 14: 171-182.

KUEHN, K.A., GESSNER, M.O., WETZEL, R.G., SUBERKROPP, K. (1999). Decomposition and CO_2 evolution from standing litter of the emergent macrophyte *Erianthus giganteus*. Microb. Ecol. 38: 50-57.

KUEHN, K.A., STEINER, D. & GESSNER, M.O. (2004). Diel mineralization patterns of standing-dead plant litter: implications for CO_2 flux from wetlands. Ecology 85: 2504-2518.

KUFEL, I. & KUFEL, L. (1988). In situ decomposition of *Phragmites australis* Trin. ex Steudel and *Typha angutifolia* L.. Ekol. Polska 36: 459-470.

KÜHL, H. & KOHL, J.-G. (1993). Seasonal nitrogen dynamics in reed beds (*Phragmites australis* (Cav.) Trin. ex. Steudel) in relation to productivity. Hydrobiologia 251: 1-12.

KVĚT, J. & WESTLAKE, D.F. (1998). Primary production in wetlands. *In* WESTLAKE, D.F., KVĚT, J. & SZCZEPAŃSKI, A. The production ecology of wetlands. Cambridge University Press, Cambridge. 78-168.

LAMOTE, V. (2003). Genetic diversity of *Phragmites australis*, *Iris pseudacorus* and *Typha latifolia* in Flanders. Ph.D. Ghent University (UGent), Ghent. 195 pp.

LARSEN, V.J. & SCHIERUP, H.-H. (1981). Macrophyte cycling of zinc, copper, lead and cadmium in the littoral zone of a polluted and a non-polluted lake. II. Seasonal changes in heavy metal content of above-ground biomass and decopmosing leaves of *Phragmites australis* (Cav.) Trin.. Aquatic Bot. 11: 211-230.

LATCH, G.C.M. (1998). Grass endophytes as a model. Sydowia 50: 213-228.

LEE,S.Y. (1990). Net aerial primary productivity, litter production and decomposition of the reed *Phragmites communis* in a nature reserve in Hong Kong: management implications. Mar. Ecol. Prog. Ser. 66: 161-173.

LUO, J., YIN, J., CAI, L., ZHANG, K. & HYDE, K. (2004). Freshwater fungi in Lake Dianchi, a heavily polluted lake in Yunnan, China. Fungal Div. 16:

LUO, Y., TACKX, M., LI F.Y., MAO, D.Q. & ZHOU, Q.X. (2002). Leaf litter ecological fate in the Schelde Estuary in Belgium. J. Environm. Sciences-China 14: 563-567.

MALINOWSKI, D.P., ALLOUSH, G.A., BELESKY, D.P. (2000). Leaf endophyte *Neotyphodium coenophialum* modifies mineral uptake in tall fescue. Plant Soil 227: 115-126.

MANN, K.H. (1988). Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. Limnol. Oceanogr. 33: 910-930.

MARKS, M., LAPIN, B. & RANDALL, J. (1994). *Phragmites australis* (*P. communis*): threats, management and monitoring. Nat. Areas J. 14: 285-294.

MASON, C.F. (1976). Relative importance of fungi and bacteria in the decomposition of *Phragmites* leaves. Hydrobiologia 51: 65-69.

MASON, C.F. & BRYANT, R.J. (1975). Production, nutrient content and decompositon of *Phragmites communis* Trin. and *Typha angustifolia* L.. J. Ecol. 63: 71-95.

MAY, R.M. (1991). A fondness for Fungi. Nature 352: 475-476.

MCLEAN, M.A., KANEKO, N. & PARKINSON, D. (1996). Does selective grazing by mites and collembola affect litter fungal community structure? Pedobiologia 40: 97-105.

MEGANCK, B. (1998). De morfologie van *Phragmites australis* (Cav.) Steud. in relatie tot hydrodynamiek, beheer en saliniteit in het Schelde-estuarium. Universiteit Gent, licentiaatsscriptie, unpublished, Gent. 97 pp.

MEIRE, P., ROSSAERT, G., DE REGGE, N., YSEBAERT, T. & KUIJKEN, E. (1992). Het Schelde estuarium: ecologische beschrijving en een visie op de toekomst. Instituut Voor Natuurbehoud & Universiteit Gent. 150 pp.

MEIRE, P., STARINK, M. & HOFFMANN, M. (1997). Integratie van ecologie en waterbouwkunde in de Zeeschelde: aanleiding tot en situering van het onderzoek milieueffecten sigmaplan (OMES). Water 95: 147-165.

MEIRE, P., YSEBAERT, T., HOFFMANN, M., VAN DEN BALCK, E., DEVOS, K., SAMANYA, R., DEREGGE, N., VAN WAEYENBERGE, J., ANSELIN, A., ROSSAERT, G. & KUIJKEN, E. (1994). Ecologisch onderzoek in de Zeeschelde door het instituut voor natuurbehoud: onderbouwing van natuurherstel en natuurontwikkeling. Biol. Jaarb. Dodonaea 62: 27-47.

MELILLO, J.M., ABER, J.D. & MURATORE, J.F. (1982). Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63: 621-626.

MELILLO, J.M., NAIMAN, R.J., ABER, J.D. & LINKINS, A.E. (1984). Factors controlling mass loss and nitrogen dynamics of plant litter decaying in northern streams. Bull. Mar. Sci 35: 341-356.

METCALFE, C.R. (1960). Anatomy of monocotyledons I. Gramineae. Clarendon Press, Oxford. 731 pp.

MEYERSON, L.A., SALTONSTALL, K., WINDHAM, L., KIVIAT, E. & FINDLAY, S. (2000). A comparison of *Phragmites australis* in freshwater and brackish water marsh environment in North America. Wetlands Ecol. Manag. 8: 89-103.

MIDDELBURG, J.J., KLAVER, G., NIEUWENHUIZE, J. & VLUG, T. (1995). Carbon and nitrogen cycling in intertidal sediments near Doel, Scheldt Estuary. Hydrobiologia 311: 57-69.

MIDDELBURG, J.J., NIEUWENHUIZ, J., LUBBERS, R.K. & VANDE PLASSCHE, O. (1997). Organic carbon isotope systematics of coastal marshes. Est. Coast. Shelf Sci 45: 681-687.

MILLE-LINDBLOM, C. & TRANVIK, L.J. (2003). Antagonism between bacteria and fungi on decomposing aquatic plant litter. Microb. Ecol. 45: 173-182.

MITSCH, W.J. & GOSSELINK, J.G. (2000). Wetlands (3rd edition). Van Nostrand Reinhold, New York. 920 pp.

MORAN, M.A., LEGOVIC, T., BENNER, R., HODSON, R.E. (1988). Carbon flow form lignocellulose - a simulation analysis of a detritus-based ecosystem. Ecology 69: 1525-1536.

MUN, H.T., NAMGUNG, J. & KIM, J.H. (2000). Mass loss and changes of nutrients during the decomposition of *Phragmites communis* at the fringe of stream. Korean J. Ecol. 23: 157-161.

MUYLAERT, K. (1999). Distribution and dynamics of protist communities in a freshwater tidal estuary. Doctoraatscriptie Universiteit Gent, Faculteit Wetenschappen, Gent, 192 pp.

MUYLAERT, K., SABBE, K. & VYVERMAN, W. (2000). Spatial and temporal dynamics of phytoplankton communities in a freshwater tidal estuary (Schelde, Belgium). Estuarine Coastal Shelf Sci. 50: 673-687.

MUYLAERT, W. (1996). Morfologisch-ecologische studie van *Phragmites australis* (Cav.) Steud. in het Schelde-estuarium. Universiteit Gent, Licentiaatsscriptie, unpublished, Gent. 120 pp.

NEWELL, S.Y. (1992). Estimating fungal biomass and productivity in decomposing litter. *In* CAROLL G.C. & WICKLOW, D.T. The fungal community. Its organisation and role in the ecosystem. 2th ed.. Marcel Dekker, New York. 521-561.

NEWELL, S.Y. (1993). Decomposition of shoots of a salt-marsh grass, methodology and dynamics of microbial assemblages. Adv. Microb. Ecol. 13: 301-326.

NEWELL, S.Y. (1994). Ecomethodology for organosomotrophs: prokaryotic unicellular versus eukaryotic mycelial. Microb. Ecol. 28: 151-157.

NEWELL, S.Y. (2001a). Spore-expulsion rates and extents of blade occupation by Ascomycetes of the smooth-cordgrass standing-decay system. Bot. Mar. 44: 277-285.

NEWELL, S.Y. (2001b). Multiyear patterns of fungal biomass dynamics and productivity within naturally decaying smooth cordgrass shoots. Limnol. Oceanogr. 46: 573-583.
NEWELL, S.Y. (2003). Fungal content and activities in standing-decaying leaf blades of plants of the Georgia Coastal Ecosystems research area. Aquatic Microb. Ecol. 32: 95-103.
NEWELL, S.Y. & BÄRLOCHER, F. (1993). Removal of fungal and total organic matter form decaying cordgrass leaves by shredder snails. J. Exp. Mar. Biol. Ecol. 171: 39-49.
NEWELL, S.Y. & FALLON, R.D. (1989). Litterbags, leaf tags, and decay of nonabscised intertidal leaves. Canad. J. Bot. 67: 2324-2327.
NEWELL, S.Y. & PORTER, D. (2000). Microbial secondary production from saltmarsh-grass shoots and its known and potential fates. *In* WEINSTEIN, M.P. & KREEGER, D.A. (EDS.) Concepts and controversion in tidal marsh ecology. Kluwer Academic Publishers, Dordrecht. 159-186.
NEWELL, S.Y. & WASOWSKI, J. (1995). Sexual productivity and spring intramarsh distribution of a key saltmarsh microbial sec. producer. Estuaries 18: 241-249.
NEWELL, S.Y., ARSUFFI, T.L. & PALM, L.A. (1996). Misting and nitrogen fertilization of shoots of a saltmarsh grass: effects upon fungal decay of leaf blades. Oecologia 108: 495-502.
NEWELL, S.Y., ARSUFFI, T.L. & PALM, L.A. (1998). Seasonal and vertical demography of dead portions of shoots of smooth cordgrass in a south-temperate saltmarsh. Aquatic Bot. 60: 325-335.
NEWELL, S.Y., ARSUFFI, T.L., KEMP, P.F. & SCOTT, L.A. (1991). Water potential of standing-dead shoots of an intertidal grass. Oecologia 85: 321-326.
NEWELL, S.Y., FALLON, R.D., RODRIGUEZ, R.M.C. & GROENE, L.C. (1985). Influence of rain, tidal wetting and relative humidity on release of carbon dioxide by standing-dead salt-marsh plants. Oecologia 68: 73-79.
ODUM, W.E. (1988). Comparative ecology of tidal freshwater and salt marshes. Ann. Rev. Ecol. Syst. 19: 147-176.
OLÁH, J. (1972). Leaching, colonization and stabilization during detritus formation. Mem. Ist. Ital. Idrobiol. 29 Suppl.: 105-127.
OLIVEIRA, R.S., DODD, J.C. & CASTRO, P.M.L. (2001). The mycorrhizal status of *Phragmites australis* in several polluted soils and sediments of an industrialised region of Northern Portugal. Mycorrhiza 10: 241-247.
OLIVER, A.C. (1953). The microfungi associated with *Phragmites communis* Trin. Growing in the Trent Valley. M. Sc. (Mycology). Thesis University of Nottingham, Nottingham.
OSTENDORP, W. (1989). 'Die-back' of reeds in Europe - a critical review of literature. Aquatic Bot. 35: 5-26.
PARBERY, D.G. (1996). Trophism and ecology of fungi associated with plants. Biol. Rev. Cambridge Philos. Soc. 71: 473-527.
PELÁEZ, P., COLLADO, J., ARENAL, F., BASILIO, A., CABELLO, A., DIEZ MATAS, M.T., GARCIA, J.B., GONZÁLEZ DEL VAL, A., GONZÁLEZ, V., GORROCHATEGUI, J., HERNÁNDEZ, P., MARTIN, I., PLATAS, G. & VICENTE, F. (1998). Endophytic fungi from plants living on gypsum soils as a source of secondary metabolites with antimicrobial activity. Mycol. Res. 102: 755-761.
PETRINI, O. (1996). Ecological and physiological aspect of host specificity in endophytic fungi. *In* REDLIN, S.C. & CARRIS, L.M. (EDS.) Endophytic Fungi in Grasses and Woody Plants. American Phytopathological Press, St.-Paul, MN. 87-100.
PETRINI, O., SIEBER, T.N., TOTI, L. & VIVET, O. (1992). Ecology, metabolite production, and substrate utilisation in endophytic fungi. Natural Toxins 1: 185-196.
PIECZYŃSKA, E. (1972). Ecology of the eulittoral zone of lakes. Ecol. Polska 20: 637-732.
POLUNIN, N.V.C. (1982). Processes contributing to the decay of reed (*Phragmites australis*) litter in freshwater. Arch. Hydrobiol. 94: 182-209.
POLUNIN, N.V.C. (1984). The decomposition of emergent macrophytes in fresh water. Adv. Ecol. Res. 14: 115-166.
POON, M.O.K. & HYDE, K.D. (1998). Biodiversity of intertidal estuarine fungi on *Phragmites* at Mai Po marshes, Hong Kong. Bot. Mar. 41: 141-155.
RAYNER, A.D.M. (1993). The fundamental importance of fungi in woodlands.. Brit. Wildlife 4: 205-215.
RAYNER, A.D.M. & TODD, D.K. (1979). Populations and community structure and dynamics of fungi in decaying wood. Adv. Bot. Res. 7: 333-420.
REICE, S.R. & HERBST, G. (1982). The role of salinity in decompostion of leaves of *Phragmites australis* in desert streams. J. Arid Environm. 5: 361-368.
REMANE, A. (1934). Die Brackwasser fauna. Verh. Dtsch. Ges. 36: 34-74.
RODEWALD, L. & RUDESCU, L. (1974). Das Schilfrohr. Die Binnengewässer Band XXVII: 1-302.

ROHFRITSCH, O. (1992). A fungus associated gall midge, *Lasioptera arundinis* (Schiner), on *Phragmites australis* (Cav.) Trin.. Bull. Soc. Bot. Fr. 139: 45-59.

ROHFRITSCH, O. (1997). Morphological and behavioural adaptations of the gall midge *Lasioptera arundinis* (Schiner) (Diptera, Cecidomyiidae) to collect and transport conidia of its fungal symbiont. Tijdschr. Entomol. 140: 59-66.

ROOTH, J.E. & STEVENSON, J.C. (2000). Sediment deposition patterns in *Phragmites australis* communities: implications for coastal areas threatened by rising sea-level. Wetlands Ecol. Manag. 8: 173-183.

ROWELL, R.M., HAN, J.S. & ROWELL, J.S. (2000). Characterization and factors effecting fiber properties. *In* FROLLINI, E., LEÃO, A.L. & MATTOSO, L.H.C. (EDS.) Natural polymers and agrofibers composites. Embrapa Instrumentação Agropecuária, São Carlos, Brazil. 115-134.

SACCARDO, P.A. (1889). Sylloge Fungorum 13. Friedländer & Sohn, Berlin.

SAIKKONEN, K., FAETH, S.H., HELANDER, M. & SULLIVAN, T.J. (1998). Fungal endophytes: a continuum of interactions with host plants. Rev. Ecol. Syst. 29: 319-343.

SAMUELS, A.L., GLASS, A.D.M., EHRET, D.L. & MENZIES, J.G. (1991). Distribution of silicon in cucumber leaves during infection by powdery mildew fungus (*Sphaerotheca fuliginea*). Canad. J. Bot. 69: 140-149.

SANGSTER, A.G. & PARRY, D.W. (1981). Ultrastructure of silica deposits in higher plants. *In* SIMPSON, T.L. & VOLCANI, B.E. (EDS.) Silican and Siliceous Structures in Biological Systems. Springer-Verlag, New York. 389.

ŜANTRŮČKOVÁ, H., PICEK, T., ŜIMEK, M., BAUER, V., KOPECKÝ, J., PECHAR, L., LUKAVSKÁ, J. & ČÍŽKOVÁ, H. (2001). Decomposition processes in soil of a healthy and a declining *Phragmites australis* stand. Aquatic Bot. 69: 217-234.

SCARTON, F., DAY, J.W., RISMONDO, A. (2002). Primary production and decomposition of *Sarcocornia fructicosa* (L.) Scott and *Phragmites australis* Trin. ex Steudel in the Po Delta, Italy. Estuaries 25: 325-336.

SCHAEFER, D., STEINBERGER, Y. & WHITFORD, W.G. (1985). The failure of nitrogen and lignin control of decomposition in a North-American desert. Oecologia 65: 382-386.

SCHIEFERSTEIN, B. (1997). Ökologische und molekularbiologische Untersuchungen an Schilf (*Phragmites australis* (Cav.) Trin. ex Steud.) im Bereich der Bornhöverder Seen. EcoSys Suppl. 22: 1-143.

SCHULTHESS, F.M. & FAETH, S.H. (1998). Distribution, abundances, and associations of the endophytic fungal community of Arizona fescue (*Festuca arizonica*). Mycologia 90: 569-578.

SHEARER, C.A. (1995). Fungal competition. Canad. J. Bot. 73: S1259-S1264.

SINCLAIR, J.B. & CERKAUSKAS, R.F. (1996). Latent infections vs. endophytic colonization by fungi. *In* REDLIN, S.C. & CARRIS, L.M. (EDS.) Endophytic Fungi in Grasses and Woody Plants. American Phytopathological Press, St.-Paul, MN. 3-29.

SINSABAUGH, R.L. & FINDLAY, S. (1995). Microbial production, enzyme activity, and carbon turnover in surface sediments of the Hudson River estuary. Microb. Ecol. 30: 127-141.

SOETAERT, K. & HERMAN, P.M.J. (1995a). Estimating estuarine residence times in the Westerschelde (The Netherlands) using a box model with fixed dispersion coefficients. Hydrobiologia 311: 215-224.

SOETAERT, K. & HERMAN, P.M.J. (1995b). Carbon flows in the Westerschelde estuary (The Netherlands) evaluated by means of an ecosytem model (MOSES). Hydrobiologia 311: 247-266.

SOETAERT, K. & HERMAN, P.M.J. (1995c). Nitrogen dynamics in the Westerschelde estuary (SW Netherlands) estimated by means of the ecosystem model MOSES. Hydrobiologia 311: 225-246.

SOETAERT, K., HOFFMANN, M., MEIRE, P., STARINK, M., VAN OEVELEN, D., VAN REGENMORTEL, S. & COX, T. (2004). Modeling growth and carbon allocation in two reed beds (*Phragmites australis*) in the Scheldt estuary. Aquatic Bot. 79: 211-234.

STANT, M.J. (1953). Variations in reed structure in relation to thatching. Kew Bull. 2: 231-238.

STATES, J.S. (1981). Useful criteria in the description of fungal communities. *In* WICKLOW, D.T. & CARROLL, G.C. The fungal community. Vol. I. Marcel Dekker, New York. 185-199.

STONE, J.K. (1987). Initiation and development of latent infections by *Rhabdocline parkeri* on Douglas-fir. Canad. J. Bot. 65: 2614-2621.

STRONG, D.R. JR. & LEVIN, D.A. (1975). Species richness of parasitic fungi of British trees. Proc. Nat. Acad. Sci. U.S. 11: 2116-2119.

STRUYF, E. & MEIRE, P. (2002). Silica cycling in a freshwater tidal marsh. *In* MEIRE, P., VAN DAMME, S. & HEIP, C. (EDS.) ESCA Local Meeting. Abstract Book. Ecological structures and functions in the Scheldt estuary: from past to future.. University of Antwerp, ESCA, Antwerp. poster 17.

SUBERKROPP, K. (1992). Aquatic hyphomycete communities. *In* CARROLL, G.C. & WICKLOW, D.T. (EDS.) The Fungal Community. Its organization and role in the ecosystem, 2nd ed. Marcel Dekker, New York. 729-747.

SUBERKROPP, K. (1995). The influence of nutrients on fungal growth, productivity, and sporulation during leaf breakdown in streams. Canad. J. Bot. Supll. 1: S1361-S1369.

SUBERKROPP, K. & CHAUVET, E. (1995). Regulation of leaf breakdown by fungi in streams: influences of water chemistry. Ecology 76: 1433-1445.

SUFFLING, R., SMITH, D.W. (1974). Litter decomposition studies using mesh bags - spillage inaccuracies and effects of repeated artificial drying. Canad. J. Bot. 52: 2157-2163.

SUSKE, J. & ACKER, G. (1987). Internal hyphae in young, symptomless needles of *Picea abies*: electron microscopic and cultural investigation. Canad. J. Bot. 65: 2098-2103.

SUSKE, J. & ACKER, G. (1989). Identification of endophytic hyphae of *Lophodermium piceae* in tissues of green symptomless Norway spruce needles by immunoelectron microscopy. Canad. J. Bot. 67: 1768-1774.

SWIFT, M.J. (1976). Species diversity and the structure of microbial communities in terrestrial habitats. *In* ANDERSON, J.M. & MACFADYEN, A. (EDS.) The role of terrestrial and aquatic organisms in decomposition processes. Blackwell Scientific, Oxford. 185-222.

SWIFT, M.J. (1982). Microbial succession during the decomposition of organic matter. *In* BURNS, R.G. & SLATER, J.H. (EDS.) Experimental Microbial Ecology. Blackwell, Oxford. 164-177.

SWIFT, M.J., HEAL, D.W. & ANDERSON, J.M. (1979). Decomposition in terrestrial ecosystems. Univeristy of California Press, Berkeley, CA.

TACKX, M., BILLIONES, R., YU, H., HANNOUTI, A., VAN MIEGHEM, R., DARO, N. (1999). Studie van de interatie tussen de waterkolom en het intertidaal in de Zeeschelde: partim kwalificatie en kwantificatie van organisch materiaal en plankton. VUB, laboratorium voor Ecologie en Systematiek, Brussel.

TACKX, M., HANNOUTI, A., DE PAUW, N., NIJS, J., VANTHOMME, R., DARO, N. (2000). Studie van de interactie tussen de waterkolom en het intertidaal in de Zeeschelde: partim kwalificatie en kwantificatie van organisch materiaal en plankton. Eindrapport verlenging 01 02 1999 - 31 01 2000. VUB, laboratorium voor Ecologie en Systematiek., Brussel. 29 pp.

TALIGOOLA, H.K. (1969). Stages in the colonization of *Phragmites communis* Trin. by microfungi. unpublished doctoral thesis, Nottingham. 153 pp.

TALIGOOLA, H.K., APINIS, A.E. & CHESTERS, C.G.C. (1972). Microfungi colonizing collapsed aerial parts of *Phragmites communis* Trin. in water. Nova Hedwigia 23: 465-472.

TALIGOOLA, H.K., APINIS, A.E. & CHESTERS, C.G.C. (1975). Microfungi colonizing the inflorescences of *Phragmites communis* Trin. Nova Hedwigia 26: 483-494.

TANAKA, Y. (1991). Microbial decomposition of reed (*Phragmites communis*) leaves in a saline lake. Hydrobiologia 220: 119-129.

TANAKA, Y. (1993). Aerobic cellulolytic bacterial-flora associated with decomposing *Phragmites* leaf-litter in a seawater lake. Hydrobiologia 263: 145-154.

TANAKA, Y. & TEZUKA, Y. (1982). Dynamics of detritus-attached and free-living bacteria during decomposition of *Phragmites communis* powder in seawater. Jnp. J. Ecol. 32: 151-158.

TANK, J. & WEBSTER, J.R. (1998). Interaction of substrate and nutrient availability on wood biofilm processes in streams. Ecology 79: 2168-2179.

TSCHARNTKE, T. (1999). Insects on common reed (*Phragmites australis*): community structure and the impact of herbivory on shoot growth. Aquatic Bot. 64: 399-410.

TURIAN, G. (1974). Sporogenesis in fungi. Ann. Rev. Phytopathol. 12: 129-137.

ÚLEHLOVÁ, B. (1978). Decomposition processes in the fishpond littoral. *In* DYKYJOVÁ, D. & KVĚT, J. (EDS.) Pond Littoral Ecosystems - Structure and Functioning. Springer, Berlin. 341-353.

VAN DAMME, S., VAN CLEEMPUT, O., MEIRE, P. (1998). Onderzoek Milieu-effecten Sigmaplan (OMES): Denitrificatie, Verslag AMIS DS7.4, Studie in opdracht van het Ministerie van de Vlaamse Gemeenschap, Vlaamse Milieumaatschappij, Bestuur Meetnetten en Onderzoek. Universiteit Gent, Laboratorium voor Toegepaste Analystische en Fysische Chemie, Gent. 174 pp.

VAN DAMME, S., YSEBAERT, T., MEIRE, P. & VAN DEN BERGH, E. (1999). Habitatstructuren, waterkwaliteit en leefgemeenschappen in het Schelde-estuarium. Rapport IN 99/24, Instituut voor Natuurbehoud, Brussel. 119 pp.

VAN DEN BERGH, E., HUISKES, A., CRIEL, B., HOFFMANN, M. & MEIRE, P. (2001). Biodiversiteit op de Schelde schorren. De Levende Natuur 102: 62-66.

VAN DER PUTTEN, W.H. (1997). Die-back of *Phragmites australis* in European Wetlands: an overview of the European research programme on Reed die-back and progression (1993-1994). Aquatic Bot. 59: 263-275.

VAN DER PUTTEN, W., VAN DIJK, C. & PETERS, B.A.M. (1993). Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature 362: 53-56.

VAN DER VALK, A.G., RHYMER, J.M. & MURKIN, H.R. (1991). Flooding and the decomposition of four emergent plant species in a prairie wetland. Wetlands 11: 1-16.

VAN DOKKUM, H.P., SLIJKERMAN, D.M.E., ROSSI, L. & COSTANTINI, M.L. (2002). Variation in the decomposition of *Phragmites australis* litter in a monomictic lake: the role of gammarids. Hydrobiologia 482: 69-77.

VÁNKY, K. (1994). European Smut Fungi. Gustav Fischer Verlag, Stuttgart. 570 pp.

VITOUSEK, P.M. & HOBBIE, S. (2000). Heterotrophic nitrogen fixation in decomposing litter: patterns and regulation. Ecology 81: 2366-2376.

WAINWRIGHT, M. (1992). The impact of fungi on environmental biogeochemistry. *In* CARROLL, G.C. & WICKLOW, D.T. (EDS.) The Fungal Community. It's Organisation and Role in the Ecosystem. Second ed.. Marcel Dekker, New York. 601-618.

WEBSTER, J. (1956). Succession of fungi on decaying cocksfoot culms. I. J. Ecol. 44: 517-544.

WEBSTER, J. (1957). Succession of fungi on decaying cocksfoot culms. II. J. Ecol. 45: 1-30.

WEBSTER, J.R. & BENFIELD, E.F. (1986). Vascular plant breakdown in freshwater ecosystems. Ann. Rev. Ecol. Syst. 17: 567-594.

WEINSTEIN, M.P. & KREEGER, D.A. (EDS.). (2000). Concepts and Controversies in Tidal Marsh Ecology. Kluwer Academic Publishers. Dordrecht.

WESTLAKE, D.F. (1968). Methods used to determine the annual production of reedswamp plants with extensive rhizomes. *In* GHILAROV, M.S. ET AL. (EDS.) Methods for productivity studies in root systems and rhizosphere organisms. Nauka, Leningrad. 226-234.

WETZEL, R.G. (1990). Land-water interfaces: metabolic and limnological regulators. Verh. Int. Verein. Limnol. 24: 6-24.

WHEELER, B.E.J. (1968). Fungal parasites of plants. *In* AINSWORTH, G.C. & SUSSMAN, A.S. (EDS.) The Fungi, Vol. 3. Academic Press, New York. 179-211.

WILLMER, C. & FRICKER, M. (1996). Stomata. Second edition. Chapman & Hall, London. 375 pp.

WILSON, D. (1995). Endophyte - the evolution of a term, a clarification of its use and definition. Oikos 73: 274-276.

WILSON, D. & CARROLL, G.C. (1994). An endophyte of Oregon white oak: infection studies of *Discula quercina*. Mycologia 86: 635-647.

WILSON, D. & CARROLL, G.C. (1997). Avoidance of high-endophyte space by gall-forming insects. Ecology 78: 2152-2163.

WINDHAM, L. (2001). Comparison of biomass production and decomposition between *Phragmites australis* (common reed) and *Spartina patens* (salt hay grass) in brackish tidal marshes of New Jersey, USA. Wetlands 21: 179-188.

WIRSEL, S.G.R. (2004). Homogenous stands of a wetland grass harbour diverse consortia of arbuscular mycorrhizal fungi. FEMS Microbiol. Ecol. 48: 129-138.

WIRSEL, S.G.R., LEIBINGER, W., ERNST, M. & MENDGEN, K. (2001). Genetic diversity of fungi closely associated with common reed. New Phytol. 149: 589-598.

WONG, M.K.M. & HYDE, K.D. (2001). Diversity of fungi on six species of Gramineae and one species of Cyperaceae in Hong Kong. Mycol. Res. 105: 1485-1491.

WRUBLISKI, D.A., MURKIN, H.R., VAN DER VALK, A.G. & NELSON, J.W. (1997). Decomposition of ermergent macrophyte roots and rhizomes in a northern prairi marsh. Aquatic Bot. 58: 121-134.

YADAV, A.S. & MADELIN, M.F. (1968). Experimental studies on microfungi from decaying *Heracleum sphondylium* and *Urtica dioica*. Trans. Brit. Mycol. Soc. 51: 261-267.

YADAV, A.S. & MADELIN, M.F. (1968). The ecology of microfungi on decaying stems of *Urtica dioica*. Trans. Brit. Mycol. Soc. 51: 249-259.

ZHOU, D. & HYDE, K.D. (2001). Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi. Mycol. Res. 105: 1449-1457.

PART 1

FUNGI IN A BRACKISH TIDAL MARSH

The complex fungal community structure associated with *Phragmites australis* in a brackish tidal marsh

Van Ryckegem, G. & Verbeken, A.
Target Mycological Research

Abstract The spatial and temporal distribution of fungal communities on living and naturally decaying above ground parts of *Phragmites australis* have been studied by direct observation of reproductive structures. Ninety-five taxa have been identified with 43 (45%) ascomycetes, 6 (6%) basidiomycetes, 46 (49%) mitosporic fungi of which 33 (35%) coelomycetes and 13 (14%) hyphomycetes. Thirty-five taxa were found on leaf blades, 77 taxa on leaf sheaths and 49 taxa on stems. Fungal succession was comparable between years. Significant differences were found in species composition between plant parts (leaf blade, leaf sheath and stems) and plant part specificity or recurrence of species is demonstrated. The fungal species matrix was analysed using cluster analysis and three-dimensional detrended correspondence analysis. Multivariate analysis demonstrated a vertical distribution pattern with different subcommunities along the vertical axis of standing reed shoots and a different subcommunity in the litter layer. These subcommunities change temporally due to successional events during the growth and decay of the reed plant. At least eleven species groups could be recognized on living and decaying above ground plant material and each group was characterized by indicator species. A high level of fungal specificity was noticed with several species restricted to a particular plant part or to a well-defined succession phase. The composition and development of the fungal community is discussed. The available data show that *P. australis* has a diverse and complex fungal community development characterized by initially several endophytic fungi which are probably turning into primary decomposers. Such a rich and complex community structure on a single host with abundant specialised species, illustrates the importance of similar studies for realistic fungal diversity estimates and for insights on the role of fungi in a well-defined ecosystem.

Introduction

Knowledge of biological processes associated with decomposing emergent macrophytes is still restricted. Recently, the increased acceptance of fungal activity during decomposition of emergent macrophytes in wetland ecosystems (Gessner et al., 1997; Komínková et al., 2000; Gessner, 2001; Findlay et al., 2002; Gessner & Van Ryckegem, 2003; Kuehn et al., 2004) and its suggested impact on host fitness (Wirsel et al., 2001, Ernst et al., 2003) has evoked a need to understand the fungal dynamics associated with the host plants. Although other organisms (i.e. bacteria and detritivores) are also involved in decomposition of common reed (*Phragmites australis* (Cav.) Trin. ex Steud. (e.g. Polunin, 1982), fungi are shown to be important agents of decay (Komínková et al., 2000; Gessner, 2001; Kuehn et al., 2004). The study of the fungal diversity and community structure is one of the basic tools for understanding the fungal role in any ecosystem (Swift, 1976). Furthermore, the knowledge of fungal organisation and species dynamics is necessary for the interpretation of carbon and nutrient patterns as mediated by fungal activity and of changes in biomass during decomposition.

Reed plant organ, substrate decay stage, habitat (local conditions) and microclimate are variables known to influence fungal composition during growth and decay of *P. australis* (Apinis et al., 1972a, 1975; Poon & Hyde, 1998; Bán et al., 2000; Van Ryckegem & Verbeken, 2005). The studied plant organs (leaf blade, leaf sheath and stem) (we considered leaf blade, leaf sheath and stem as the plant 'organs' in our study; the term organ will be used in this sense) differ chemically and anatomically (Rodewald & Rudescu, 1974; Armstrong et al., 1996) and hence represent distinct niches with particular microecological and physiological conditions (Petrini et al., 1992). Reed quality, as defined by anatomical structure and chemical composition, influences the decomposition process and the breakdown rate of the different plant organs and defines their suitability as a habitat for decomposers (Park, 1976; Hietz, 1992; Newell et al., 1996; Gessner, 2000). The stem is characterized by more sclerenchymatous tissues and thicker epidermal cell walls than the leaves (Metcalfe, 1960; pers. obs.); leaf sheaths contain usually abundant aerenchyma channels which are absent in leaf blades (Armstrong et al., 1996). Chemical differences between the tissue types are multiple and generally mean higher ash, silica, nitrogen and phosphorus concentrations in the leaf blades than in the stem tissue at the start of senescence (Granéli, 1990; Van Ryckegem et al., 2005). Leaf sheaths generally fall in between for nitrogen and phosphorus but have a higher amount of ash and silica compared to blades (Granéli, 1990; Gessner, 2000). Although overall carbon content of the plant organs is similar, the proportion of structural plant polymers differs between plant organs (Van Ryckegem & Verbeken, 2005; unpublished data). The most obvious differences were observed in the cellulose concentrations being highest in stems, intermediate in leaf sheaths and lowest in leaf blades. With the lignin concentration nearly similar in all plant organs, this results in a higher cellulose:lignin proportion for stems compared to leaves. Anatomically, standing reed culms vary along their vertical axis with stomatal frequency increasing with height (Willmer & Fricker, 1996) and cuticle and epidermal cell wall thickness generally increasing towards the top of the plants with increased sun exposure (Webster, 1956). This might affect fungal penetration, as might the concentration of phytoliths (silica) - possibly deleterious for fungal entry (Samuels et al., 1991). The highest concentration of phytoliths was found in the highly photosynthetic upper parts of the shoots (Deleebeeck, 2000). Once a fungus entera its host, further anatomical differences along the longitudinal axis of the shoots could influence fungal growth. For example, within the stems an internal ring of sclerenchyma is thickest near the base and thinner or incomplete near the top of the stems. Hence, the refractory sclerenchyma cells will probably slow down internal penetration for some fungi more efficiently at the base than at the top (Bosman, 1985). Moreover plant organs vary in their chemical composition (other than nutrient content) and physical properties (e.g. fiber length) depending on their position on the plant and the age of the plant (Rowell et al., 2000). For several plants (not determined for *P. australis*), the lignin, glucose and xylose content higher at the bottom than at the top (Rowell et al., 2000).

Distinct differences in microclimatic conditions such as temperature and humidity are observed in the canopy of a reed stand if wind speed is not too high. This microclimate creates buffered conditions in the middle and basal canopy (Rodewald & Rudescu, 1974).

There are several ways of addressing the analysis of a fungal community, one of them being the study of species in time and space on the substrate, as conducted here. This way we delineated, at a specific time, the partitioning of fungal species that co-exist in physically separable communities. These unit communities are spatial and temporal associations within the whole fungal community on reed, and depend on the presence of a variety of niches determined by either the chemical characteristics or/and the physical environment of the different resource units (*sensu* Swift, 1976). Other essential levels of community research are the nutritional base of each species (States, 1981), the biomass and the productivity of the involved species on the resource (e.g. Gessner et al., 1997). To the best of our knowledge no specific studies addressed the enzymatic capacity of the natural phragmiticolous mycoflora. However, Bosman (1985) presented microscopic prove of soft-rot cavities in stems of common reed and several fungal genera common on *P. australis* are able to assimilate lignocellulose (e.g. *Phaeosphaeria*, *Mycosphaerella*, *Buergenerula*, *Passeriniella*, *Stagonospora* and *Helicomyces*) (Bergbauer & Newell, 1992; Bergbauer et al., 1992a, b; Newell et al., 1996).

We recorded fungal presence by means of direct observation of sporulation structures on the resource plant. The advantages and drawbacks of using direct versus indirect observation are discussed by multiple authors (e.g. Bills & Polishook, 1990; Van Ryckegem & Verbeken, 2005) and are probably best summarized in the words of Garrett (1952): "With the plate count one identifies what one cannot see (i.e. *in situ*), whereas with the direct method one sees what one cannot identify". In an ideal case, the highest resolution of a fungal community study is obtained by a combination of direct and indirect microscopic tools and genetic tools; comparability of these techniques was demonstrated and discussed by Buchan et al. (2002).

In the present study we examined the development of the fungal community on common reed during growth till nearly complete decay on different plant organs and in different microhabitats within a reed stand by direct observation of fungal reproductive structures. The results were interpreted by means of multivariate analysis and key species for each of the recognized groups were assigned.

Methods

Site description and field procedures

Our study area ('Doel' 51° 21' N, 4° 14' E) is located in a brackish, mesohaline tidal marsh of the Scheldt-estuary 53.9 km inland, situated between the Dutch-Belgian border and the 'Verdronken Land van Saeftinghe' in the Netherlands. Salinity (as chlorinity) of tidal exchange water varies seasonally, with maximum values during dry periods in the summer (e.g. 6864 mg Cl^-/l in 1999) and an average salinity of 3270 mg Cl^-/l in 1999 (n = 24). The 50 ha large marsh consists mainly of a monospecific stand of *P. australis* with a wide belt of *Scirpus maritimus* L. at the margin. The marsh is polluted with heavy metals (Pb, Cu, Zn, Cd, Hg), and tidal exchange water is characterized by (seasonally shifting) high nitrogen ($N0_3^-$ varied in 2001 between 3.7-6.3 mg/l) and PO_3^{-4} loads [av. 0.16 mg/l (n =12, in 2001)], the latter scoring 1-2 orders of magnitudes higher than in unpolluted situations.

The pH of the tidal exchange water was found to be 7.5- 8 (data from Flemish Environment Agency, 1999-2001: VMMNR154100; Van Damme et al., 1999).

Two adjacent sample plots of 6 m^2 (3 x 2 m) were established to be monitored in two successive years. In plot 1 and 2 monitoring started respectively in May 2000 and in May 2001 and in both plots samples were collected every 4 weeks for 24 months. Both plots are considered to share similar characteristics (Table 1). The plots have been fenced (1.4 m high and mesh 1 cm^2) in order to prevent input of reed with unknown age. At the start of each cohort sampled, the plots where mown and the material was removed (January 2000 in plot 1 and January 2001 in plot 2).

Four microhabitats being basal, middle and top canopy and the litter layer, were selected in our study plots and followed during plant growth and decay.
Based on a cumulative species recovery experiment at the start of the inventory (see Van Ryckegem & Verbeken, 2005b), the sample size was set at fifteen litter culms (stems and leaf sheaths), ten litter leaves and ten replicas of all plant organs in the canopy.

Standing shoots were sampled as follows: monthly 10 shoots were cut at bottom level and divided, if possible, into 3 standardized, 30 cm long parts: basal part, middle part and upper part (excluding inflorescence). Each part consisted of attached leaf blades (if still present) and both stem and leaf sheath (if still present). Ten leaf blades were randomly selected from the middle and top canopy. Only the basal and middle parts of the stems were screened as the upper parts of the culms mainly consist of tightly packed leaf sheaths. In July 2001 and 2002, after 15 months in a standing dead position, 70 still standing culms in each plot were marked at the base with red flagged cable ties, to be sure of the age of the dead sampled culms.

In order to follow litter layer decay, initial samples were directly collected from the marsh surface. However, to be sure of the age of future samples we enclosed ten leaf blades and fifteen 30 cm long culm pieces from the last growing season, separately within the plots in plastic litterbags with 4 mm mesh, 35 x 20 cm. Plant material was collected from the litter layer in the two plots during the last week of November 2000 (plot 1) or 2001 (plot 2).

Each plant organ was sampled for different periods in their natural position (Table 2): for the stems (St) 21 months of standing colonization and decay, and 18 months of litter decay. The mycota on the leaf sheath (Ls) were followed during maximum 18 months of standing colonization and decay, and for 13 months in the litter layer. Leaf blades (L) were screened during 6 months in the canopy and subsequently for 9 months in the litter layer.

All field samples were put in plastic bags and brought directly to the laboratory to be stored at 4 °C. Within 2 weeks after collecting, they were screened for fungal presence under a dissecting microscope (magnification 180×); leaf blades, stems and leaf sheaths were processed separately. All observations are based on the presence of mature fruiting structures formed *in situ*. It was not feasible to identify all taxa to species level, as for several genera, monographs or up to date identification keys are not available. Furthermore some genera contain numerous species and/or consist of species-complexes difficult to unravel by a non-specialist or without molecular work. Each taxon was described and illustrated (Van Ryckegem, 2005).

Table 1. Characterization of sampling site. Marsh of 'Saeftinghe', a brackish tidal marsh in the Scheldt estuary (Belgium).

Inundation frequency (%) [$]	Average height of inundation (cm)[£]	Sediment ation (mm/year) [!]	Reed characteristics			Reed Biomass g/m² (n = 6) [£]
			Stem diam. first internode (mm) (n = 60)[£]	Culm height (cm) (n = 10)[†]	Density of living culms #/m² (n = 6)[£]	
15.2	17	34	4.0 ± 0.2	196 ± 21	191 ± 65	879 ± 270

[$] Percentage of flooding whit high tides (Meganck, 1998).

[£] Data from Meganck (1998). Reed characteristics estimated at the end of the growing season (10 September 1997) by harvesting all above-ground living reed matter within 0.25 m² quadrats; biomass ≈ annual above net above-ground production.

[!] Based on: sedimentation = 157.44x(flooding frequency) + 10.029, $R^2 = 0.518207$, *P*-level: 0.000001 (Van Damme et al., 1999).

[†] Own measurement (2001)

The screening of the samples resulted in a relative occurrence of the fungal taxa on a scale of 10 for the standing samples and litter leaves while a relative occurrence on a scale of 15 for the litter culms was obtained. Before data analysis the latter scores were transformed to a scale of 10 as the rest of the occurrence data.

Data analysis

To analyse the presence of fungal taxa at different plant organs we used a generalized linear model (GLM) procedure which can be adapted in cases where traditional models based on the normal distribution are not suitable. Using the SAS macro Glimmix (SAS Statistical Package version 8.2, SAS 1999) we applied a Binomial distribution with logit transformation of the probability parameter. Factors 'organ', 'plots' and the two-factor interaction were included as fixed effects and were tested by F-tests, adjusting the denominator degrees of freedom by Satterthwaite's procedure. Factors 'species' and the two-way interaction with fixed factor 'organ' were included as random effects. Significance of the 'species*organ' interaction was obtained from a likelihood ratio test. Pair wise comparisons of the presence of fungal taxa between organs were Bonferroni-corrected (Rice, 1990).

Three related multivariate statistical techniques were used to analyse the data: cluster analysis (CA), detrended correspondence analysis (DCA) and indicator species analysis (ISA). They constitute somewhat different approaches to explore the dataset, and when implemented together the techniques can be used to complement, supplement and evaluate the other analyses (Økland, 1996). Raw data were used for multivariate analysis. Cluster analysis was used to identify natural groupings of samples based on similarity in fungal assemblages; Bray-Curtis distance measure and the group average method were used. Clusters were tested for significance with the multi-response permutation procedure (MRPP), testing the hypothesis that there is no difference between the groups identified by

the cluster analysis (Biondini et al., 1988). Euclidian distance and weighting of groups by n.[sum(n)]$^{-1}$ setup was used. Because we were making multiple comparisons, *P*-values were adjusted using a Bonferroni correction (Rice, 1990). Gradients in the dataset were reconstructed using the indirect DCA algorithms. Default settings were used. Relative Euclidean correlation was calculated to express the proportion of variance explained by the DCA-axes (McCune & Mefford, 1999).

Indicator species analysis was used to contrast performance of individual species across the groups found by the previous two techniques. The indicator value (IV) for a species was determined by combining relative frequency and relative abundance in a given group and can range from 0 (no indication) to 100 % (perfect indication, meaning the species was present in all samples in the group and was absent from all samples in other groups) (Dufrêne & Legendre, 1997). Indicator species were assigned if they had an indicator value higher than 25% and a Monte Carlo simulation (1000 runs) with high significance ($P \leq 0.005$) of the IV. This supposes that an indicator species is present in at least 50% of the samples of one group and that its relative occurrence in that group reaches at least 50% (Dufrêne & Legendre, 1997). All multivariate statistical analyses were performed with PC-ORD version 4.26 (McCune & Mefford, 1999).

Subsequent analyses of the DCA-clusters include the calculation of species richness per cluster, alpha diversity per cluster (Shannon's diversity index: $H' = -\sum_{i}^{S} p_i \ln p_i$, where S = # of species in the cluster, $p_i = n_i/N$, n_i = number of records of species i, N = total number of records collected) and beta diversity (the Jaccard coefficient as qualitative index $S_j = a/(a+b+c)$, where a = # of species shared between two clusters, b = # of species restricted to the first cluster, c = # of species restricted to the second cluster). Analysis of variance of the alpha diversity data (no transformations) with the Tukey HSD test (Zar, 1996), for post hoc multiple comparisons, were performed with S-PLUS (version 6.1 for Windows, professional edition, Insightful Corp. 2002).

Table 2. Dataset characteristics for both series. 139 samples investigated in one plot, three plant organs, standing decomposition (dark grey) and litter decomposition (light grey).

		M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A
	Month[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Leaf blade	Top																								
	Middle																								
	Base[2]																								
	Litter																								
Leaf sheath	Top																								
	Middle																								
	Base[3]																								
	Litter																								
Stem	Top[4]																								
	Middle																								
	Base																								
	Litter																								

[1] first month = May of the year X, last month (24) = April of the year X + 2, X = plot of the year 2000 or 2001

[2] leaf blades at the base of shoots were not included as they are soon dropped

[3] base samples of leaf sheaths include the bud scales tightly surrounding basal node of stems

[4] top samples of stems were not included (see text)

Results

General dataset characteristics

The whole dataset comprises 3976 fungal records (513 records on leaf blades, 2516 records on leaf sheaths and 947 records on stems), representing 95 taxa. Forty-three (45%) were ascomycetes, 6 (6%) basidiomycetes and 46 taxa (49%) presumably belong with asexual ascomycetes (mitosporic fungi). The latter group is comprised of 33 (35%) coelomycetes and 13 (14%) hyphomycetes. Thirty-five taxa were found on leaves, 77 on leaf sheaths and 49 on stems. The distribution of the major fungal groups (ascomycetes, basidiomycetes, coelomycetes and hyphomycetes) on the different plant organs and heights in the canopy is shown in Fig. 1. Graphs show the species richness, excluding 20 taxa which were found only once or twice during the entire study, reducing the species richness to 33 taxa from leaves, 41 from stems and 65 from leaf sheaths. The overall interpretation of the graphs is not influenced by this simplification except for stems, where there was a decrease in ascomycete species richness with 7 taxa. Hence, our complete species list, suggests more pronounced ascomycete taxa richness on stems compared to that of asexual coelomycetes.

Table 3. Test of fixed and random effects on the presence of fungal species on different plant organs (leaf blade, leaf sheath and stem).

Fixed effects		
Organ	$F_{2,137} = 19.76$	$P = 0.0001$
Plot	$F_{1,218} = 3.75$	$P = 0.054$
Organ*Plot	$F_{2,216} = 1.56$	$P = 0.21$
Random effects		
Species*Organ	$\chi_1^2 = 25.8$	$P = 0.0001$

The higher abundance of ascomycetes (Fig. 1b), illustrates the overall higher occurrence of teleomorphic phases on stems. The opposite is true for leaf sheaths, where, on average, a relatively higher occurrence of conidiomata was found (Fig. 1b). However, the taxa richness is comparable for both groups (Fig. 1a). Basidiomycetes and hyphomycetes (Fig. 1a), show the highest species richness on leaf sheaths but their relative occurrence was highest on leaf blades (Fig. 1b).

Based on total occurrence of the species, the plant organs show an overall significant difference (Kruskal-Wallis, df = 2, $P < 0.001$). When plant organs are compared in pairs, all but the combination of leaf blades and stems had no significant difference in species occurrence (Wilcoxon rank-sum test, Bonferroni correction (α /3): leaf blade-leaf sheath, $P < 0.001$; stem-leaf sheath, $P < 0.001$; stem-leaf blade, $P = 0.087$).

Based on presence-absence data the species composition significantly varied between organs ($\sigma^2 = 5.19$, Table 3). The species composition did not significantly differ between the two plots of reed sampled (Table 3). However, given the strong (albeit non-significant) trend (Table 3) we decided to keep this factor in the model when testing for differences between organs. The organ*plot interaction was not significant and was

removed from the final model. Species richness differed significantly between organs (Table 3) except for leaf blades and stems (F = 2.55, *P* = 0.33), with more species present on leaf sheaths compared to leaf blades (F = 58.58, *P* = 0.0001) and in leaf sheaths compared to stems (F = 20.53, *P* = 0.0001).

The number of taxa within each fungal group is remarkably similar in the different microhabitats (basal, middle and top canopy and litter layer) (Fig. 1c). Total taxa richness is comparable for the two dominant groups (ascomycetes and the coelomycetes) except for the lower ascomycete species richness in the top canopy. Moreover, there is an increasing occurrence of coelomycetes per sample investigated with increasing height in the reed stand (Fig. 1d). In correspondence with the species richness, ascomycete occurrence per sample is comparable between the litter layer and basal and middle canopy while few records are scored in the top canopy.

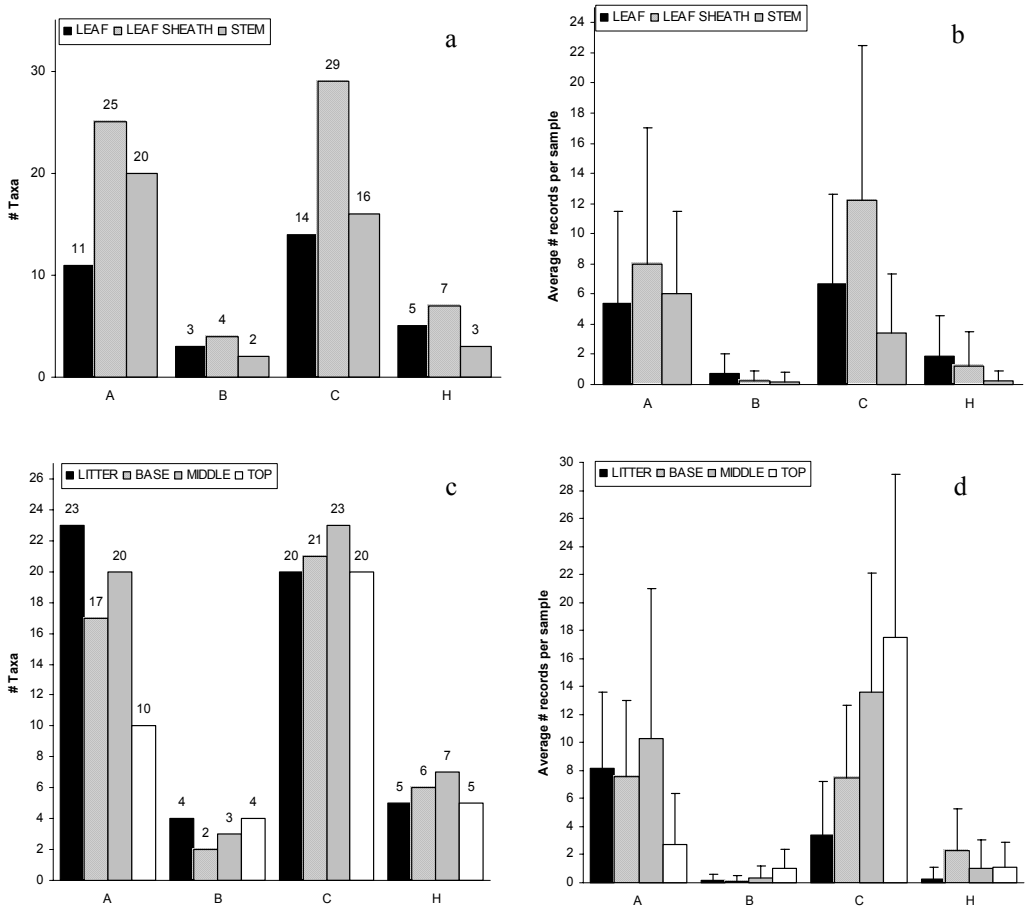


Figure 1. Distribution of the major fungal groups on the different plant organs (leaf blade, leaf sheath and stem) (a, b) and on different heights (basal, middle and upper canopy and litter layer) (c, d) in a brackish tidal marsh based on all taxa (75) recorded more than twice during the study. A = ascomycetes; B = basidiomycetes; C = coelomycetes; H = hyphomycetes. a) Total species richness on the different plant organs within each group. b) Average number of records per sample collected on the different plant organs within each group. c) Species richness on the different heights along a standing shoot and in the litter layer within each group. d) Average number of records per sample collected at a certain height within each group.

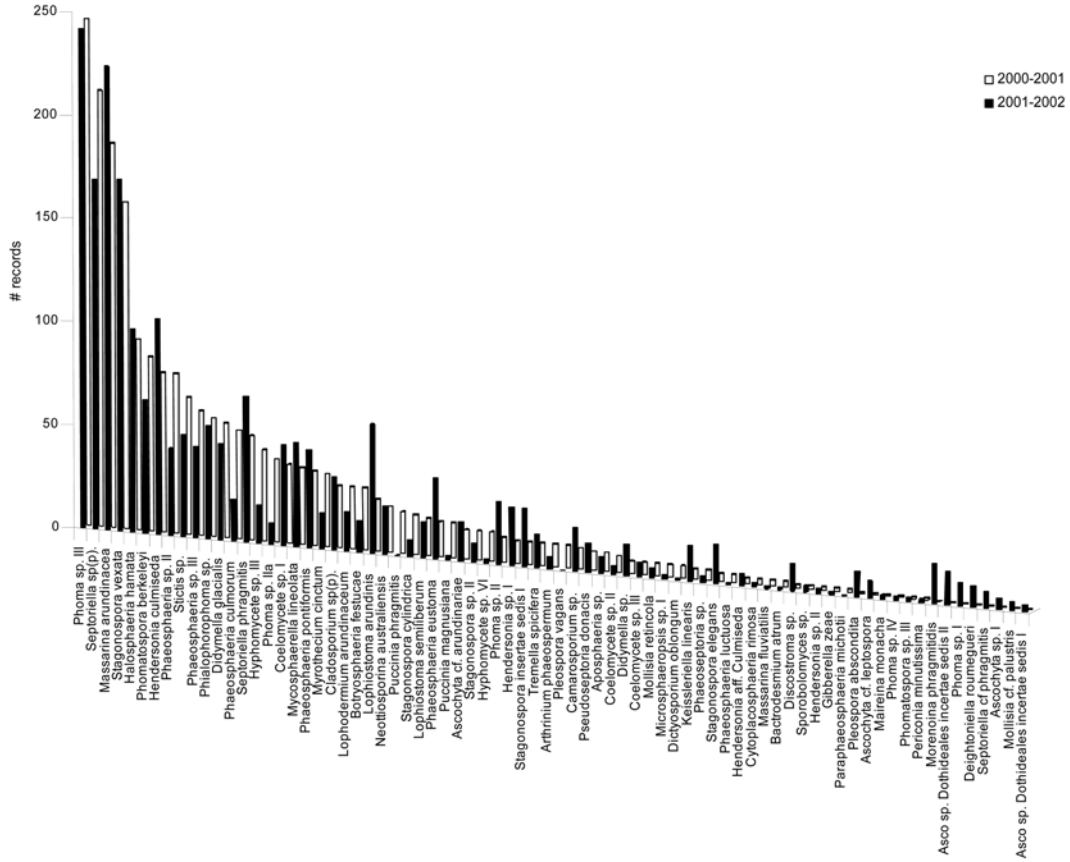


Figure 2. Species frequency distribution compared between the two successive plots with one year interval. All taxa (75) recorded more than twice during the study are shown.

Only eleven taxa are recorded more than 100 times (i.e. > 2.5% of the total number of records) and *Phoma* sp. III stands for 11.7% of the records. Forty-four taxa (46%) are found less than ten times. The average species richness on the reed samples, excluding samples without fungal colonisation (47 samples), is almost 6 (SD ± 3.7). The average number of records in one sample is 17 (SD ± 13.6). The frequency distribution within the two plots is similar for most species (Fig. 2)

Classification & Ordination

The two plots of reed followed with one year interval show high similarity, both based on presence-absence data of species (Jaccard similarity 0.695) and when comparing species abundance data (Bray-Curtis similarity 0.806). The total number of records (2102 versus 1874), the taxa distribution (Fig. 2) and the species number (80 versus 81) are also comparable. Furthermore, the results of the generalized linear model showed no significant effect from series on the species presence on the different plant organs (Table 3). These observations make it justifiable to combine both plot datasets.

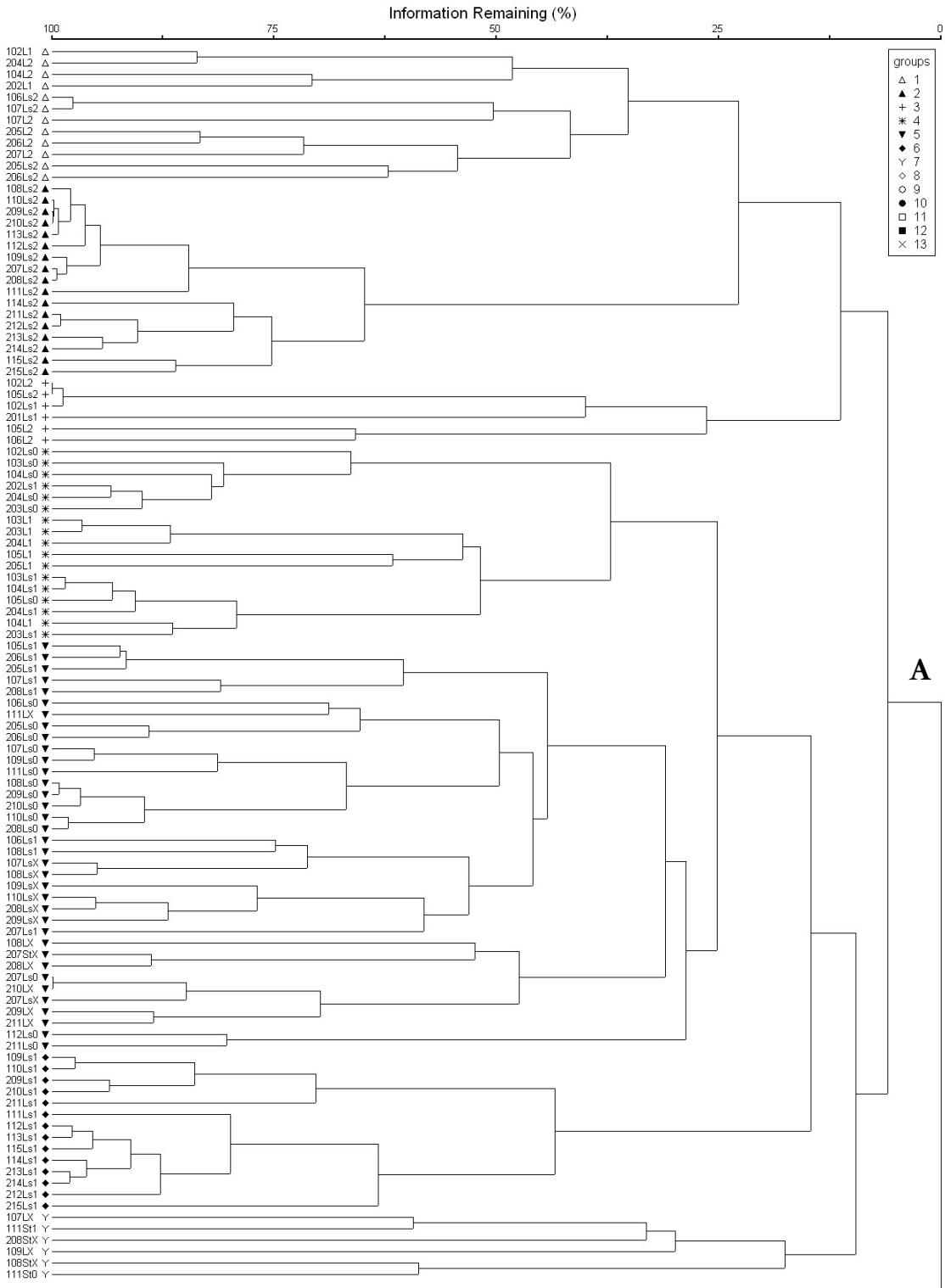
In total 278 samples were screened (Table 2) (139 from each plot) for fungal presence but on 47 samples no fungi were found, leaving 231 samples. Additionally the

dataset was reduced by leaving out all species which were only recorded once or twice (on a total of 3976), by elimination of samples which consisted of only one record and by exclusion of one obvious outlier sample (identified after preliminary cluster analysis), leaving 75 taxa and 209 samples for further statistical analysis. The excluded outlier was a leaf sample exposed to the litter for one month, which had six taxa with low frequency more typical for the top canopy leaf sheaths.

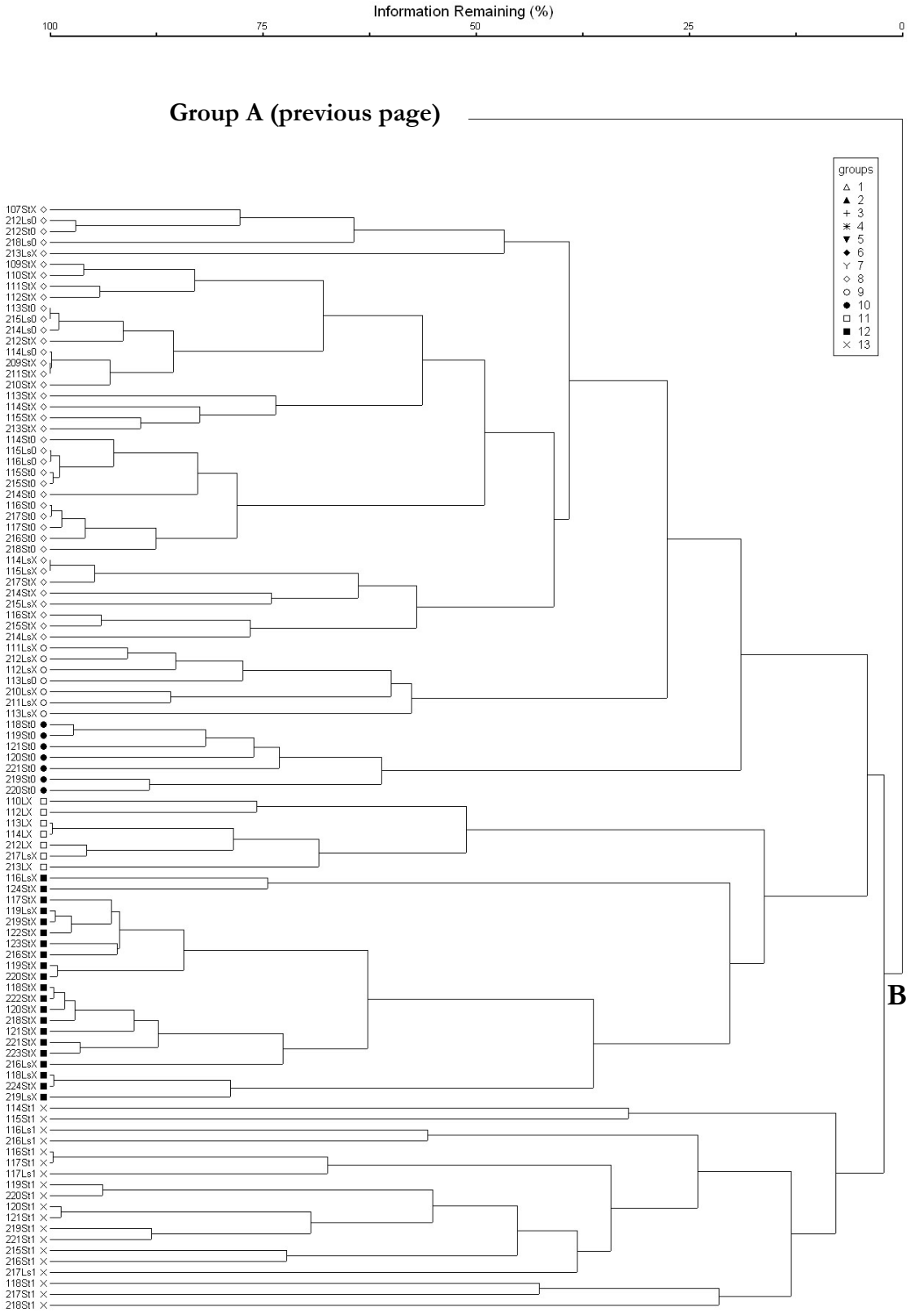
Cluster Analysis

Cluster analysis (Fig. 3) identified two large groups (A & B) and within these groups, 13 smaller subgroups based on similarities in fungal assemblages. Group A includes leaf samples (blades and sheaths) in the canopy together with samples freshly exposed in the litter layer; a few stem samples early in succession are also included in this cluster. Group B holds all other stem samples, from initial till last phases of decomposition and leaf blade and leaf sheath samples in a later phase of decomposition. Eleven subgroups can be interpreted easily, while two subgroups (number 3 and 7) are rest groups (Fig. 3): subgroup **1**: leaf blade and leaf sheath samples from the top and middle canopy during early colonisation; subgroup **2**: a tight cluster with all leaf sheath samples from the top canopy during standing decay; subgroup **3**: a rest group of samples close to group 1; subgroup **4**: early colonisation samples of leaf blades and leaf sheaths, characteristic for the middle and basal canopy; subgroup **5**: with leaf blade and leaf sheath samples maximally exposed for 5 months in the litter layer and basal and middle leaf sheath samples on moribund or senescent standing reed shoots (start decomposition); subgroup **6**: a tight cluster of leaf sheath samples from the middle canopy during decomposition; subgroup **7**: a rest group of samples close to group 5, with leaf blades and stem samples initially colonized in the litter layer; subgroup **8:** first phase of fungal sporulation on the base of standing stems and stems in litter layer, together with some late decay samples of basal canopy and litter layer leaf sheaths; subgroup **9**: a transitional cluster (between subgroup 5 and subgroup 8) of leaf sheath samples, characterized by samples decomposing in the litter layer for at least 2 months; subgroup **10**: with basal canopy stem samples in a late phase of standing decomposition; subgroup **11**: with leaf blades in the litter layer at the end of their decomposition; subgroup **12**: with stem litter layer samples at the end of the decay and subgroup **13:** with leaf sheath and stem samples from the middle canopy in a late phase of decomposition.

A multiresponse permutation procedure (MRPP) showed that the overall difference amongst the groups was highly significant (overall $P < 0.00001$). Furthermore, even when Bonferroni correction was applied (78 comparisons, α set at 0.00064), all of the subgroups compared showed significantly different species composition (data not shown).



Group B (next page)


Information Remaining (%)
100
75
50
25
0
Group A (previous page)
groups
1
2
3
4
5
6
7
8
9
10
11
12
13
B

DCA-ordination

The DCA groupings of the species data of the 209 samples correspond well with the groups identified by cluster analysis (Fig. 3) with the exception of the small subgroups 3 and 7. Those subgroups were scattered respectively within group 1 and 5 in the initial DCA scattergrams (and supported by TWINSPAN analysis, data not shown). Based on this information, groups 3 and 7 were predestined in all further DCA plots to subgroup 1 and 5 respectively.

DCA-analysis (Fig. 4, 5e, 5f) resulted in three interpretable axes explaining 48.2% of the variation in the species composition. The first axis has a considerably higher eigenvalue (0.801) and hence more explanatory power than the two subsequent axes with eigenvalues at 0.490 and 0.424. High eigenvalues together with a large length of gradient for all axes (6.199, 3.988 and 4.396 respectively) indicate a distinct β-diversity along all axes, and suggest that all axes should be considered in a fungal community interpretation. Axis 1 separates clusters A and B (Fig. 5a) corresponding largely to a separation of leaf blade and leaf sheath samples on one hand from stem samples on the other hand (Fig. 5b) and the axis is positively correlated with time (succession) (Fig. 5d). Axis 2 is also correlated with a time factor and along this axis a separation of the subgroups in cluster A is best resolved. Axis 3 shows highest explanatory power for subgroups in cluster B (Fig. 5e, f). Some of the observed effects are summarized below.

Plant organ effect

Axis 1 largely separates stems from leaves (Fig. 5b). Leaf blades and leaf sheaths are characterized by a similar species composition in the canopy and hence samples from both substrates cluster together. Group 5 (Fig. 4), contains samples in a state of initial decay in the litter layer (leaf blades, leaf sheaths and stems), without apparent plant organ effect. This group 5 also forms the transitional assemblage between both large clusters A and B. In the litter layer, the final phase in decomposition of the leaf sheaths (included in group 8) (Fig. 4) shows high similarity in species composition to the initial saprotrophic communities found on basal stem parts and litter layer stems. In a later stage of decay the fungal communities from basal and litter layer stems develop their own characteristic community: respectively group 10 and 12 (Fig. 4). The final stage of leaf blade decay in the litter layer (group 11) is also separated from the other litter layer communities (Fig. 4).

← **Figure 3**. Dendrogram from cluster analysis of combined data from both plots with relative occurrence data of 75 taxa and 209 samples. Two primary groupings A & B and thirteen clusters are identified. Sample code exists of four character states. The first figure indicates the series number being 1 or 2; the second number stands for month of collection (between 1 – 24), see Table 2; the letter code indicates plant organ: L = leaf blade, Ls = leaf sheath, St = stem; the last code stands for height in the canopy (0 = basal, 1 = middle, 2 = top) or litter layer (X). Scaled by percentage information remaining in the clusters.

Spatial effect: distribution in the reed stand

A vertical distribution of species on the reed plant is shown. This is clear for top canopy leaf blades (group 2), and well illustrated for canopy leaf sheaths. Those leaf sheath show a different species composition in the basal (group 5), middle (group 6) and top (group 2) canopy.

Comparable, standing dead basal (group 10) and middle (group 13) regions of stems are recognized by their unique species composition. Furthermore, the third axis (Fig. 5e, f) spreads out group B samples and stresses the difference between basal canopy and litter layer samples of stems.

Temporal effect: succession

DCA- axes 1 and 2 are both corresponding with a decay process gradient (Fig. 5d, arrows; Fig. 5f). Axis 1 shows a succession of fungal communities during decay of leaf blades; a succession of stems and leaf sheaths in the lower canopy; and a successional gradient in the litter layer. Axis 1 shows a pattern of initial fungal colonisation in the canopy on living shoots, gradually turning into a period of standing decay and a final phase of decomposition in the litter layer. Axis 2 is negatively correlated with a decay process gradient of leaf sheaths decomposed in the canopy: all samples in a later phase of standing decay are found in the lower half of the DCA plot (Fig. 4, 5d). Standing fungal communities associated with leaf sheaths from the top and middle canopy follow each a separate succession pattern (Fig. 5d). For leaf blades, this standing decay is very short in time and a standing decay gradient similar to the one for leaf sheaths could not be detected. Successional trends during stem decay are also shown in Fig. 4 and 5d. Initially, litter layer stem samples and basal stem samples show similar species composition (group 8), but as decay proceeds both of these microhabitats develop their own characteristic fungal community. Successional trends on standing stems are less pronounced.

Alpha and beta diversity among DCA clusters

Shannon's diversity index (H), a composite index of species richness and abundance, shows the highest taxa diversity for group 6 (leaf sheaths, standing dead, middle canopy) and 9 (leaf sheaths, litter layer) with high taxa richness and overall high evenness of the taxa abundances within the samples (Fig. 6). The lowest taxa diversity was seen in group 11, which is characterized by several leaf blade samples in the final phase of decay in the litter layer. Several of these leaves were colonized by only one taxon. Groups 5 and 8, clustering litter and basal standing samples in their initial decay phase, showed, in spite of their highest taxa richness (Table 4), a lower and highly variable diversity. This reflects the low evenness, as these two groups constitute a heterogenic assemblage, because litter layer samples from different origin enter the litter layer. Initially, it is possible to retrieve all typical terrestrial fungi in the litter layer. However, these taxa soon cease sporulation and for a short period only a few taxa can be recovered from the litter layer [indicated by the diversity flags reaching zero in groups 5 and 8 (Fig. 6)]. Within two months other taxa adapted to the litter layer conditions start to sporulate.

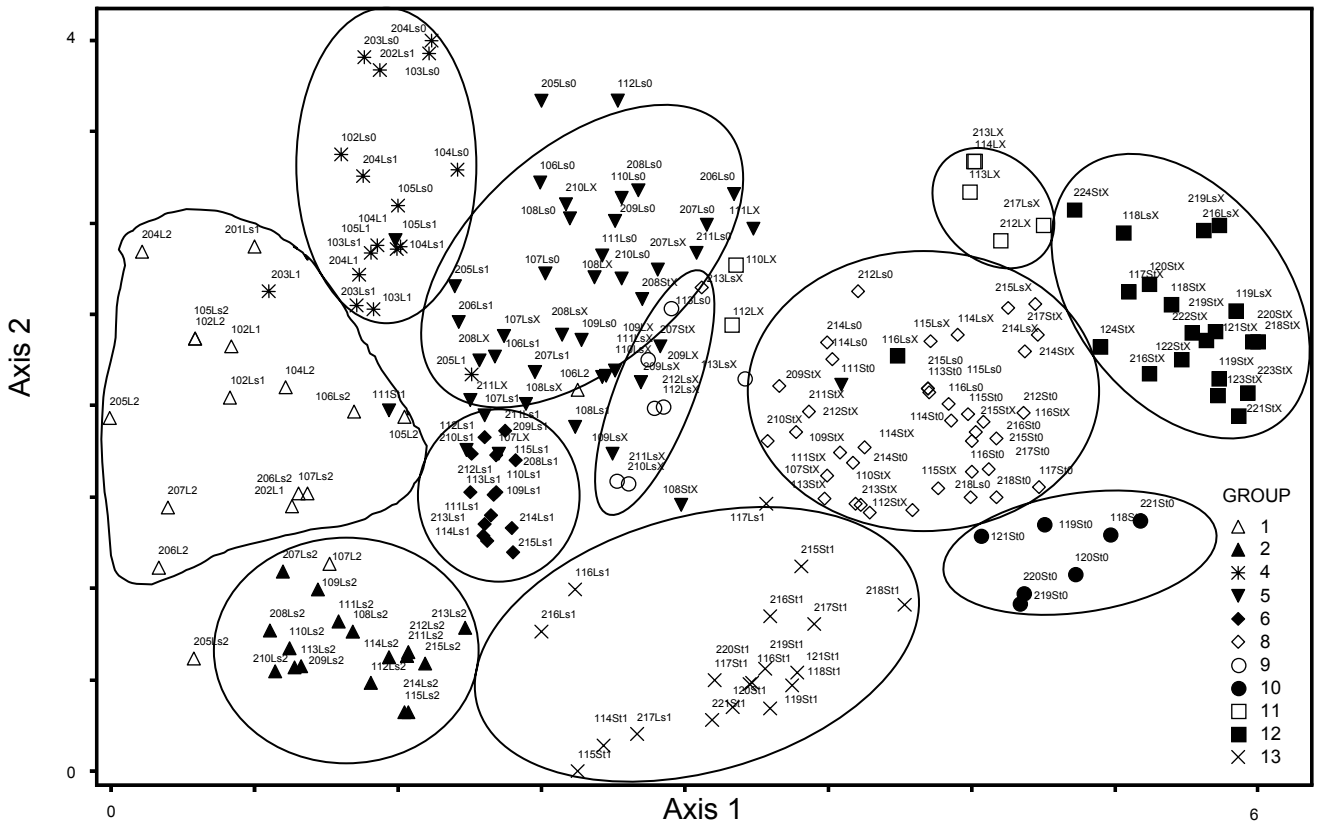


Figure 4. First two ordination axes of a DCA of species occurrences. Samples are plotted and coded by cluster groupings defined in Fig. 3 (same dataset). Cluster 3 and 7 in CA were coded in DCA as respectively belonging to group 1 and 5 (see text). Axes scaled in SD units.

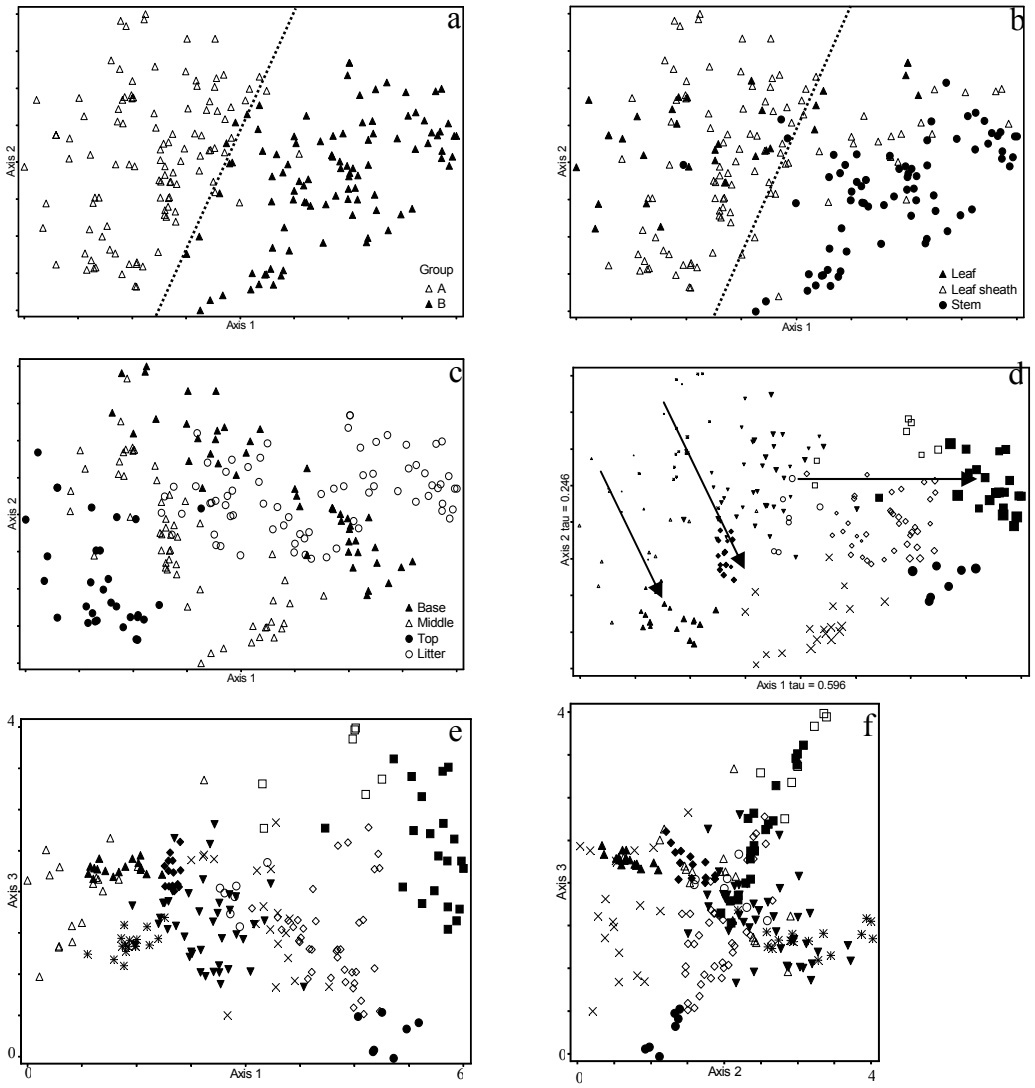
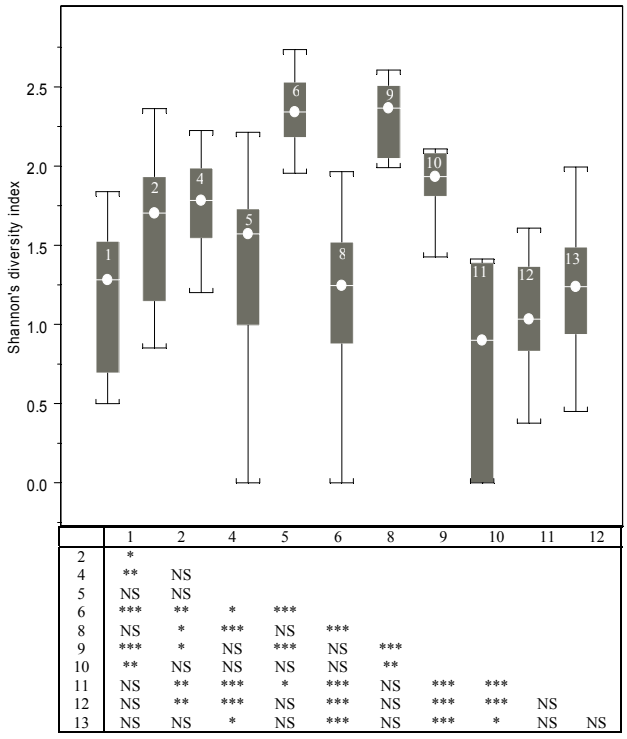


Figure 5. DCA ordinations of samples. a) Overlay plot of DCA (axes 1 & 2) with primary clusters A & B defined by CA in Fig. 3. b) Overlay plot of DCA (axes 1 & 2) with plant organs. c) Overlay plot of DCA (axes 1 & 2) with height of samples in the canopy or litter layer collection. d) Plot of DCA (axes 1 & 2) with samples coded as in Fig. 4 showing the relationship of sampling date to the ordination of samples. The size of the symbol is related to the date – smallest symbols are the earliest sampling dates; largest are the latest sampling dates within the study period. Kendall rank correlation (tau) with each axis is given. Arrows indicate different temporal patterns in dataset. e) Plot of DCA (axes 1 & 3) samples coded as in Fig. 3 and 4. f) Plot of DCA (axes 2 & 3) samples coded as in Fig. 3 and 4. Axes are scaled in SD units.

Table 4. The Jaccard similarity (%) matrix, based on presence-absence data used as a qualitative measurement for β-diversity among the DCA-groups. Groups are ordered by the position of the centroid of the clusters along the first DCA-axis. Similarities higher than 30% are marked in bold. Species richness of the groups is indicated between brackets in the table heading.

	1 (24)	**2 (34)**	**4 (25)**	**6 (33)**	**5 (43)**	**9 (26)**	**13 (25)**	**8 (38)**	**11 (8)**	**10 (21)**
2	**41.5**									
4	**42.9**	**33.4**								
6	29.5	**59.5**	**31.1**							
5	**32**	**52**	**36**	**50**						
9	18.6	**35.6**	29.3	**46.3**	**53.3**					
13	22.5	**34.1**	27.5	**34.9**	**34**	**44.5**				
8	17	20	23.1	22.4	**37.9**	**35.4**	**40**			
11	14.3	16.7	13.3	13.9	19.1	20.7	22.2	18		
10	7.1	12.3	6.8	8	16.7	14.3	27.8	**47.5**	11.5	
12	8.8	6.8	8.3	9.5	12.2	17.6	18.8	27.5	**31.3**	**30.8**



	1	2	4	5	6	8	9	10	11	12
2	*									
4	**	NS								
5	NS	NS								
6	***	**	*	***						
8	NS	*	***	NS	***					
9	***	*	NS	***	NS	***				
10	**	NS	NS	NS	NS	**				
11	NS	**	***	*	***	NS	***	***		
12	NS	**	***	NS	***	NS	***	***	NS	
13	NS	NS	*	NS	***	NS	***	*	NS	NS

Figure 6. Box plot of alpha diversity (untransformed) expressed by Shannon's diversity index showing the median and quartiles of each DCA-group (fungal unit communities). Cluster abbreviations as in Fig. 4. Below ANOVA, Tukey HSD (untransformed): NS, not significant; *, $p < 0.05$; ** $p < 0.01$; ***, $p < 0.001$.

The Jaccard coefficient was used as a qualitative measurement for β-diversity (high similarity between groups indicates a low species turn-over between them). Table 4 shows the highest similarities of adjacent groups along the first DCA-axis and decreasing similarity along the axis is showing the species turn-over. The highest similarity, based on species composition, was found between group 2 and group 6. Cluster analysis and ordination

show a clear separation between the two groups based on taxa relative occurrence. The low similarities between group 11 and the other groups show the characteristic, impoverished species composition of leaf blades in a final stage of decay.

Indicator species analysis

The characteristic taxa for the groups as generated by CA, but with group 3 lumped in group 1 and group 7 in 5, conform to the ordination results are presented in figure 7. More taxa were found to be characteristic for a specific group (lowest hierarchical level) compared to multigroup assemblages (higher hierarchical levels). This indicates a high degree of overall specialization of the involved taxa. The first dichotomy in our dataset is supported by four indicator taxa both for group A & B (Fig. 7). Although, none of the taxa reach their maximal IV, the two groups represent the core groups for separating initial colonization, early and standing dead decay of leaf blades and leaf sheaths (Group A) from stem decay and last phases of leaf blade and leaf sheath decay (Group B). Within group A, subgroup 1_2, containing samples from standing initial colonisation and initial decay in the top canopy, is characterized by *Septoriella* sp(p). On green to moribund leaf blades and sheaths in the top canopy *Cladosporium* sp(p). is recognized as indicator. Group 2 (dead leaf sheaths decomposing in the top canopy) is characterized by *Hendersonia culmiseda*, *Septoriella* sp(p). and *Stagonospora elegans*. The other branch of the cladogram holds group 4_5_6 and is characterized by the overall presence of *Phoma* sp. III, a typical colonizer of middle canopy leaves, especially leaf sheaths. Because of its persisting (and the ability to form?) conidiomata it can be found frequently during initial decay in the litter layer (group 5). Group 6, with leaf sheaths in middle canopy, is charachterized by several species such as *Phialophorophoma* sp., *Stictis* sp., *Phaeosphaeria culmorum*, Coelomycete sp. I, *Lophodermium arundinaceum*, *Camarosporium* sp., *Pleospora abscondita*, *Didymella glacialis* and *Mycosphaerella lineolata*. Before this species rich community develops, the first colonization and the initial decay (group 4), of leaf sheaths in the basal and middle canopy, are characterized by *Phaeosphaeria* spp., *Didymella* sp., *Ascochyta* cf. *arundinariae* and high recurrence of *Phoma* sp. III and *Cladosporium* sp(p). Group 5 is a cluster of leaf sheath samples from the basal canopy, few leaf sheath samples from the middle canopy and some newly exposed litter layer samples of all plant organs. This group 5 is clearly heterogeneous (see above, Fig. 6, Table 4), and is characterized by only one (poor) indicator species: Hyphomycete sp. III.
Group 13, with standing dead stem samples in the middle canopy is characterized by several indicator taxa: *Botryosphaeria festucae*, *Stagonospora vexata*, *Phoma* sp. II and *Keissleriella linearis*. The latter species is dominant on the standing middle height stems after 22 months of standing decay. However it was not selected by the ISA because too few standing samples were included (Van Ryckegem & Verbeken, 2005c). Group 11_12 clusters respectively leaf blades and stems in a late phase of decay, each of these groups being well characterized by an indicator species. Group 11 is typified by *Phomatospora berkeleyi* and group 12 by *Halosphaeria hamata*. Group 8_9_10 incorporates leaf sheath and stem litter samples and basal standing dead decay of stems, all microhabitats in a reed stand were we find *Massarina arundinacea* as an indicator species.

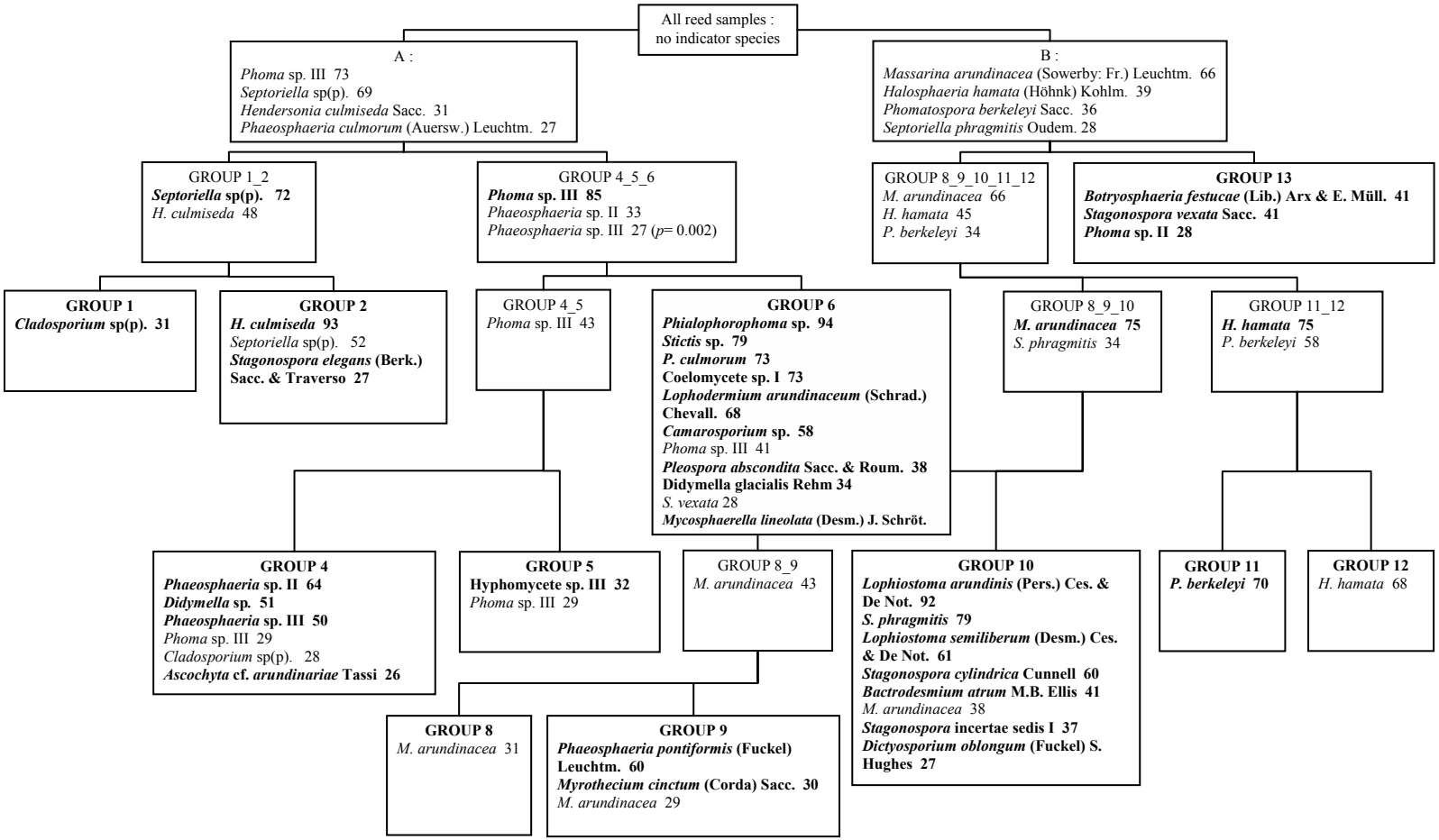


Figure 7. Sample clusters and hierarchy as obtained with CA (Fig. 3) but with group 3 and 7 respectively lumped in group 1 and 5 (Fig. 4), with associated indicator species and indicator values. All species with and indicator value > 25 and Monte-Carlo p-values ≤ 0.005. Maximum indicator values for a species are printed bold.

Basal, standing dead stems (group 10) are characterized by a typical fungal community with indicators species *Lophiostoma arundinis*, *Septoriella phragmitis*, *Lophiostoma semiliberum*, *Stagonospora cylindrica*, *Bactrodesmium atrum*, *Stagonospora* incertae sedis I, *Dictyosporium oblongum*. Group 8 contains initial phases of fungal decay on stems and groups them with late decay of leaf sheaths (mainly leaf sheaths sampled from the basal canopy). Although *M. arundinacea* is most characteristic at a higher hierarchical level in the analyses, it is also the best indicator for group 8. After a few months in the litter layer leaf sheaths develop a typical mycoflora clustered in group 9 and well- characterized by *Phaeosphaeria pontiformis* and less by *Myrothecium cinctum*. The latter species is also found on leaf sheaths in the basal canopy.

Discussion

Methodology

During this study no significant differences were observed between the two plots of common reed followed with one year interval (Table 3; 90% of the plot couples – sampled in same month but with one year interval – are in the same CA group). This shows that the observed patterns are consistent between years in a specific reed stand (see Van Ryckegem & Verbeken, 2005b,c). Furthermore, if direct observation of fungal communities was not a reliable method, a significant amount of unexplained variation should have appeared from the two successive year surveys.

We realize that the methods and scaling used in a fungal community study will inevitably bias the results and conclusions. Direct observation of the sequential sporulation of fungal taxa does not necessarily imply real successional events. Effective successional events are the result of the spatial replacement of one species by another (Fryar, 2002). However, after fructification, mycelia might still be active or at least static and consolidating their niche for longer periods (Frankland, 1998). The scoring of sporulation on a host is not a guaranty that the recorded dominant taxa during a certain phase are really the important taxa. It is possible that other fungal taxa are cryptic but nonetheless participate in the decay process. Testing this would require other methods than direct observation (e.g. genetic tools). The quantity (here estimated as relative occurrence) of sporulation structures formed is not necessarily correlated with species biomass (Wardle et al., 1993). Although it may be reasonable to expect that abundant sporulation is at least roughly correlated with total mycelial production or state of maturity for a given species, this has not yet been thoroughly tested (see Hawker, 1966). Furthermore, the often complex life cycles of fungi, characterized by pleomorphic sporulation makes it difficult to draw conclusion about the spatial and temporal distribution of the holomorphic species. Such questions are future challenges and should be addressed by a combination of genetic screening, indirect and direct observations.

Crucial steps in fungal community description are determining the exact replicate number and the size of unit communities in relation to the research objectives (States, 1981). Sample size was determined by a cumulative recovery experiment (Van Ryckegem &

Verbeken, 2005b) and proved to be sufficiently large. Besides the replica number, the physical size of the unit sample is another topic to consider. A possible smaller unit community niche than sampled during this study was shown by Apinis et al. (1972a, 1975). They demonstrated that endophytic fungal species colonization and distribution on a young, green leaf blade differed between base and apex. During this study, a preliminary survey revealed no distinct community patterns between leaf apex and base of senescent leaf blades. Additionally, Apinis et al. (1975) noticed a different species composition for standing dead stem nodes and internodes. However, observations suggest that the arrangement of leaf sheaths compared to stems could have an influence on the observed patterns. Leaf sheaths tightly surround the internodes, leaving only the top section (or a part) of the stem internode and the next node free for fungal colonization and sporulation. This means that fungal colonization is retarded and sporulation is prevented of the stem part underneath the leaf sheath. This does not imply that there are no species showing preference for nodes or internodes, but the results of Apinis et al. (1975) could merely indicate a temporal or spatial effect more than a plant part effect.

In some studies, visual evidence shows that some endophytes occupy extremely limited domains within plant tissues, confined to the lumens of single cells (Stone, 1987; Suske & Acker,1987, 1989; Cabral et al., 1993; Carroll, 1995) and thus it is conceivable that our approach, using direct observation of reproductive structures, is too imprecise to define fungal subcommunities which could even develop on a much smaller scale.

Composition of the fungal community

Eleven taxa (i.e. 12%) account together for 60% of all observations (Fig. 2, the first most abundant eleven taxa ordered in the graph), while the majority of the taxa occurs rarely. This pattern of few very common species and a long tail of species with only one or two records is common in fungal species diversity, both for endophytic and saprotrophic communities (Webster, 1956; Swift, 1976; Petrini et al., 1992). Moreover, in this study, this pattern is consistent within all defined subcommunities and the tendency to overall low frequency of the taxa is demonstrated by high β-diversity between sample clusters differentiated spatially and temporally. This implies that more specialized species can be represented by relatively few observations (compared to the generalists) but still have a considerable importance in a subcommunity. The idea of bias towards a narrow niche differentiation (specialists) is also reflected by the indicator species analysis that revealed few generalists. From the eleven abundant taxa (each more than 2.5 % of total records), eight are considered to be generalists as they have high IVs on the higher hierarchical levels. Two species (*Phialophorophoma* sp. and *Stictis* sp.) are specialists and restricted almost exclusively to the leaf sheath in the middle canopy (group 6). Indicator values of taxa in the canopy are conservative. This is caused by the inclusion of initial litter samples (groups 5 and 8) in the ISA, in which we could theoretically retrieve all the canopy taxa after falling into the litter layer. These heterogeneous reed fragments (Fig. 6), containing a mixture of fungal species, all originated from different standing dead communities. Those initial litter layer samples are poorly characterized by specific fungal species. The majority of canopy

taxa soon disappeared and is replaced within two months by more specific estuarine fungi (Van Ryckegem & Verbeken 2005b,c).

The recorded species (see Fig. 2) show a broad taxonomic distribution with twenty-nine teleomorphic genera. The most common teleomorphic (all ascomycetes) genera are *Phaeosphaeria*, *Massarina*, *Lophiostoma*, *Phomatospora* and *Mycosphaerella*. Twenty-three anamorphic genera were identified. The most common anamorphic genera are *Phoma*, *Stagonospora*, *Septoriella*, *Hendersonia*, *Phialophorophoma, Myrothecium* and *Cladosporium*.

Teleomophic taxa (51%) and anamorphic taxa (49%) are equally well-represented, although the number of records is slightly higher for the anamorphic taxa (56 %). Within the asexual fraction, coelomycetes are better represented than hyphomycetes, an observation which seems to be common for grass fungi (Wong & Hyde, 2001). Furthermore, the distribution of the large taxonomic groups differs between plant organs studied (Fig. 1a) and changes during the decomposition of the reed (Van Ryckegem & Verbeken, 2005b,c; Van Ryckegem et al., 2005).

Several taxa found during this study could be considered as endophytic as shown by moist incubation of very young reed parts (Apinis et al., 1972a,b, 1975) or by indirect isolation methods (plating surface sterilized material or by DNA extraction) (Apinis et al., 1972; Peláez et al., 1998; Wirsel et al., 2001). *Alternaria alternata*, *Epicoccum purpurascens* and *Torula herbarum* are often isolated abundantly after indirect isolation from *Phragmites* but sporulate rarely in natural conditions (Van Ryckegem pers. obs.). *Arthrinium phaeospermum*, *Septoriella phragmitis* and *Stagonospora elegans* are other presumed endophytic taxa among other genera retrieved during this study, mentioned to have endophytic representatives on *Phragmites: Cladosporium*, *Epicoccum*, *Phoma*, *Septoria*, *Phaeosphaeria*, *Hendersonia*, *Ascochyta*, *Microsphaeropsis*, and *Fusarium*. Genera with endophytic representatives found during this study but not reported before as endophytes of *P. australis* are *Anthostomella*, *Mycosphaerella* and *Didymella* (Petrini, 1986). Sporulation of those endophytic taxa on leaves (blades and sheaths) and stems seems to be dominant on moribund plant parts and/or during the first two phases of decay (Van Ryckegem & Verbeken, 2005b,c). This suggests an important role of endophytic fungi during the first phases of decay (see also Boddy & Griffith, 1989; Bills, 1996; Kowalski & Kehr, 1996; Parbery, 1996; Petrini, 1996; Zhou & Hyde, 2001). The results also suggest that endophytes have potentially a high impact on the outcome and the development of all subcommunities observed and hypothetically are the main actors in the establishment of the mature fungal communities. Endophytes should not necessarily sporulate very fast on the dying host. For example the appearance of the first sporulation structures on stems is a slow process and characterized by the appearance of *Massarina arundinacea* several months after plant death (Apinis et al., 1972b; 1975; Van Ryckegem & Verbeken, 2005c). This species develops slowly (Van Ryckegem & Verbeken, 2005c) and is probably endophytic (Apinis et al., 1972b). Other stem endophytes, among them *Stagonospora elegans* and *Septoriella phragmitis,* are part of the mature community. On leaf sheaths many endophytes sporulate on the host in spring time and partly constitute the mature communities. The endophytic phase seems to give these fungi an advantage in exploiting the dead host and consolidate their niche on the host plant before secondarily saprophytes can. Most secondarily saprotrophs are considered to sporulate on the host in latter successional phases (Van Ryckegem & Verbeken, 2005b,c).

Plant organ effect and species specificity

The main factor determining the fungal community structure during a decay series of reed seems to be the plant organ as indicated by the cluster analysis. This is also supported by the statistical analysis showing significant differences between all plant organs based on overall species recurrence (Table 3). Specific species composition of the different plant organs is more pronounced if based on recurrence data (*sensu* Zhou & Hyde (2001)) of the species than based on presence-absence data. This indicates a higher specificity for a certain plant organ notwithstanding taxa can occur on different plant organs. This corresponds with results in other studies (e.g. Lodge, 1997; Arnold et al., 2000).

Leaf blades are less characterized by specific fungal communities than leaf sheaths and stems. Only the last phase of leaf blade decay is characterized by a specific, species poor assemblage. Based on the reduced dataset, all except for two taxa occurring on leaf blades (33 taxa) were also found on leaf sheaths. The lower specificity and the community development of fungi on leaf blades is probably in close relationship with the short period of standing decay of leaf blades. New leaf blades are formed months after the leaf blades from previous year's growth are dropped, making direct transfer of spores from decaying leaf blades impossible. This direct canopy cycle is not interrupted for leaf sheaths and stems with a longer standing decay. It seems that freshly formed leaf blades in spring are inoculated with fungal spores mainly originating from leaf sheaths in the canopy. It is noticed that these specific species, common between leaf sheaths and leaf blades, are still sporulating on sheaths in spring when young leaf blades are appearing as a potential substrate (Fig. 8) (see also Van Ryckegem & Verbeken, 2005b).

Only fifteen taxa were in common between leaf blades and stems. Eight taxa were unique on stems, sixteen were unique on the leaf sheaths and one species was restricted to leaf blades. Thirty-two species were retrieved both from the leaf sheaths and from the stems and fourteen species were found on all plant organs. A remarkable observation was the significantly higher species richness on the leaf sheath, a plant organ which is often ignored in floristic and decomposition studies. The higher species richness (compared to leaf blades) is explained through a prolonged period of standing decay for the leaf sheaths; their association with stems makes it more likely that they are colonized by more typical stem fungi in the last stages of decay. Furthermore, the leaf sheaths were available in the litter layer a few months longer than leaf blades increasing the chance to get colonized with litter layer fungi. To test this effect we compared leaf blades and leaf sheaths over the same period when leaf blades were still attached to the plant. Comparison shows a significant difference in species relative occurrence (Wilcoxon rank sum test: $P = 0.0061$) between leaf blades and leaf sheaths. However, this difference is mainly due to the specific mature community on litter layer samples of both plant organs (Wilcoxon rank sum test: $P = 0.0001$). This is further demonstrated by the fact that a selection of only standing samples proved not to be significantly different (Wilcoxon rank sum test: $P = 0.29$). If we include standing leaf sheath samples for two months longer until they are — as the leaf blades — completely senescent, both plant organs compared show an increasingly different mycoflora (Wilcoxon rank sum test: $P < 0.05$). Inflorescences were not intensively studied during this study, but some inventories showed that except from *Claviceps* and a rare

parasite on the ergot (*Cerebella andropogonis* Ces.) all common species occurring on the panicles were also common on leaf sheaths (see also Taligoola et al., 1975).

The only exclusive species for leaf blades found more than once was *Deightoniella roumegueri*, a parasite (Bán et al., 2000). However, some species show a higher recurrence on leaf blades compared to leaf sheaths. For example the rust *Puccinia phragmitis* had a high recurrence on leaf blades, while a related species *P. magnusiana* mainly formed telia on the leaf sheaths. Fungal species recurrence on a specific plant organ was shown for both endophytic and saprotrophic phragmiticolous taxa (Apinis et al., 1972a,b, 1975; Taligoola et al., 1972, 1975) and further demonstrated in this study. Specific species were recognized if the taxon had at least 10 records during the study with a recurrence of more than 95% of the records on the emphasized plant organ. On the leaf sheaths the specific taxa were *Discostroma* sp. (on the tough basal bud scales of the young reed shoots), *Lophodermium arundinaceum*, *Mycosphaerella lineolata*, *Phaeosphaeria culmorum*, *Pleospora abscondita*, *Stictis* sp., *Camarosporium* sp., Coelomycete sp. I, Coelomycete sp. III, *Hendersonia culmiseda*, *Phialophorophoma* sp., Hyphomycete sp. III, Hyphomycete sp. VI, *Myrothecium cinctum*. Species with a high specificity for stems were *Keissleriella linearis*, *Lophiostoma arundinis*, *Lophiostoma semiliberum*, *Stagonospora cylindrica* and *Stagonospora* incertae sedis I.

Possible reasons for the high recurrence or even specificity on different plant organs are the differences in inoculation potential on the different plant organs, nutritional requirements of the fungi, the ability of fungi to utilize different resources or the presence of modifier molecules such as phenols and tannins known to have inhibitory effect on the activity of fungi (Swift, 1976; Hudson, 1986; Adaskaveg et al., 1991). Within our reed host several potential variables have been suggested to be important such as chemical and anatomical variation between plant organs (Metcalfe, 1960; Rodewald & Rudescu, 1974; Gráneli, 1990; Armstrong et al., 1996; unpublished data). Once in the litter layer, nutritional differences between plant organs are probably of less importance as dissolved nutrients are readily available from the tidal exchange water (Sinsabaugh et al., 1993). However, this supplementary nutrient availability from the exchange water may strengthen the fungal community differences between standing (middle and upper part culms) and basal standing – litter decay, as the standing fungal communities develop in a more oligotrophic habitat while the other habitats could be characterized as an eutrophic habitat.

The physical contact between stem and leaf sheaths probably makes it possible for some species to make the transfer to one or the other. The importance of this phenomenon compared to direct germination of spores is unknown. The exchange of species growing from one organ to the other was most obvious for leaf sheaths in a late phase of decay, getting colonized by fungi growing out of the stem towards the leaf sheaths. Some taxa have the ability to protrude the leaf sheaths with long ostioles or form fruit bodies which could be interpreted as superficial on the stems within the leaf sheath tissue (e.g. *Stagonospora elegans*).

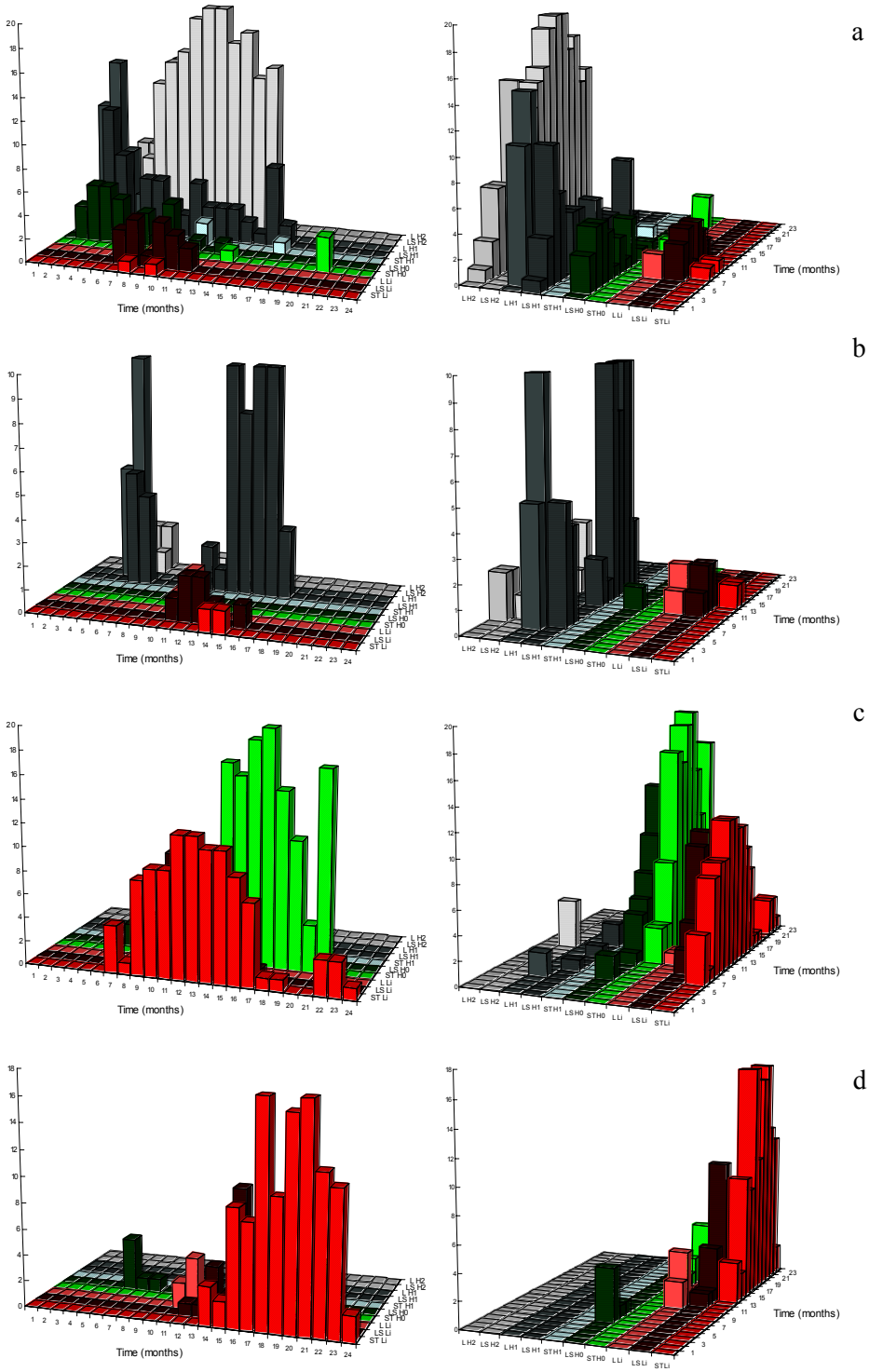


Figure 8. Distribution and occurrence of some selected species during the 2 year study period on *Phragmites australis*. With X and Y axes representing time in months or plant organ coded by height in the canopy + litter layer samples (see table 2 and code as in Fig. 3); axis Z shows number of records of the species (notice the different scaling). a. *Septoriella* sp(p).; b. *Didymella glacialis*; c. *Massarina arundinacea*; d. *Halosphaeria hamata*.

To what extent non-systemic endophytes migrate between green or newly senescent plant organs is unknown. Notwithstanding being a plausible colonization pathway (Webster, 1956; Halmschlager et al., 1993; Ernst et al., 2003), it is thought to be less important in colonization compared to spore transfer directly on the plant organ (Kaneko & Kaneko, 2004). Mycelial migration of fungi from the earlier senescent leaf blades to the living or moribund sheaths could be a logical route for saprotrophic fungi, but until now undocumented. Moreover, it is unknown to which degree the abscission zone blocks outgrow to the sheaths.

Vertical distribution: host physiology, structure and micro-environmental conditions

Spatial arrangement of fungal communities and the specific distribution of species (both endophytic and saprotrophic) (Fig. 8) on the reed shoots can be related to vertical physiological differences (e.g. senescence pattern with resorption of carbohydrates), chemical (e.g. nutrients and fiber content) and anatomical differences within plant organs (e.g. Webster, 1956; Bosman, 1985; Willmer & Fricker, 1996; Deleebeeck, 2000; Rowell et al., 2000). Furthermore, microclimatic differences and the direct influence of tidal exchange water on the basal part of standing shoots (Webster, 1956; Rodewald & Rudescu, 1974; Van Ryckegem & Verbeken, 2005a) will cause vertical gradients in a reed stand. All of the above factors together create distinct micro-ecological habitats along the vertical axis of the reed shoots which generates distinct niches for fungal species (Van Ryckegem et al. 2005; Van Ryckegem & Verbeken 2005b,c).

Vertical distribution of fungal communities on standing dead plants is well established (Webster, 1956; Hudson & Webster, 1958; Yadav & Madelin, 1968; Gessner, 1977; Kohlmeyer & Volkmann-Kohlmeyer, 2001) and has been described previously for *P. australis* (Apinis et al., 1972a, 1975; Poon & Hyde, 1998; Van Ryckegem & Verbeken, 2005). However, the distinct zonation of several fungal communities as suggested here by CA & DCA is a consequence of the sampling method and in reality the subcommunities are probably more gradually changing along the vertical axis of the shoots. Preliminary survey of standing shoots completely screened on 15 cm sections, suggested a wider growth range along the vertical axis of species restricted in this study to the top and middle 30 cm, showing a gradually changing fungal composition and a zone where both top and middle species could be retrieved. However, basal communities influenced by tidal exchange water, showed to be restricted to the lower 30 cm of the shoots. Gradual changes in time of fungal appearance on the moribund tissues in different vertical zones on the shoots could be caused by the physiological state of the plant which is characterized by a bottom-top senescence of the leaves (blade and sheath) and a top-bottom process for stems (Granéli, 1990). However, observing the gradual decay pattern of the different plant organs as reflected in species appearance on the organs was unclear because species compositions differ markedly between the different heights in the canopy. On the leaves (blade and sheath) some taxa, occurring both in the upper and middle region of the shoots, sporulated sooner in the middle canopy (Fig. 8a, *Septoriella*).

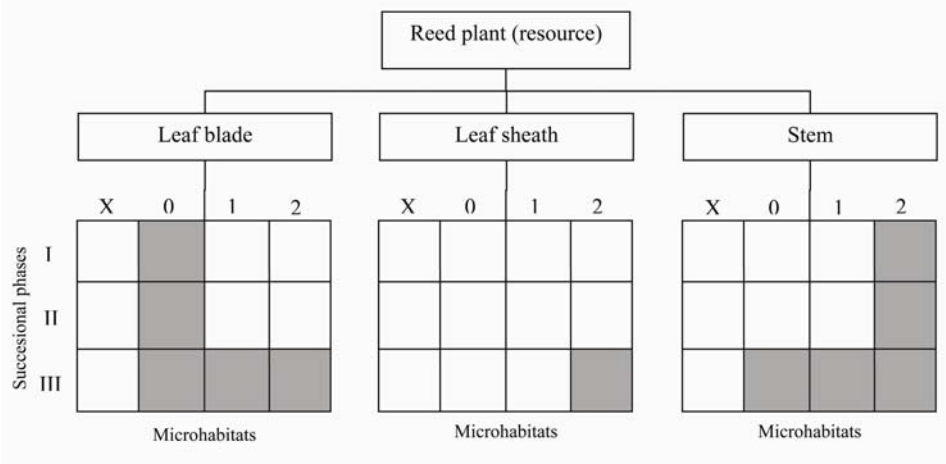


Figure 9. Diagram illustrating the spatial and temporal partitioning of fungal unit communities in a brackish tidal reed stand. Separate unit communities are characterized in each of the boxes; those unit communities characterize the different subcommunities in time (successional phases) and space (microhabitats) on the different plant organs sampled. Microhabitat acronyms: 0 = basal canopy, 1 = middle canopy, 2 = top canopy, X = litter layer. For successional phases see text. Unsampled unit communities during this study are indicated by grey coloured boxes.

Temporal effect

Fungal communities can be unique during certain seasons and during certain stages of succession (Frankland, 1998; Tokumasu, 1998; Yanna et al. 2001), a process evoked by the growth and senescence pattern of the host plant. Fungal fructification shows a succession on the different plant organs (Fig. 4, 5d). The timing of the transition of standing dead litter towards the litter layer is presented as if it was a momentary occasion, while in natural circumstances this is a gradual and continuous process. Episodic events such as storms or heavy winds were found to have a considerable impact (pers. obs.). The detailed aspects of species succession have been discussed in Van Ryckegem & Verbeken (2005 b,c).

The fungal community

Fungal community establishment on *P. australis* is complex. Community structure is characterized by dynamic consortia of fungi distinct between each plant organ and in different microhabitats. At least three successional (temporal) phases are discerned on each of the plant organs in each of the microhabitats (Van Ryckegem & Verbeken, 2005b,c; Van Ryckegem et al. in prep.]. A first phase (Phase I) corresponding with initial colonization on young, moribund and initially senescent plant organs. A second phase (Phase II) corresponding with the establishment of a species diverse, mature community and a final phase (Phase III) characterized as an impoverished community on decomposed plant material. The first successional phase in the litter layer is not characterized as an initial community but considered to be a transitional stage (Phase X). This phase shows a turnover of species characterized by a loss of terrestrial taxa and the establishment of

estuarine species (Van Ryckegem & Verbeken, 2005b,c). Assuming a three step successional pattern in each microhabitat (3 heights in the canopy and the litter layer) on all plant organs (leaf blades, leaf sheath and stems) we could theoretically have recognized 36 fungal unit communities (Fig. 9). However several units remained unsampled because the proportion of the habitat was small in the reed stand (e.g. the lower canopy leaves fall off quickly or upper canopy stems are very thin) or the study was too short in time to sample the units. Eventually we sampled 25 fungal unit communities and in the most complex situation we could have revealed those 25 fungal units after multivariate analysis. However, if we consider the whole community analysis (Fig. 3, 4), we recognized eleven groups. Several subcommunities show a similar species composition and are grouped after multivariate analysis. The resolution between subcommunities, based on species composition, was the lowest during the first successional phases (inclusive the transitional phase in the litter layer) and for basal and litter communities in general. However, if subdatasets were created, including species matrices on each plant organ separately, we found a clear distinction between all microhabitats sampled (Van Ryckegem & Verbeken, 2005b,c; Van Ryckegem et al. 2005). This indicates that subcommunities are better characterized by a specific species composition than could be demonstrated by analysing the whole fungal community in one dataset.

The results demonstrate that random distribution of fungi on a host is limited. This is supported by the high similarity between the two plots followed with one year interval and by a comparison of two plots within the same year followed for eight months (results not shown).

Apinis et al. (1972a) and Taligoola et al. (1972) recognized five successional stages in the canopy (instead of three) and two stages of aquatic litter decay. Fungal colonization in the canopy was divided in two phases on the living plant (the young and mature plant) and three stages on the standing dead plant. The initial phases recognized by Taligoola (1969) on the living host demonstrate the sequential arrival of new species on the plant. These subphases in initial successsion were not recognized during this study; if present they probably have to be demonstrated by indirect observation and plating of plant organs. Further direct comparisons and discussion of Taligoola's work is difficult as taxonomic delineation of the taxa is unclear (also in Taligoola (1969)), phenology of the taxa is often influenced by incubation or indirect observation after plating and characterization of the defined stages is poor. Notwithstanding these difficulties, some general patterns observed by Apinis et al. (1972a) were congruent with our results such as the observed differences between plant organs, microhabitats and the strong trend of successional sporulation in different phases.

Conclusions

In conclusion we can state that fungal community structure on above ground organs of *P. australis* is complex and species rich. These distinct fungal subcommunities are characteristic for the studied ecosystem, a brackish tidal marsh and are determined by specific physicochemical circumstances. This is concluded by combining results from this study with previous research results demonstrating that changing environmental conditions result in different species compositions (Van Ryckegem & Verbeken, 2005a). It is argued

that minor differences in characteristics of the reed plant and the surrounding environment will have major repercussions on fungal communities occurring on one host plant and for fungal diversity estimates in general.

Fungal community structure on *P. australis* appears rather complex compared to many other emergent macrophytes e.g. *Typha* (Pugh & Mulder, 1971) or *Spartina alterniflora* (Gessner, 1977; Buchan et al., 2002). Notwithstanding the species poor communities, differences between leaf blades and sheaths are also prevalent on *Spartina alterniflora* (Newell & Wasowski, 1995). A comparable high diversity and a complex fungal community are found on *Juncus roemerianus* (Kohlmeyer & Volkmann-Kohlmeyer, 2001). Comparable to *P. australis*, *J. roemerianus* is characterized by different successional species assemblages during the long period of standing decay; taxa that show a vertical distribution along the leaves and a specific litter layer mycoflora (Kohlmeyer & Volkmann-Kohlmeyer, 2001). The reasons for those differences in species richness and community complexity between different hosts are unclear and need to be resolved.

Acknowledgements

We are indebted to Prof. Dr. Luc Lens (Ghent University, Research group terrestrial ecology, Ghent, Belgium) for statistical advice and for programming the SAS analysis. The research was funded by the Institute for the Promotion of Innovation by Science and Technology in Flanders, Belgium (to G.V.R.).

References

ADASKAVEG, J.E., BLANCHETTE, R.A. & GILBERTSON, R.L. (1991). Decay of date palm wood by white-rot and brown-rot fungi. Canad. J. Bot. 69: 615-629.

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1972a). Colonisation of *Phragmites communis* leaves by fungi. Nova Hedwigia 23: 113-124.

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1972b). Microfungi colonizing submerged standing culms of *Phragmites communis* Trin.. Nova Hedwigia 23: 473-480.

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1975). Microfungi colonizing nodes and internodes of aerial standing culms of *Phragmites communis* Trin.. Nova Hedwigia 26: 495-507.

ARMSTRONG, J., ARMSTRONG, W., BECKETT, P.M., HALDER, J.E., LYTHE, S., HOLT, R. & SINCLAIR, A. (1996). Pathways of aeration and the mechanisms and beneficial effects of humidity- and Venturi-induced convections in *Phragmites australis* (Cav.) Trin. ex Steud.. Aquatic Bot. 54: 177-197.

ARNOLD, A.E., MAYNARD, Z., GILBERT, G.S., COLEY, P.D. & KURSAR, T.A. (2000). Are tropical fungal endophytes hyperdiverse? Ecol. Letters 3: 267-274.

BÁN, R., ENGLONER, A.I. & VIRANYI, F. (2000). Occurrence of fungal pathogens in relation to reed quality. Z. Pflanzenkrankh. Pflanzenschutz. 107: 258-266.

BERGBAUER, M. & NEWELL, S.Y. (1992). Contribution to lignocellulose degradation and DOC formation from a salt marsh macrophyte by the ascomycete *Phaeosphaeria spartinicola*. FEMS Microb. Ecol. 86: 341-348.

BERGBAUER, M., EGGERT, C. & KALNOWSKI, G. (1992a). Biotreatment of pulp mill bleachery effluents with the coelomycetous fungus *Stagonospora gigaspora*. Biotechnol. Lett. 14: 317-322.

BERGBAUER, M., MORAN, M.A. & HODSON, R.E. (1992b). Decomposition of lignocellulose from a freshwater macrophyte by aero-aquatic fungi. Microb. Ecol. 23: 159-167.

BILLS, G.F. (1996). Isolation and analysis of endophytic fungal communities from woody plants. *In* REDLIN, S.C. & CARRIS, L.M. (EDS.) Endophytic Fungi in Grasses and Woody Plants. American Phytopathological Press, St.-Paul, MN. 31-65.

BILLS, G.F. & POLISHOOK, J.D. (1990). Microfungi from *Carpinus caroliniana*. Canad. J. Bot. 69: 1477-1482.

BIONDINI, M.E., MIELKE JR., P.W., BERRY, K.J. (1988). Data-dependent permutation techniques for the analysis of ecological data. Vegetatio 75: 161-168.

BODDY, L. & GRIFFITH, G.S. (1989). Role of endophytes and latent invasion in the development of decay communities in sapwood of angiospermous trees. Sydowia 41: 41-73.

BOSMAN, M.T.M. (1985). Some effects of decay and weathering on the anatomical structure of the stem of *Phragmites australis* Trin. ex Steud.. Int. Ass. Wood. Anat. Bull. 6: 165-170.

BUCHAN, A., NEWELL, S.Y., MORETA, J.I.L. & MORAN, M.A. (2002). Analysis of internal transcribed spacer (ITS) regions of rRNA genes in fungal communities in a southeastern U.S. Salt Marsh. Microb. Ecol. 43: 329-340.

CABRAL, D., STONE, J.K. & CARROLL, G.C. (1993). The internal mycobiota of *Juncus* spp. microscopic and cultural observations of infection patterns. Mycol. Res. 97: 367-376.

CARROLL, G.C. (1995). Forest endophytes: pattern and process. Canad. J. Bot. 73 (Suppl. 1): 1316-1324.

DELEEBEECK, N. (2000) Phytolits of common reed (*Phragmites australis* (Cav.) Steudel (Poaceae)) along the Schelde and Durme (Belgium). Unpublished master thesis (in Dutch). Ghent University, Gent, part 1, 73 pp.

DUFRÊNE, M. & LEGENDRE, P. (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67: 345-366.

ERNST, M., MENDGEN, K.W. & WIRSEL, S.G.R. (2003). Endophytic fungal mutualists: seed-borne *Stagonospora* spp. enhance reed biomass production in axenic microcosms. Molec. Pl.-Microbe Interact. 16: 580-587.

FINDLAY, S.E.G., DYE, S. & KUEHN, K.A. (2002). Microbial growth and nitrogen retention in litter of *Phragmites australis* compared to *Typha angustifolia*. Wetlands 22: 616-625.

FRANKLAND, J.C. (1998). Fungal succession - Unraveling the unpredictable. Mycol. Res. 102: 1-15.

FRYAR, S.C. (2002). Fungal succession or sequence of fruit bodies?. Fungal Div. 10: 5-10.

GARRETT, S.D. (1952). The soil fungi as a microcosm for ecologists. Sci. Progress 40: 436-450.

GESSNER, M.O. (2000). Breakdown and nutrient dynamics of submerged *Phragmites* shoots in the littoral zone of a temperate hardwater lake. Aquatic Bot. 66: 9-20.

GESSNER, M.O. (2001). Mass loss, fungal colonisation and nutrient dynamics of *Phragmites australis* leaves during senescence and early decay. Aquatic Bot. 69: 325-339.

GESSNER, M.O. & VAN RYCKEGEM, G. (2003). Water fungi as decomposers in freshwater ecosystems. *In* BITTON, G. (ED.) Encyclopedia of Environmental Microbiology. Wiley, New York. (online edition: DOI: 10.1002/0471263397.env314).

GESSNER, M.O., SUBERKROPP, K. & CHAUVET, E. (1997). Decomposition of plant litter by fungi in marine and freshwater ecosystems. *In* WICKLOW, D.C. & SÖDERSTRÖM, B. (EDS.) The mycota IV, Environmental and microbial relationships. Springer-Verlag, Berlin Heidelberg. 303-322.

GESSNER, R.V. (1977). Seasonal occurrence and distribution of fungi associated with *Spartina alterniflora* from a Rhode Island estuary. Mycologia 69: 477-491.

GRANÉLI, W. (1990). Standing crop and mineral content of reed, *Phragmites australis* (Cav.) Trin. ex Steud., in Sweden - management of reed stands to maximize harvestable biomass. Folia Geobot. Phytotax. 25: 291-302.

HALMSCHLAGER, VON, E., BUTIN, H. & DONAUBAUER, E. (1993). Endophytische pilze in Blättern und Zweigen von *Quercus petraea*. Eur. J. Forest Pathol. 23: 51-63.

HAWKER, L.E. (1966). Environmental influences on reproduction. *In* AINSWORTH, G.C. & SUSSMAN, A.S. (EDS.) The Fungi. Vol. 2. Academic Press, New York. 435-469.

HIETZ, P. (1992). Decomposition and nutrient dynamics of reed (*Phragmites australis* (Cav.) Trin. Ex Steud.) litter in Lake Neusiedl, Austria. Aquatic Bot. 43: 211-230.

HUDSON, H.J. (1986). Fungal Biology. Edward Arnold (Publishers), London. 298 pp.

HUDSON, H.J. & WEBSTER, J. (1958). Succession of fungi on decaying stems of *Argopyron repens*. Trans. Brit. Mycol. Soc. 41: 165-177.

KANEKO, R. & KANEKO, S. (2004). The effect of bagging branches on levels of endophytic fungal infection in Japanese beech leaves. Forest Pathol. 34: 65-78.

KOHLMEYER, J. & VOLKMANN-KOHLMEYER, B. (2001). The biodiversity of fungi on *Juncus roemerianus*. Mycol. Res. 105: 1411-1412.

KOMÍNKOVÁ, D., KUEHN, K.A., BÜSING, N., STEINER, D. & GESSNER, M.O. (2000). Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of a large lake. Aquatic Microb. Ecol. 22: 271-282.

KOWALSKI, T. & KEHR, R.D. (1996). Fungal endophytes of living branch bases in several European tree species. *In* REDLIN, S.C. & CARRIS, L.M. (EDS.) Endophytic Fungi in Grasses and Woody Plants. American Phytopathological Press, St.-Paul, MN. 67-86.

KUEHN, K.A., GESSNER, M.O., WETZEL, R.G., SUBERKROPP, K. (1999). Decomposition and CO2 evolution from standing litter of the emergent macrophyte *Erianthus giganteus*. Microb. Ecol. 38: 50-57.

KUEHN, K.A., LEMKE, M.J., SUBERKROPP, K. & WETZEL, R.G. (2000). Microbial biomass and production associated with decaying leaf litter of the emergent macrophyte *Juncus effesus*. Limnol. Oceanogr. 45: 862-870.

KUEHN, K.A., STEINER, D. & GESSNER, M.O. (2004). Diel mineralization patterns of standing-dead plant litter: implications for CO2 flux from wetlands. Ecology 85: 2504-2518.

LODGE, D.J. (1997). Factors related to diversity of decomposer fungi in tropical forests. Biodiv. Conserv. 6: 681-688.

MCCUNE, B. & MEFFORD, M.J. (1999). PC-ORD for Windows. Multivariate Analysis of Ecological Data. Version 4.0.. MjM Software, Gleneden Beach, Oregon, U.S.A.

MEGANCK, B. (1998) The morphology of *Phragmites australis* (Cav.) Steud. in relation to hydrodynamics, management and salinity in the Scheldt estuary. Unpublished master thesis (in Dutch). Ghent University, Gent, 97 pp.

METCALFE, C.R. (1960). Anatomy of monocotyledons I. Gramineae. Clarendon Press, Oxford. 731pp.

MITSCH, W.J. & GOSSELINK, J.G. (2000). Wetlands (3rd edition). Van Nostrand Reinhold, New York. 920 pp.

NEWELL, S.Y. & WASOWSKI, J. (1995). Sexual productivity and spring intramarsh distribution of a key saltmarsh microbial sec. producer. Estuaries 18: 241-249.

NEWELL, S.Y., ARSUFFI, T.L. & PALM, L.A. (1996). Misting and nitrogen fertilization of shoots of a saltmarsh grass: effects upon fungal decay of leaf blades. Oecologia 108: 495-502.

ØKLAND, R.H. (1996). Are ordination and constrained ordination alterntive or complimentary strategies in general ecological studies. J. Veg. Sci. 7: 289-292.

PARBERY, D.G. (1996). Trophism and ecology of fungi associated with plants. Biol. Rev. Cambridge Philos. Soc. 71: 473-527.

PARK, D. (1976). Carbon and nitrogen levels as factors influencing fungal decomposers. *In* ANDERSON, J.M. & MACFADYEN, A. (EDS.) The role of terrestrial and aquatic organisms in decomposition processes. Blackwell Scientific, Oxford. 41-59.

PELÁEZ, P., COLLADO, J., ARENAL, F., BASILIO, A., CABELLO, A., DIEZ MATAS, M.T., GARCIA, J.B., GONZÁLEZ DEL VAL, A., GONZÁLEZ, V., GORROCHATEGUI, J., HERNÁNDEZ, P., MARTIN, I., PLATAS, G. & VICENTE, F. (1998). Endophytic fungi from plants living on gypsum soils as a source of secondary metabolites with antimicrobial activity. Mycol. Res. 102: 755-761.

PETRINI, O. (1986). Taxonomy of endophytic fungi of aerial plant tissues. *In* FOKKEMA, N.J. & VAN DEN HEUVEL, J. (EDS.) Microbiology of the phyllosphere. Cambridge University Press, Cambridge, UK. 175-187.

PETRINI, O. (1996). Ecological and physiological aspect of host specificity in endophytic fungi. *In* REDLIN, S.C. & CARRIS, L.M. (EDS.) Endophytic Fungi in Grasses and Woody Plants. American Phytopathological Press, St.-Paul, MN. 87-100.

PETRINI, O., SIEBER, T.N., TOTI, L. & VIVET, O. (1992). Ecology, metabolite production, and substrate utilisation in endophytic fungi. Natural Toxins 1: 185-196.

POLUNIN, N.V.C. (1982). Processes contributing to the decay of reed (*Phragmites australis*) litter in freshwater. Arch. Hydrobiol. 94: 182-209.

POON, M.O.K. & HYDE, K.D. (1998b). Evidence for the vertical distribution of saprophytic fungi on senescent *Phragmites australis* culms at Mai Po marshes. Bot. Mar. 41: 285-292.

RICE, W.R. (1990). A consensus combined P-value test and the family-wide significance of component tests. Biometrics 46: 303-308.

RODEWALD, L. & RUDESCU, L. (1974). Das Schilfrohr. Die Binnengewässer Band XXVII: 1-302.

ROWELL, R.M., HAN, J.S. & ROWELL, J.S. (2000). Characterization and Factors Effecting Fiber Properties. *In* FROLLINI, E., LEÃO, A.L. & MATTOSO, L.H.C. (EDS.) Natural Polymers and Agrofibers Composites. Embrapa Instrumentação Agropecuária, São Carlos, Brazil. 115-134.

SAMUELS, A.L., GLASS, A.D.M., EHRET, D.L. & MENZIES, J.G. (1991). Distribution of silicon in cucumber leaves during infection by powdery mildew fungus (*Sphaerotheca fuliginea*). Canad. J. Bot. 69: 140-149.

SINSABAUGH, R.L., ANTIBUS, R.K., LINKINS, A.E., MCCLAUGHERTY, C.A., RAYBURN, L., REPERT, D. & WIELAND, T. (1993). Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecology 74: 1586-1593.

STATES, J.S. (1981). Useful criteria in the description of fungal communities. *In* WICKLOW, D.T. & CARROLL, G.C. The fungal community. Vol. I. Marcel Dekker, New York. 185-199.

STONE, J.K. (1987). Initiation and development of latent infections by *Rhabdocline parkeri* on Douglas-fir. Canad. J. Bot. 65: 2614-2621.

SUSKE, J. & ACKER, G. (1987). Internal hyphae in young, symptomless needles of *Picea abies*: electron microscopic and cultural investigation. Canad. J. Bot. 65: 2098-2103.

SUSKE, J. & ACKER, G. (1989). Identification of endophytic hyphae of *Lophodermium piceae* in tissues of green symptomless Norway spruce needles by immunoelectron microscopy. Canad. J. Bot. 67: 1768-1774.

SWIFT, M.J. (1976). Species diversity and the structure of microbial communities in terrestrial habitats. *In* ANDERSON, J.M. & MACFADYEN, A. (EDS.) The role of Terrestrial and aquatic organisms in decomposition processes. Blackwell Scientific, Oxford. 185-222.

TALIGOOLA, H.K. (1969). Stages in the colonization of *Phragmites communis* Trin. by microfungi. unpublished doctoral thesis, Nottingham. 153 pp.

TALIGOOLA, H.K., APINIS, A.E. & CHESTERS, C.G.C. (1972). Microfungi colonizing collapsed aerial parts of *Phragmites communis* Trin. in water. Nova Hedwigia 23: 465-472.

TALIGOOLA, H.K., APINIS, A.E. & CHESTERS, C.G.C. (1975). Microfungi colonizing the inflorescences of *Phragmites communis* Trin. Nova Hedwigia 26: 483-494.

TOKUMASU, S. (1998). Fungal succession on pine needles fallen at different seasons: the succession of interior colonizers. Mycoscience 39: 409-416.

VAN DAMME, S., YSEBAERT, T., MEIRE, P. & VAN DEN BERGH, E. (1999). Habitatstructuren, waterkwaliteit en leefgemeenschappen in het Schelde-estuarium. Rapport IN 99/24, Instituut voor Natuurbehoud, Brussel. 119 pp.

VAN RYCKEGEM, G., GESSNER, M.O. & VERBEKEN, A. (2005). Fungi on leaf blades of *Phragmites australis* in a brackish tidal marsh: diversity, succession and leaf decomposition. Microb. Ecol. (submitted).

VAN RYCKEGEM, G. & VERBEKEN, A. (2005a). Fungal diversity and community structure on *Phragmites australis* (Poaceae) along a salinity gradient in the Scheldt estuary (Belgium). Nova Hedwigia (in print)

VAN RYCKEGEM, G. & VERBEKEN, A. (2005b). Fungal ecology and succession on *Phragmites australis* in a brackish tidal marsh. I. Leaf sheaths. Fungal Div.

VAN RYCKEGEM, G. & VERBEKEN, A. (2005c). Fungal ecology and succession on *Phragmites australis* in a brackish tidal marsh. II. Stems. Fungal Div.

WARDLE, D.A., PARKINSON, D. & WALLER, J.E. (1993). Interspecific competitive interactions between pairs of fungal species in natural substrates. Oecologia 94: 165-172.

WEBSTER, J. (1956). Succession of fungi on decaying cocksfoot culms. I. J. Ecol. 44: 517-544.

WHEELER, B.E.J. (1968). Fungal parasites of plants. *In* AINSWORTH, G.C. & SUSSMAN, A.S. (EDS.) The Fungi, Vol. 3. Academic Press, New York. 179-211.

WILLMER, C. & FRICKER, M. (1996). Stomata. Second edition. Chapman & Hall, London. 375 pp.

WIRSEL, S.G.R., LEIBINGER, W., ERNST, M. & MENDGEN, K. (2001). Genetic diversity of fungi closely associated with common reed. New Phytol. 149: 589-598.

WONG, M.K.M. & HYDE, K.D. (2001). Diversity of fungi on six species of Gramineae and one species of Cyperaceae in Hong Kong. Mycol. Res. 105: 1485-1491.

YADAV, A.S. & MADELIN, M.F. (1968). The ecology of microfungi on decaying stems of *Urtica dioica*. Trans. Brit. Mycol. Soc. 51: 249-259.

YANNA, HO, W.H. & HYDE, K.D. (2001). Fungal communities on decaying palm fronds in Australia, Brunei, and Hong Kong. Mycol. Res. 105: 1458-1471.

ZAR, J.H. (1996). Biostatistical analysis (3rd edition). Prentice-Hall, Upper Saddle River, New Jersey. 662 pp.

ZHOU, D. & HYDE, K.D. (2001). Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi. Mycol. Res. 105: 1449-1457.

Fungal ecology and succession on *Phragmites australis* in a brackish tidal marsh. I. Leaf sheaths

Adapted from: Van Ryckegem, G. & Verbeken, A. (2005). Fungal ecology and succession on *Phragmites australis* in a brackish tidal marsh. I. Leaf sheaths. Fungal diversity 19 (in press)

Abstract Direct observation of fungal succession and community development on leaf sheaths of *Phragmites australis* have been studied over a period of 19 months in a brackish tidal marsh of the river Scheldt (The Netherlands). Seventy-seven taxa were identified: 33 ascomycetes (43%); 31 coelomycetes (40%); 9 hyphomycetes (12%) and 4 basidiomycetes (5%). Four microhabitats were screened, the top, middle and basal communities along the vertical axis of standing reed shoots and a community in the litter layer. Fungal community structure analyzed by multivariate analysis showed that all microhabitats are characterized by different mycota. Detrended correspondence analysis (DCA) of leaf sheath samples suggests the importance of a spatial separation (microhabitat) in explaining species variation between samples. Within each of those microhabitats DCA indicated a specific temporal pattern (succession). Fungal succession (community development) was described by a sequence of three phases in fungal sporulation. For the different microhabitats and all successional stages indicator species were assigned. The importance of screening host plants *in situ* and the use of indicator species analysis for fungal communities is addressed. The effect of seasonality on fungal succession is discussed.

Introduction

Bacteria have traditionally been considered to be the main contributors to macrophyte decomposition (Mason, 1976; Benner et al., 1986). However, there is an increased awareness of the importance of fungi in nutrient cycling in reed dominated wetlands (Newell, 1996; Komínková et al., 2000; Findlay et al., 2002; Gessner and Van Ryckegem, 2003). A high fungal diversity is known from *Phragmites australis* from both tropical (Poon and Hyde, 1998a; Wong and Hyde, 2001) and temperate (Apinis et al., 1972; Taligoola et al. 1975; Wirsel et al., 2001; Van Ryckegem and Verbeken, 2005) regions. Hawksworth (2001) stressed the need for long term succession studies of fungi on hosts in order to obtain a better understanding of fungal diversity. Determining key species and their dynamics during the decay of a resource (*sensu* Swift, 1976) is an important step in recognizing the several successional phases during plant breakdown. Furthermore, a contrasting species composition between sample units might offer clues for the complex decay pattern mediated by fungi associated with a single host. Monitoring fungal succession by direct observation requires careful interpretation (Fryar, 2002; Jones and Hyde, 2002). Fungal succession is based on appearance of sporulating structures and could be attributed to mycelial replacement of one species by another ie. real succession (Rayner and Todd, 1979; Fryar, 2002) or sporulation due to events such as nutrient depletion, species specific life cycle differences, competition effects, or other interactions between species or the biotic and abiotic environment (e.g. Frankland, 1992, 1998; Gessner et al., 1997).

Within the Scheldt estuary site specific conditions (mainly salinity and substrate quality differ) have been found to influence fungal community composition (Van Ryckegem and Verbeken, 2005). Site specificity has been noted by Apinis et al. (1972) and Poon and Hyde (1998b). Spatial and temporal factors have also been shown to influence fungal community development within one site (Apinis et al., 1972, 1975; Poon and Hyde, 1998b).

This paper records the succession of sporulating fungi on standing leaf sheaths of *P. australis* and those in the litter layer, during plant's growth and decomposition. The fungal community is characterized by multivariate analysis and indicator species for each of the recognized clusters.

Materials and methods

The study was situated in a brackish reed stand of the Scheldt estuary near Doel (51° 21' N, 4° 14' E). The tidal marsh is located 53.9 km inland. It is situated between the Dutch-Belgian border and the 'Verdronken Land van Saeftinghe' (The Netherlands), the largest tidal nature reserve along the Scheldt. The vegetation in our study area consists of a monotypic reed stand. The tidal exchange water floods the marsh with a frequency of 15.2% of the high tides with a flooding height of 17 cm on average and is characterized by a seasonally shifting salinity of 2455 ± 1510 mg $Cl^- l^{-1}$ (average ± STDEV, N = 19 in the year 2002, data from the Flemish Environment Agency) (see Van Ryckegem and Verbeken (2005) for additional environmental and reed characteristics).

The sampling comprised two cohorts of fungal succession of equal duration but with a year interval. This was to check for between year variations. Two plots of 6 m^2 (3 × 2 m) were therefore fenced (1.4 m high and mesh 1 cm) in order to prevent input of reed with unknown age in the litter layer. At the start of each survey, the plots where mown and the material removed (in January 2000 for plot 1 and January 2001 for plot 2). The plots were adjacent and considered to share similar characteristics. Standing shoots were sampled as follows: monthly 10 shoots were cut at bottom level and divided, if possible, into 3 standardized, 30 cm long parts: basal part, middle part and upper part (excluding inflorescence). Each part consisted of both stem and leaf sheath (if still present). In July, the year after emergence, 70 standing culms in each plot were marked at the base with red flagged cable ties, to ensure the age of dead culms. In order to follow litter decay, sampling commenced in November (both cohort-years) when the culms start senescencing and gradually enter the litter layer (see Table 3). Culm pieces considered to represent middle sections were randomly collected from the marsh surface. To be sure of the age and residence time of samples in the litter layer, we enclosed fifteen 30 cm long culm pieces (stems + leaf sheaths) from the last growing season, in plastic litterbags with a 4 mm mesh, 35 × 20 cm. Culms, representing *ca.* the middle section of snapped shoots were collected from the litter layer during the last week of November 2000 (plot 1) or 2001 (plot 2). Both plots were sampled monthly starting from May 2000 (plot 1) or May 2001 (plot 2) during 19 months for leaf sheath monitoring. The mycota were followed during 17 and 18 months in

standing position in the middle and basal regions respectively and 13 months in the upper zone. Fungal sporulation was monitored for 13 months in the litter layer. The screening of the samples resulted in a relative occurrence of the fungal taxa on a scale of 10 for the leaf sheaths in a standing position, while a relative occurrence on a scale of 15 for the litter culms was obtained. Before data analysis the latter scores were transformed to a scale of 10 as a matter of conformity. All field samples were placed in plastic bags and returned to the laboratory and stored at 4°C. Samples were screened for fungal presence within 2 weeks after collecting by means of a dissecting microscope (magnification × 180). Before screening, the samples were gently wetted to make subepidermal fruit bodies more visible. All observations are based on the presence of mature fruiting structures (judged on maturity of spores or conidia) formed *in situ*. Each unique taxon was described and illustrated (Van Ryckegem, 2005).

The following indices were calculated to assess the importance of fungal taxa in the subcommunities (see Tables 1, 2).

$$\%\ \text{abundance of a taxon } X_m = \frac{\sum records\ of\ taxon\ X_m}{\sum records\ of\ all\ taxa_m} * 100$$

$$\%\ \text{recurrence of a taxon } X_m = \frac{\sum records\ of\ taxon\ X_m}{\sum records\ of\ taxon\ X} * 100$$

$$\%\ \text{occurrence of a taxon } X_{m,t} = \frac{\sum records\ of\ taxon\ X_{m,t}}{number\ of\ plant\ parts\ investigated} * 100$$

With *m* the microhabitat (top, middle and basal height of standing shoots or litter layer) where the taxa were collected and *t* the time (months).

Both plots were compared using the Jaccard coefficient as qualitative index S_j = a/(a+b+c), where a = # of species shared between two series, b = # of species restricted to the first series, c = # of species restricted to the second series and the Bray-Curtis (Sørensen) coefficient as quantitave index S_{bc} = 2s/(a+b), where s is the sum of shared abundances and a and b are the sums of abundances in individual sample units. Species diversity was calculated by Shannon's diversity index: $H' = -\sum_i p_i \ln p_i$,
where S = # of species in the community, $p_i = n_i/N$, n_i = number of records of species i, N = total number of records collected.

Fungal species composition on reed leaf sheaths was investigated using cluster analysis (CA) to identify groupings of samples based on similarity in fungal assemblages (whole dataset, raw data) using the Bray-Curtis distance measure and group average method.

Gradients in the dataset were reconstructed using the indirect detrended correspondence analysis (DCA) algorithms based on raw data and without down-weighting of rare taxa in the final ordination.

Indicator species analysis (ISA) was used to ascertain which species were responsible for the differences among the groups found in the cluster analysis (Dufrêne and Legendre, 1997). The indicator value (IV) for a taxon was determined by combining relative frequency and relative abundance in a given group. This value can range from 0 (no indication) to 100 (perfect indication, meaning the species was present in all samples in the group and was absent from all samples in other groups). Like Dufrêne and Legendre (1997) we arbitrarily chose a threshold level of 25% IV, and a Monte Carlo simulation (1000 runs) was used to determine significance ($P \leq 0.01$) of species IVs.

The dataset used for CA, DCA and ISA was simplified by excluding species which were only found once or twice during the study. Furthermore, all samples with only one or two records were removed from the dataset. Also after preliminary ordinations, two samples (201LS1, 217LS1) were removed from the dataset because of their aberrant behaviour. The first sample was a collection with a single dominant species, and the second had abnormal high species richness with multiple rare species. Such outliers mask potential information in ordination by compressing the other ordination output (e.g. Manly, 1994). The dataset matrix used for CA, DCA and ISA consisted of 103 samples and 53 taxa.

To study the relationship between the DCA-axes and a temporal factor we evaluated their correlation with the sample date by Kendall rank correlation (Legendre and Legendre, 1998). Differences in species composition found in the four microhabitats (subcommunities) were tested for significance using the multi-response permutation procedure (MRPP) a nonparametric method testing the hypothesis that there is no difference between the groups identified by a clustering method (Biondini et al., 1988). Euclidian distance and weighting of groups by $n.[sum(n)]^{-1}$ setup was used. Because we made multiple comparisons, *P*-values were adjusted using a Bonferroni correction (Rice, 1990). CA, MRPP, DCA and ISA were performed with PC-ORD version 4.26 (McCune and Mefford, 1999). For statistical tests we used the statistical package of S-plus (version 6.1 for Windows, professional edition, Insightful Corp. 2002).

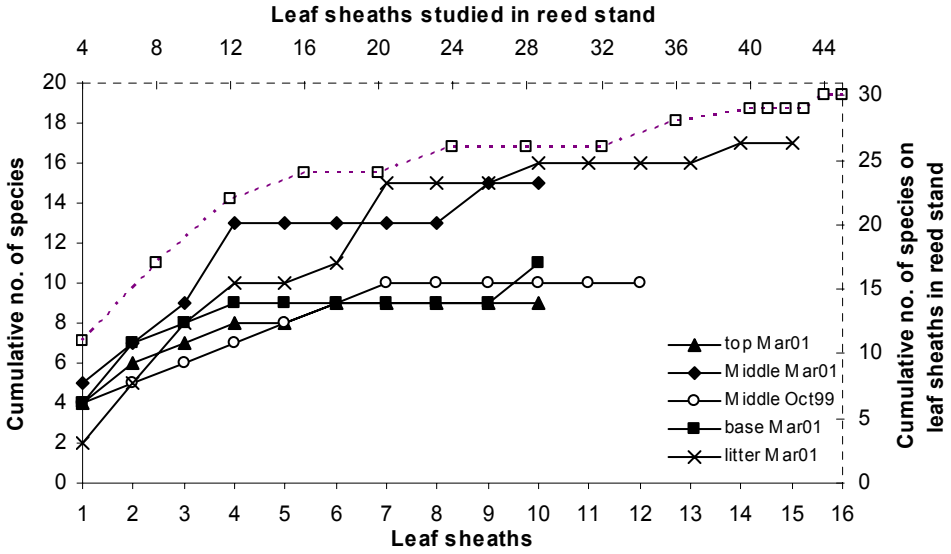


Figure 1. Cumulative number of species forming sporulation structures on leaf sheaths. Primary axes, with data in full line, show results from the October 1999 sampling — used to set the number of replicate samples at ten for standing reeds — compared with March 2001 samples with the highest species richness on leaf sheaths found during the study. Secondary axes, with data in dashed line (□), show the cumulative species number in the reed stand on leaf sheaths if one collection is considered to consist of leaf sheaths collected at three heights in the canopy and one in the litter layer.

Results

Sample size

Fig. 1 shows the species area curves on leaf sheaths in different microhabitats and in different successional stages. Preliminary screening of October 1999 leaf sheath collections, from the middle part of standing moribund culms, was used to set the sample size at ten for standing culms. This level of replication is sufficient to control for variability caused by seasonality, succession and unknown differences between microhabitats. Because all curves reach a plateau before ten replicate sheaths were screened (Fig. 1). Repeating the procedure for the other plant parts, a similar or lower sample size is suggested.

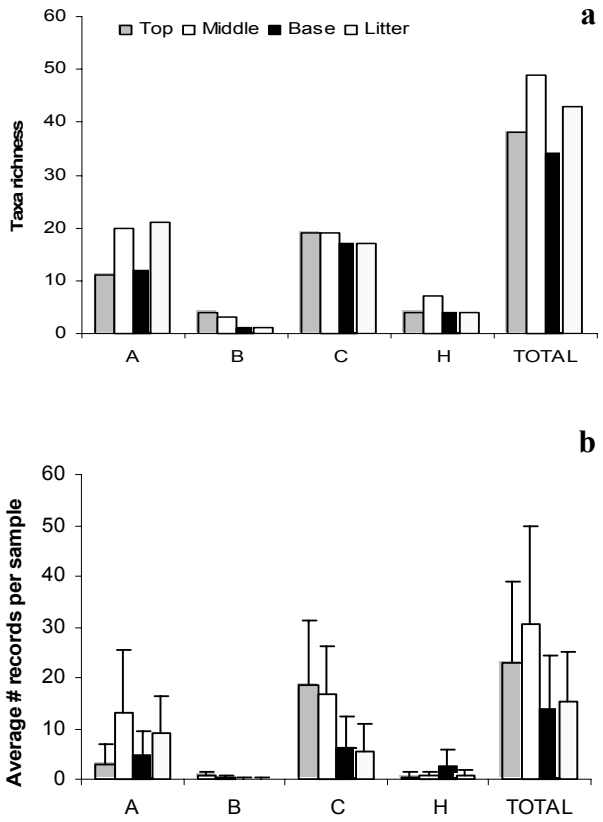


Figure 2a. Taxa richness and distribution of the major (pseudo)systematic groups on leaf sheaths of *Phragmites australis* in the different microhabitats investigated. **b.** Average number of records per sample occasion in the different microhabitats investigated. The same column coding for both panels.

In the litter layer the number of required sample replicates was determined by screening culms of varied age and time of decay (October 1999 (not shown)). The results of the mixed litter layer sample showed a curve that levelled off at twelve leaf sheaths. Because of the high level of uncertainty of age distribution in the October 1999 sample and the unknown seasonal and successional variability, the experimental sample size was set at fifteen litter layer sample units. This sample size proved to be large enough if plotted for leaf sheaths sampled in the litter layer at the time of highest species richness during decomposition (Fig. 1).

In total 122 leaf sheath samples were screened for fungal presence (see Table 3), rescaled to a total of 1220 individual leaf sheath sections screened (each month and in each subcommunity ten leaf sheaths) to calculate fungal occurrence.

For analysis, the two data sets were pooled, this seemed justified because of the high similarity between the two plots based on species composition (Jaccard similarity: 69%) and comparing species records between the two plots (Bray-Curtis similarity: 79%). Both plots had the same distribution as tested with a Mann-Whitney U test (P = 0.2064). Furthermore, the

results of the cluster analysis showed that 81% of all leaf sheath samples compared at one year intervals, clustered in the same group identified (see Fig. 3).

Fungal diversity

During growth and decomposition of common reed 2516 records were made belonging to 77 fungal taxa. In general many taxa were rare, ie. encountered only once or twice (Table 1). The screened leaf sheaths comprised 33 ascomycetes (43%), 31 coelomycetes (40%), nine hyphomycetes (12%) and only four basidiomycetes (5%). Coelomycetes and ascomycetes accounted for 93% of all records demonstrating the low presence of hyphomycetes and basidiomycetes (Figs 2a,b). Three genera account for 56% of all fungal records on *P. australis* leaf sheaths. These include *Stagonospora* s.l. (11 taxa), *Phoma* (4 taxa) and *Phaeosphaeria* (6 taxa). *Stagonospora* s.l. was divided into several smaller genera [*Stagonospora* s.s. (5 taxa) (*sensu* Sutton, 1980), *Septoriella* spp. (2 taxa) (*sensu* Sutton, 1980; Nag Raj, 1993) and *Hendersonia* (4 taxa) (invalid name, see Wakefield 1939, but for practical cause used to indicate *Stagonospora* spp. s.l. with brown conidia without obvious slime appendage and solitary conidiomata].

Of the four microhabitats (subcommunities) the middle canopy leaf sheath showed the highest taxa richness (49 taxa), while the lower and upper canopy leaf sheaths had 34 and 38 taxa respectively. The number of taxa found on the decomposing leaf sheaths in the litter layer was 43. A comparison of the distinct fungal groups within different microhabitats (Fig. 2b) revealed a significant difference in ascomycete occurrence between middle and top shoot sections (Wilcoxon rank-sum test $P = 0.0063$; with α set at 0.0083 when Bonferroni corrected), and between middle and basal leaf sheaths (Wilcoxon rank-sum test $P = 0.022$, but not significant if Bonferroni corrected). Relative occurrence of coelomycetes, as measured by sporulation frequency, appeared to be substantially higher (although not significant) in the middle and upper canopy compared to the basal canopy and litter layer leaf sheaths (Fig. 2b).

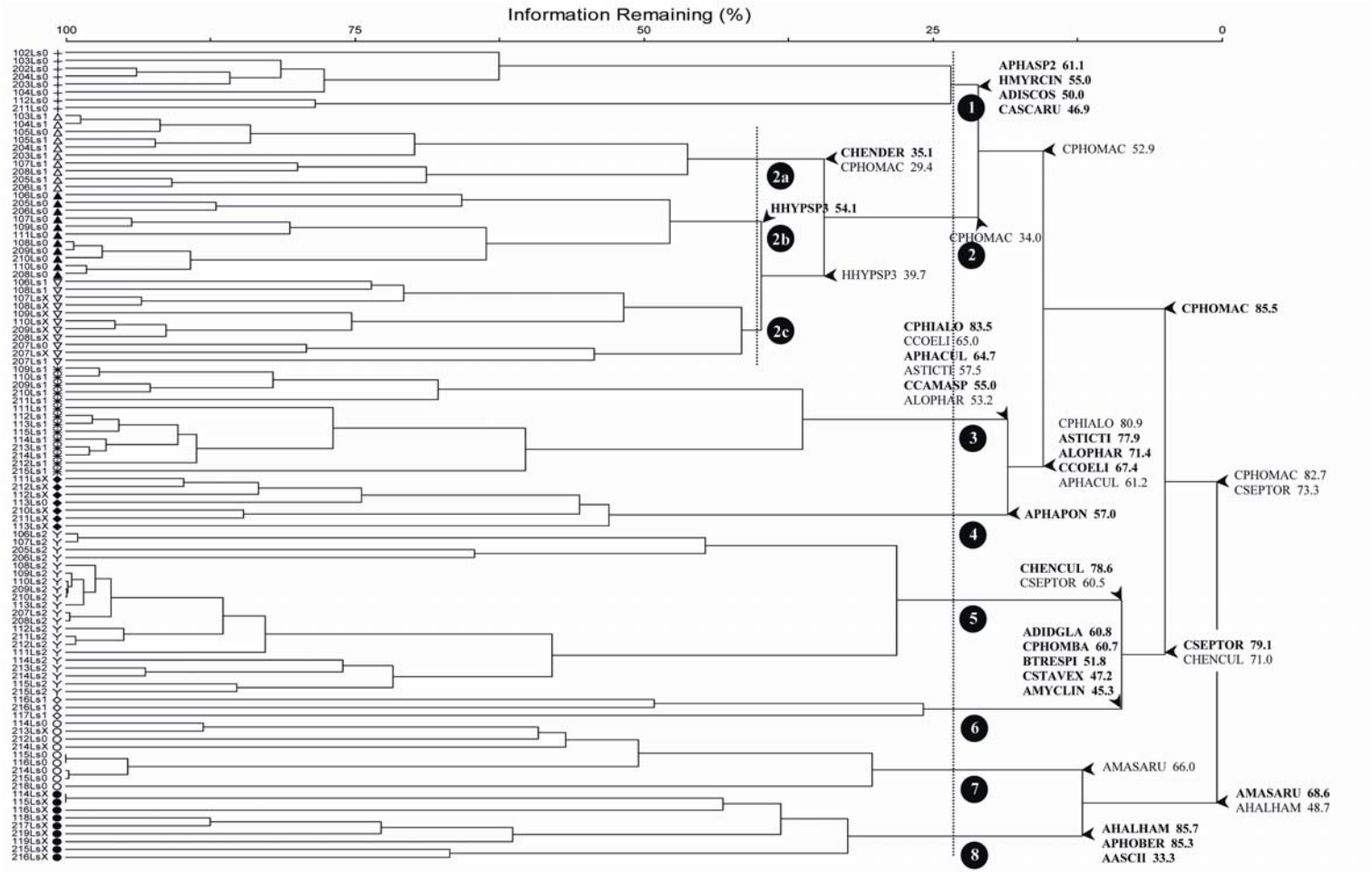


Figure 3. Dendrogram from cluster analysis of the pooled data from plot 1 and 2 with 53 taxa and 103 samples included. Sample code exists of four character states. The first figure indicates the plot number being 1 or 2; the second number stands for month of collection (between 1-19), see Table 3; the letter code 'ls' stands for leaf sheath sample and the last figure indicates the microhabitat (0 = base, 1 = middle, 2 = top and X = litter layer) the samples were taken. Indicator species with indicator values are shown for the groups generated by cluster analysis. All species with indicator value > 25 and Monte-Carlo *P*-values < 0.01 are included. The maximum indicator values for a species are printed in bold. Acronyms abbreviations in Table 1. Dendrogram is scaled with the percentage information remaining in the branches.

Table 1. Taxa list and frequency distribution of all taxa found on leaf sheaths of *Phragmites australis* during growth and decomposition. Data for the two successive series pooled. % abundance (% ab.)[1] is the proportion of records of a taxon on the total number of records of all taxa. Taxa acronyms code for taxa names in Figures 3 and 7. The first letter of the acronym codes for the pseudo-systematic position of a taxon: A = ascomycetes; B = basidiomycetes; C = coelomycetes; H = hyphomycetes.

Taxa	Acronyms	# rec.	% ab.[1]	Taxa	Acronyms	# rec.
Phoma sp. III	CPHOMAC	381	15.1	*Phaeoseptoria* sp.	CPHAEOS	8
Septoriella sp(p).	CSEPTOR	281	11.2	*Didymella* sp.	ADIDYME	7
Hendersonia culmiseda Sacc.	CHENCUL	169	6.7	*Hendersonia* aff. *culmiseda* Sacc.	CHENCFC	7
Stagonospora vexata Sacc. sensu Diedicke	CSTAVEX	163	6.5	*Microsphaeropsis* sp. I	CMICSP1	7
Massarina arundinacea (Sowerby: Fr.) Leuchtm.	AMASARU	118	4.7	*Septoriella phragmitis* Oudem.	CSEPPHR	5
Stictis sp.	ASTICTI	115	4.6	Hendersonia sp. II	CHENDII	4
Phaeosphaeria sp. II	APHASP2	101	4.0	*Mollisia* cf. *palustris* (Roberge ex Desm.) P. Karst.	AMOLPAL	4
Phialophorophoma sp.	CPHIALO	100	4.0	*Stagonospora* sp. II	CSTAINB	4
Coelomycete sp. I	CCOELI	81	3.2	*Ascochyta* cf. *leptospora* (Trail) Hara	CASCLEP	4
Phaeosphaeria culmorum (Auersw.) Leuchtm.	APHACUL	71	2.8	*Ascochyta* sp. I	CASCOCI	3
Mycosphaerella lineolata (Roberge ex Desm.) J. Schröt.	AMYCLIN	69	2.7	*Cytoplacosphaeria rimosa* (Oudem.) Petrak s.l.	CCYTRIM	3
Didymella glacialis Rehm	ADIDGLA	69	2.7	*Periconia minutissima* Corda	HPERMIN	3
Hyphomycete sp. III	HHYPSP3	62	2.5	*Phoma* sp. IV	CPHOMAD	3
Phaeosphaeria pontiformis (Fuckel) Leuchtm.	APHAPON	56	2.2	*Sporobolomyces* sp.	BSPOROB	3
Phaeosphaeria sp. III	APHASP3	56	2.2	*Arthrinium phaeospermum* (Corda) M.B. Ellis		2
Phomatospora berkeleyi Sacc.	APHOBER	55	2.2	Asco sp. Dothideales incertae sedis I		2
Myrothecium cinctum (Corda) Sacc.	HMYRCIN	52	2.1	*Gibberella zeae* (Schwein.) Petch		2
Lophodermium arundinaceum (Schrad.) Chevall.	ALOPHAR	48	1.9	*Nectria graminicola* Berk. & Broome		2
Phaeosphaeria eustoma (Fuckel) L. Holm s.l.	APHAEUS	38	1.5	*Paraphaeosphaeria michotii* (Westend.) O.E. Erikss.		2
Halosphaeria hamata (Höhnk) Kohlm.	AHALHAM	37	1.5	*Phaeosphaeria luctuosa* (Niessl) Otani & Mikawa		2
Ascochyta cf. *arundinariae* Tassi	CASCARU	30	1.2	*Puccinia phragmitis* (Schumach.) Körn.		2
Camarosporium sp.	CCAMASP	30	1.2	*Stagonospora* incertae sedis III		2
Neottiosporina australiensis B. Sutton & Alcorn	CNETAUS	23	< 1	*Alternaria alternata* (Fr.) Keissl.		1
Phoma sp. IIa	CPHOMBA	22		*Apiospora montagnei* Sacc.		1
Phoma sp. II	CPHOMAB	21		*Aposphaeria* sp.		1
Hendersonia sp. I	CHENDER	17		*Botryosphaeria festucae* (Lib.) Arx & E. Müll.		1
Discostroma sp.	ADISCOS	15		*Camarosporium feurichii* Henn.		1
Asco sp. Dothideales incertae sedis II	AASCII	13		*Fusarium* sp. III		1
Coelomycete sp. III	CCOELIII	13		*Lewia infectoria* (Fuckel) M.E. Barr & E.G. Simmons		1
Hyphomycete sp. VI	HHYPSP6	13		*Massarina fluviatilis* Aptroot & Van Ryck.		1
Pleospora abscondita Sacc. & Roum.	APLEABS	13		*Massarina* sp. III		1
Puccinia magnusiana Körn.	URPUCMA	13		*Mollisia hydrophila* (P. Karst.) Sacc.		1
Pseudoseptoria donacis (Pass.) B. Sutton	CPSEDON	12		*Mollisia retincola* (Rabenh.) P. Karst.		1
Stagonospora elegans (Berk.) Sacc. & Traverso	CSTAELE	12		*Periconia digitata* (Cooke) Sacc.		1
Coelomycete sp. II	CCOELII	11		*Phomatospora* sp. IV		1
Cladosporium sp(p).	HCLADOS	10		*Schizothecium hispidulum* (Speg.) Lundq.		1
Pleospora vagans Niessl	APLEVAG	10		*Septoria* sp.		1
Tremella spicifera Van Ryck., Van de Put & P. Roberts	BTRESPI	10		*Stagonospora* incertae sedis I		1
Morenoina phragmitidis J.P. Ellis	AMORPHR	9				

[1] another index, the % occurrence of a taxon is not presented. This measure, which indicates the proportion of all leaf sheaths colonized by a specific taxon, was about double of the presented percentages for abundance.

Spatial and temporal characterization of the subcommunities

Cluster analysis (Fig. 3) of the samples results in seven interpretable groups and one small rest group. The groups are mainly separated by spatial and temporal characteristics and correspond well to the natural subcommunity units sampled. Group 1 – a cluster of mainly basal leaf sheath samples with an initial fungal colonization on living sheaths. Group 2 – a large cluster of 3 smaller subcommunities comprising all initial species assemblages except for the samples from the top canopy. Group 2a are samples from the middle canopy showing initial fungal colonization on living, moribund and newly dead leaf sheaths. Group 2a is significantly different from Group 2b (Bonferroni corrected, MRPP, $P < 0.001$). The latter clusters all samples from moribund or dead leaf sheaths from the basal canopy. Group 2c predominantly consists of samples that had recently fallen into the litter layer. Group 3 – clusters all samples from the middle canopy height in a later phase of fungal community development. Group 4 – is a small cluster of transitional samples between the early and the late successional samples in the litter layer (Group 8). Group 5 – is a tight cluster of top canopy leaf sheaths with subclusters showing a temporal gradient. Group 6 – is a rest group of middle leaf sheaths. Group (7+8) – is the outgroup of the cluster dendrogram and illustrates the distinct species assemblages on leaf sheaths influenced by tidal exchange water in a last phase of decay. Group 7 – clusters basal leaf sheaths in a final phase of decay together with some litter samples showing comparable species composition. Group 8 – clusters leaf sheath samples in the litter layer in a final phase of decay.

DCA (Fig. 4) resulted in two interpretable axes with both axes having a high eigenvalue (axis 1: 0.69; axis 2: 0.42) and thus a high explanatory power. Axis 3 with an eigenvalue of 0.22 explained little of the observed variation in our leaf sheath dataset. The high eigenvalues and a large length of gradient (axis 1: 5.962; axis 2: 3.615) indicate a distinct β-diversity along both axes. An after-the-fact evaluation of the variation explained by the axes by relative Euclidean correlation (McCune and Mefford, 1999) estimated that 38% of the variation was explained by axis 1 and 14% by axis 2. DCA sample clusters (Fig. 4) are concurrent with the groups delimited by cluster analysis. A strong spatial component in fungal community structuring is illustrated in Fig. 4 showing the delimitation of the different microhabitats sampled in a reed stand. Axis 1 represents the spatial component, separating top, middle and (basal + litter) samples from each other. The latter two microhabitats show more resemblance to each other than the aerial reed parts, however along the second axis these basal samples are largely separated from the litter samples with the exception of the basal leaf sheath samples in the final phase of decay.

Table 2. Sequence in fungal sporulation on leaf sheaths of *Phragmites australis* during growth and decomposition in four different microhabitats. Only taxa with more than 10 records (39 taxa) during the entire study are shown with their number of records, percentages abundance (calculation based on all taxa) and percentage recurrence for each of the different microhabitats. The percentages of frequency of occurrence of the taxa in each month sampled starting from May (see Table 3) and for each microhabitat sampled. Data from the two plots are pooled, 20 leaf sheaths screened per sampling time per microhabitat. Microhabitats without taxa records were omitted. 'Gr' codes for the pseudo-systematic position of a taxon: A = ascomycetes; B = basidiomycetes; C = coelomycetes; H = hyphomycetes.

Gr	SPECIES	Micro-habitat	∑ records	% ab.	% rec.	% occurrence (months)																	
						M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N
A	*Discostroma* sp.	base	15	3	100	10	15	45	5														
H	*Cladosporium* sp(p).	top	1	0	10					5													
		middle	7	1	70	10	5	10									5	5					
		base	2	0	20		10																
C	*Septoriella* sp(p).	top	173	29	62					30	65	75	80	95	100	100	85	90	70	75			
		middle	62	6	22	5	10	55	35	0	25	25	15	10	25	15	15	15	10	5	35	10	
		base	29	6	10		20	25	25	20	5	15	5	20	0	5	0	5					
		litter	17	4	6							15	20	5	20	15	10						
C	*Phoma* sp. II	middle	7	1	33	5																30	
		base	12	3	57							5	0	25	0	25	0	5					
		litter	2	1	10												5	0	0	5			
A	*Phaeosphaeria* sp. II	middle	32	3	32			25	40	35	5						2	3	3	3			
		base	64	14	64		90	80	60	35	10	5				15	15	5	5				
		litter	4	1	4											15	0	5					
C	*Phoma* sp. III	top	15	3	4						10	15	15	10	5	5	10	5					
		middle	176	17	46			60	90	100	75	80	70	75	85	85	70	45	15	25	5		
		base	132	27	35		40	55	50	65	80	60	75	70	65	55	20	20	5				
		litter	58	15	15							60	60	65	45	40	10	10					
H	*Myrothecium cinctum*	base	45	10	87		10	45	30	15	15					30	50	30					
		litter	7	2	13										5	10	20						
C	*Ascochyta* cf. *arundinariae*	top	2	0	7														10				
		middle	1	0	3																5		
		base	25	5	87		5	45	45	10	15			5	0	5							
		litter	1	0	3													5					
A	*Phaeosphaeria* sp. III	top	5	1	9						5					20							
		middle	41	4	73			45	45	25	5			5		5	15	25	20	5	5	5	
		base	5	1	9			5	10	5	0	5											
		litter	5	1	9								5				10	10					
C	*Hendersonia* sp. I	top	8	1	47					5	15									20			
		middle	8	1	47			10	5	20	5												
		base	1	0	6					5													

Table 2 continued.

Gr	SPECIES	Micro-habitat	Σ records	% ab.	% rec.	% occurrence (months)																	
						M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N
C	*Neottiosporina australiensis*	top	13	2	57								10	5	5	0	15	10	10	10				
		middle	8	1	35						5	0	5	10	15	5								
		base	1	0	4			5																
		litter	1	0	4									5										
C	Coelomycete sp. III	top	7	1	54					5	5	10	5	10										
		middle	6	1	46				10	15	5													
A	*Phaeosphaeria eustoma*	top	19	3	50											15	5	45	25	5				
		middle	16	2	42	5						10					10	10	30	10	5			
		base	3	1	8			15																
A	*Didymella glacialis*	top	7	1	10						5					5	5	0	5	15				
		middle	53	5	78					25	20					10	5	50	40	50	50	15		
		base	1	0	1											5								
		litter	7	2	10											5	10	10	5	0	5			
C	*Hendersonia culmiseda*	top	148	25	88							25	80	85	95	85	100	100	95	75				
		middle	14	1	8					15	5	15	20	10	5									
		litter	7	2	4								10	10	10	0	5							
C	*Pseudoseptoria donacis*	top	12	2	100					5	5							30	20					
A	*Phaeaosphaeria pontiformis*	middle	12	1	21					5	0	5				5	0	10	10	10	15			
		base	4	1	7													10	10					
		litter	40	10	71							5	10	45	30	35	50	15	0	10				
A	*Halosphaeria hamata*	base	6	1	17						20	5	5											
		litter	30	8	83												5	5	20	10	50	10	10	40
A	*Phaeosphaeria culmorum*	top	15	3	21						10	0	5	5	5	0	10	15	20	5				
		middle	47	4	66						5	10	5	25	15	40	30	45	45	15				
		litter	9	2	13									15	5	10	15							
A	*Massarina arundinacea*	top	4	1	3													20						
		middle	5	0	4							5				5			10		5			
		base	53	11	46						10				5	20	25	35	50	70	40	0	10	
		litter	53	14	46									10	20	50	55	45	35	40	5	5		
H	Hyphomycete sp. III	top	1	0	2													5						
		middle	3	0	5								5				5	5						
		base	49	11	79						5	30	40	50	45	60	15							
		litter	9	2	15								15	10	5	10	5							
B	*Puccinia magnusiana*	top	10	2	77						5	10	15	0	5	10	5							
		middle	3	0	23								10			5								

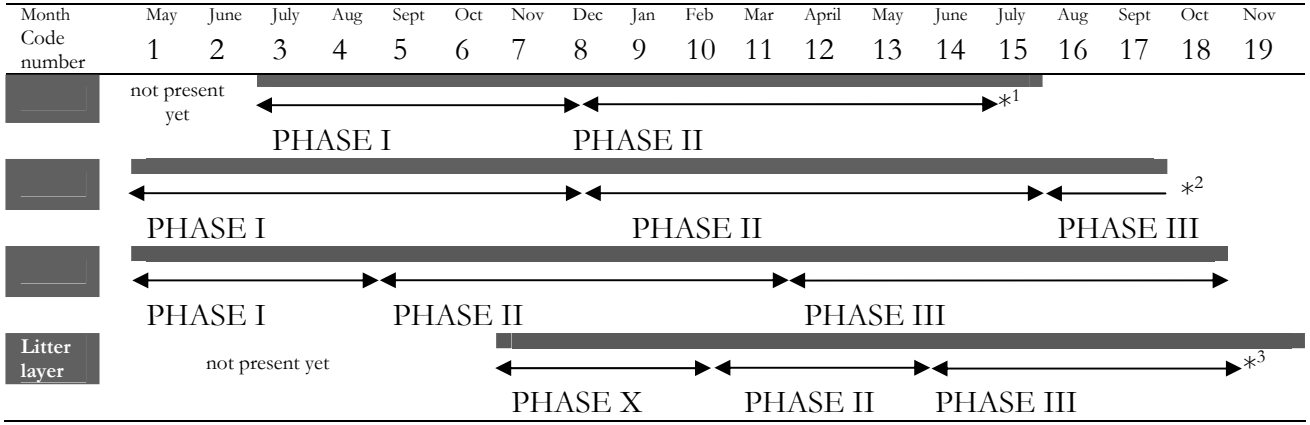
Table 2 continued.

Gr	SPECIES	Micro-habitat	∑ records	% ab.	% rec.	% occurrence (months)																	
						M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N
A	*Phomatospora berkeleyi*	middle	9	1	18						5	5	15									20		
		base	1	0	2							5												
		litter	41	11	80							5	5	5	0	5	10	30	15	45	35	25	10	15
A	*Stictis* sp.	middle	93	9	81						5	5	5	10	5	50	100	100	100	80	5			
		base	6	1	5											15	0	15						
		litter	16	4	14									5	0	25	40	10						
A	*Pleospora vagans*	top	1	0	10														5					
		middle	9	1	90						5	5			10	10			15					
C	*Phoma* sp. IIa	top	1	0	5								5											
		middle	12	1	55													5			30	25		
		base	4	1	18						5			5	10									
		litter	5	1	23							5	0	5	10	5								
C	*Stagonospora vexata*	top	61	10	37									10	0	45	60	45	75	70				
		middle	77	7	47							5	20	50	30	45	30	55	40	20	70	20		
		base	1	0	1													5						
		litter	24	6	15							5	10	25	15	30	15	10	5	5				
C	Coelomycete sp. I	top	14	2	18											15	5	15	35					
		middle	58	6	73							5	10	55	55	40	35	50	30	10				
		litter	8	2	10								5	5	5	15	5	5						
A	*Pleospora abscondita*	top	1	0	8															5				
		middle	12	1	92							5	5			10	15	10	10	5				
C	Coelomycete sp. II	top	8	1	73												10			30				
		middle	2	0	18														5	5				
		base	1	0	9							5												
C	*Camarosporium* sp.	top	2	0	7											5			5					
		middle	26	2	87								5	15	40	15	20	15	20					
		litter	2	1	7								5					5						
C	*Stagonospora elegans*	top	11	2	92								5			5	0	15	10	20				
		base	1	0	8									5										
C	*Phialophorophoma* sp.	top	4	1	4														10	10				
		middle	89	9	91									15	45	70	85	85	100	40	5			
		litter	5	1	5											10	10	5						
B	*Tremella spicifera*	top	2	0	20											5	5							
		middle	5	0	50									5	5						10	5		
		base	2	0	20											10								
		litter	1	0	10											5								

Table 2 continued.

Gr	SPECIES	Micro-habitat	∑ records	% ab.	% rec.	% occurrence (months)																	
						M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N
A	*Lophodermium arundinaceum*	middle	41	4	85										5	30	35	55	40	40				
		base	2	0	4													10						
		litter	5	1	10											10	15							
H	Hyphomycete sp. VI	top	9	2	69											15			30					
		middle	4	0	31											15			5					
A	*Mycosphaerella lineolata*	top	17	3	25														35	50				
		middle	51	5	74												5	50	80	40	80			
		litter	1	0	1													5						
A	Asco sp. Dothideales incertae sedis II	litter	13	3	100															20	30			15

Table 3. Sampling design and the different stages in fungal succession on leaf sheaths of *P. australis* recognized in our study. The complete dataset exists of two successional series with one year interval, with series 1 started in May 2000 and series 2 in May 2001.



*1 There are indications that phase III of fungal succession starts in month 15.
*2 Lose observations on leaf sheath remains showed an ongoing – decreasing – sporulation till January the next year when the leaf sheaths were almost completely decomposed.
*3 Probably a fourth phase of fungal succession, characterized by common soil fungi, is established.

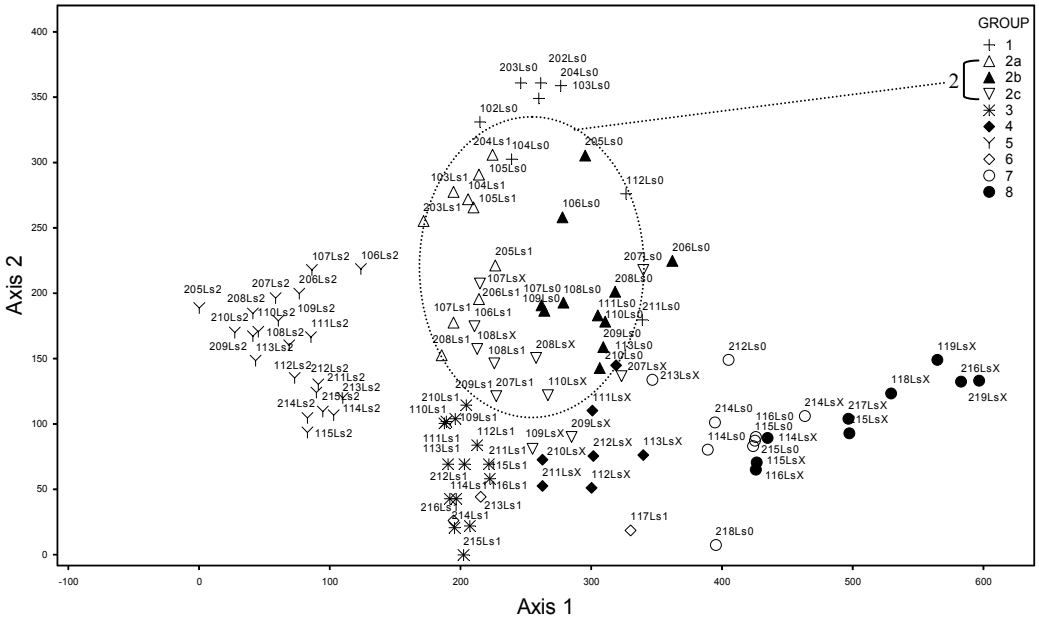


Figure 4. First two ordination axes of a DCA based on species occurrences on leaf sheaths of *Phragmites australis* with 53 taxa (raw data) and 103 samples included. Sample grouping symbols and acronyms are the same as in the cluster analysis (Fig. 3). Axes are scaled in SD units (x 100).

Those leaf sheaths have almost identical fungal assemblages to those in the litter layer. Axis 2 shows a strong negative correlation with the sampling date (Kendall rank), representing a clear successional gradient in the fungal community development in the canopy (Fig. 6). Although axis 1 shows a weaker successional trend, as indicated by the lower correlation results, it appears to be associated with a temporal aspect for the litter layer samples (Fig. 6). In Fig. 7 a biplot of samples and taxa shows the taxa scores along axis 1 and 2. All of the sample scores represent a reciprocal calculation with species scores and are plotted as the centroid of the species present in the samples. This means that species plotted near samples show affinity to these samples. Species plotted at the margins of the ordination are generally rare species (McCune and Grace, 2002).

Indicator species analysis (ISA) reveals contrasting performance of several species in sample groups generated by CA. Those indicator taxa are plotted on the CA-diagram (Fig. 3). ISA can generate indicators at several levels of the community hierarchy. At the highest level (ie. all samples) of the cluster dendrogram, group (7+8) is the first to be separated from the rest of the leaf sheath samples. Indicator species for this group are *Massarina arundinacea* and *Halosphaeria hamata*. Within group (7+8) *M. arundinacea* is more a generalist compared to *H. hamata* as the former reaches the highest IV for the whole group; while *H. hamata* is more a specialist as it reaches highest IV in the litter layer (group 8).

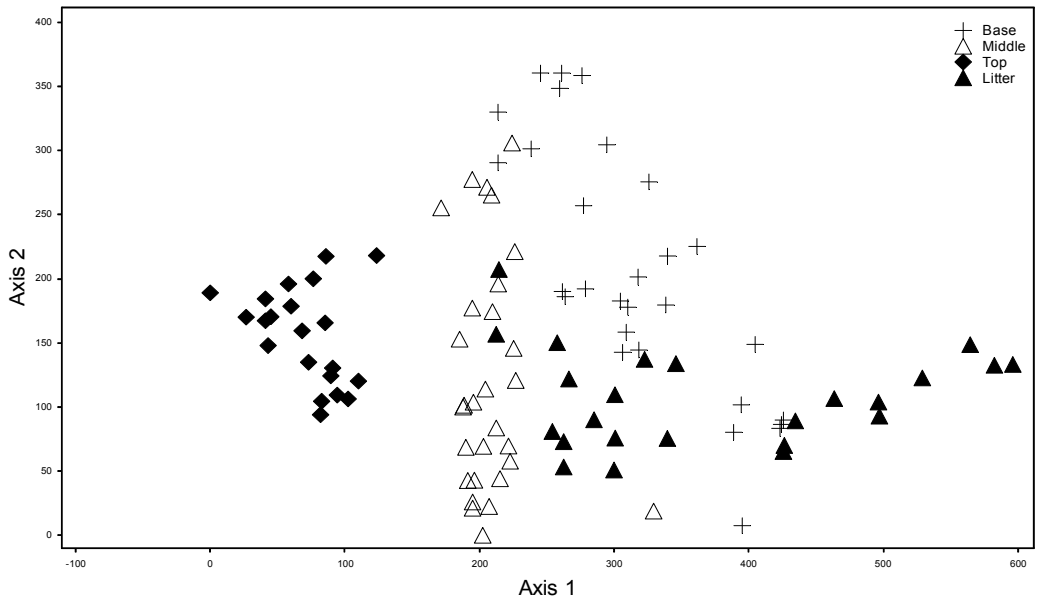


Figure 5. DCA plot identical to fig. 4 with the leaf sheath samples from *Phragmites australis* coded by the subcommunity they originated from. Axes are scaled in SD units (x 100).

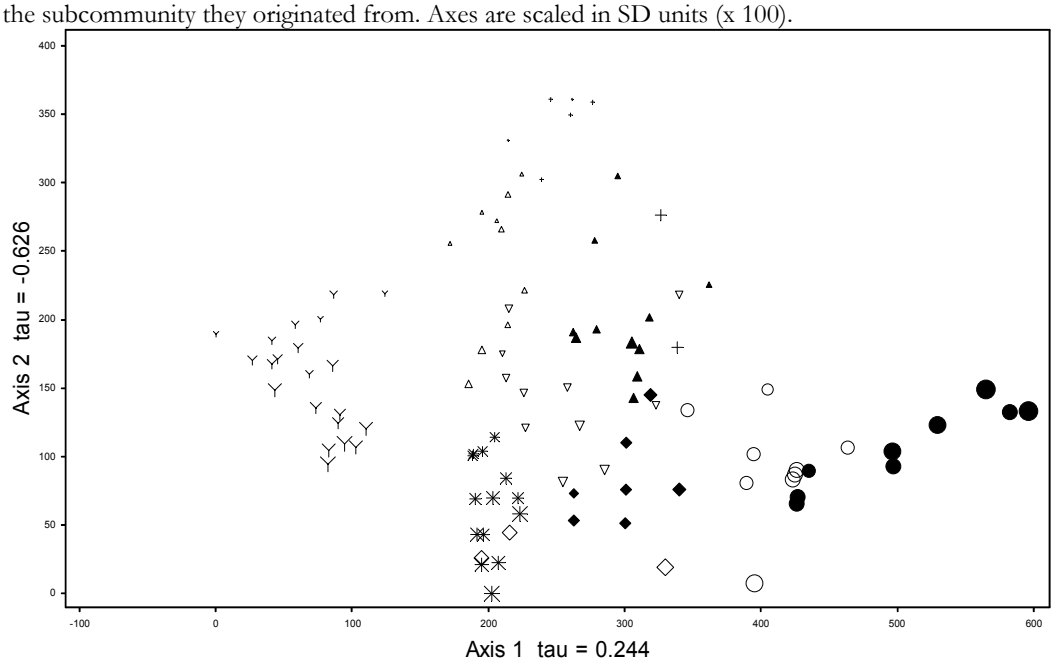


Figure 6. DCA plot identical to fig. 4 of leaf sheath samples from *Phragmites australis* showing the temporal correlation of the first two ordination axes with Kendall rank correlation (tau) indicated. Smallest symbols indicate earliest samples in the successional community development. Axes are scaled in SD units (x 100). Symbols are coded similar to fig. 4.

These litter samples in a final phase of decay are further characterized by the presence of *Phomatospora berkeleyi* and Asco sp. Dothideales incertae sedis II. The best indicator for group 7, basal leaf sheaths in a final phase of decay, was *M. arundinacea*. Although *P. berkeleyi* is a good indicator for leaf sheaths (and leaf blades, unpublished data) in late stage of decay in the litter layer, it was also observed higher up in the canopy on recently senescent sheaths (Table 2; unpublished data). General, eurytopic indicators for all canopy samples and litter layer leaf sheaths recently fallen on the sediment are *Phoma* sp. III and *Septoriella* sp(p). *Phoma* sp. III is mainly indicative for the basal and middle canopy leaf sheaths during the early colonization, while *Septoriella* sp(p). is a typical colonizer of the upper moribund and dead leaf sheaths of common reed. The latter was however, occasionally found in the middle canopy (group 2a and 6), and in particular it had a high % occurrence on green middle canopy leaf sheaths (Table 2). The third level in cluster hierarchy showed highest IV for *Didymella glacialis*, *Phoma* sp. IIa, *Tremella spicifera*, *Stagonospora vexata* and *Mycosphaerella lineolata* (group 6). *Hendersonia culmiseda* is a specific indicator for the entire subcommunity of top canopy leaf sheath (group 5) (Table 4). Group (3+4) clustering the middle leaf sheaths in a second phase of fungal colonization and some leaf sheath litter layer samples are characterized by *Stictis* sp., *Lophodermium arundinaceum* and Coelomycete sp. I. On a lower level group 3, clustering solely the middle leaf sheaths, is characterized more specifically by *Phialophorophoma* sp., *Phaeosphaeria culmorum* and *Camarosporium* sp. Group 4 is specifically characterized by *Phaeosphaeria pontiformis* although it has a number of species in common with group 3 but in a lower frequency. Group 1, containing living green and moribund leaf sheath at the basal culm part, is characterized by *Phaeosphaeria* sp. II, *Myrothecium cinctum, Discostroma* sp. and *Ascochyta* cf. *arundinariae*. Group 2 and its subgroups show few good indicator taxa. In general group 2 and 2a are well characterized by *Phoma* sp. III with group 2a further typified by *Hendersonia* sp. Within group (2b+2c) Hyphomycete sp. III is the most typical taxon, with highest indicative power for group 2b. Initial litter layer samples (group 2c) showed no good indicator taxa.

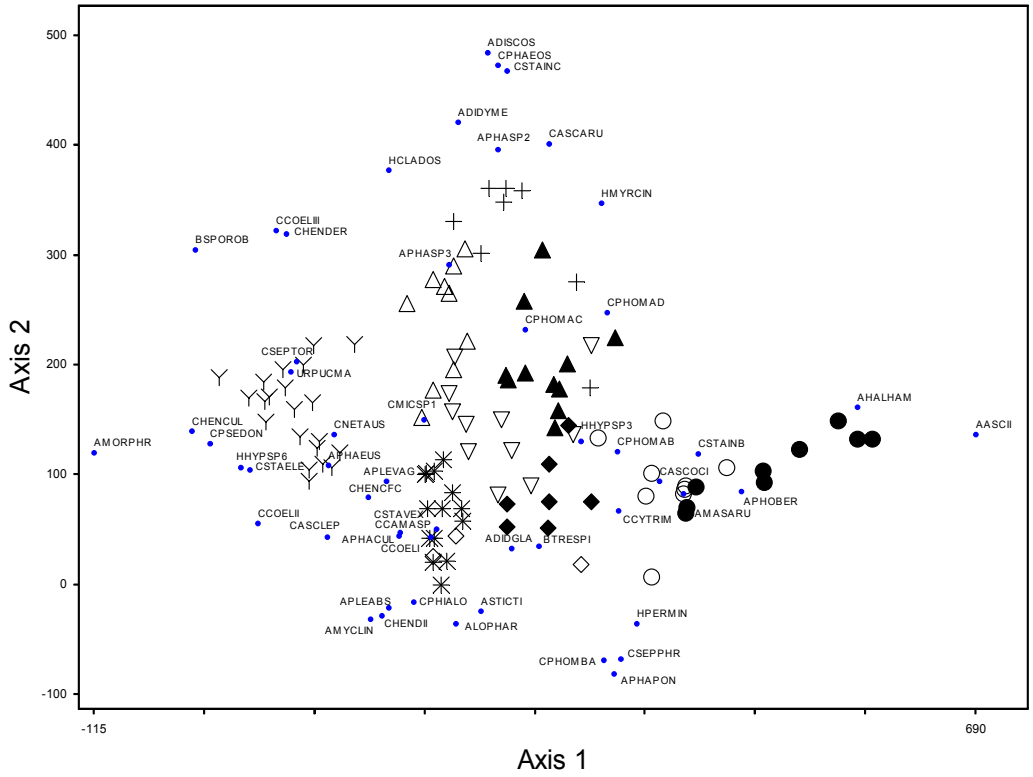


Figure 7. First two ordination axes of a DCA biplot showing *Phragmites australis* leaf sheath samples (103) (Figs 3, 4) and the species distribution (53 taxa). Acronyms as in Table 1. Symbols are coded similar to fig. 4.

Table 4. Indicator taxa with indicator value (IV) on *Phragmites australis* leaf sheaths identified for the four different subcommunities sampled: top, middle and basal height leaf sheaths along the vertical axis of standing shoots and litter layer leaf sheath

Top		**Middle**		**Base**		**Litter**	
Taxa	IV	Taxa	IV	Taxa	IV	Taxa	IV
Hendersonia culmiseda	77.2	*Phialophorophoma* sp.	45.8	Hyphomycete sp. III	28.9	*Phomatospora berkeleyi*	58.9
Septoriella sp(p).	69.0	*Stictis* sp.	43.8	*Ascochyta* cf. *arundinariae*	28.9	*Phaeosphaeria pontiformis*	43.8
		Didymella glacialis	40.3	*Phaeosphaeria* sp. II	28.5	*Halosphaeria hamata*	42.9
		Phoma sp. III	39.4			*Massarina arundinacea*	31.4
		Phaeosphaeria culmorum	37.1				
		Phaeosphaeria sp. III	35.7				
		Coelomycete sp. I	34.5				
		Camarosporium sp.	28.8				
		Lophodermium arundinaceum	28.8				

Table 5. Jaccard similarity between different microhabitats screened for fungal sporulation on leaf sheaths of *Phragmites australis*.

	Litter	Base	Middle	Top
Litter	100			
Base	46	100		
Middle	48	40	100	
Top	35	30	62	100

Discussion

The chosen number of replicates of the sample unit was evaluated by plotting the species area curves for the month with highest species richness during the entire study (Fig. 1). In each of the investigated microhabitats those curves reached a plateau before the chosen replicate size was reached. If we consider a pooled species area curve including all sample units collected in one month, we could argue that we over-sampled to have a general idea of the fungal community. This comment is however only valid where all microhabitats in a reed stand are included during sampling. This is illustrated by the dotted line in Fig. 1. This species area curve was compiled by recognizing a sample as consisting out of a litter layer sheath and 1 sheath from each of three heights monitored in the canopy. Such a collection is a representative sample of all different types of leaf sheaths available in a reed stand in March. No fresh shoots have emerged and leaf sheaths from the previous growing season have (almost) completely decomposed (pers. obs.). The 45 leaf sheaths screened in March are considered to give a representative picture of fungal species richness. The curve shows that after screening 16 leaf sheaths, four from each microhabitat, we had already found 80% of all fungal species sporulating on leaf sheaths in March. A replicate size of four is considered as a minimum for describing fungal community development in a specific microhabitat. Thus, to have a fairly complete view on fungal richness and composition on a single host, it seems important to invest more effort in screening a high number of potential microhabitats and less on the number of sample units. In investigating fungal diversity on submerged wood samples it was found that the asymptote was reached after examination of *ca.* 100 samples of bamboo and 70 wood sample, with 30 samples considered optimal for sampling the most common species (Cai et al., 2003).

The natural decay pattern of *P. australis* consists of an initial standing decomposition phase and a secondary phase of breakdown in the litter layer. The importance of studying plant decay in this natural sequence has been stressed by Newell (1993) and others (e.g. Gessner, 2000). The criticism by the above authors mainly originated from the practice of unnaturally clipping of plant parts and incubating them (often in litterbags) on the soil surface or hanging them in an aerial position. However, the natural time in standing position and the physical contact with the whole plant are important for plant resorption processes (Granéli, 1990; Gessner, 2001). Therefore, decomposition studies manipulating reeds in an unnatural way

describe breakdown rates and nutrient patterns probably not existing in field conditions. Furthermore, this study demonstrates the importance of natural decay patterns with specific fungal microhabitats along the vertical axis of a reed shoot, an aspect noticed before (Apinis et al. 1972, 1975; Poon and Hyde, 1998b; Van Ryckegem and Verbeken, 2005). Each of the four studied microhabitats seemed to develop a unique fungal species composition (MRPP overall $P < 0.0001$, all six comparisons of microhabitats were highly significant $P < 0.0001$ even when Bonferroni corrected). This implies a natural vertical taxa distribution and a different taxa composition in the litter layer (Table 5). Such a vertical zonation of fungal taxa seems to be a feature in various reed wetlands and not restricted to tidal systems (e.g. Apinis et al. 1972; Poon & Hyde, 1998b; Van Ryckegem & Verbeken, 2005).

Species richness differed between the vertically recognized microhabitats. We observed lower species richness in the upper canopy. This corresponds to the results of Poon & Hyde (1998b). Although the latter authors did not state specifically the part of the plant screened (stem or leaf sheath), their low species richness in the upper canopy probably corresponds to the low number of taxa on upper leaf sheaths. This is deduced from the fact that leaf sheaths form the major fraction of plant material in the top canopy. However, the observed dominance of coelomycetes on top leaf sheaths in this study (Fig. 2b) was not recorded during the screening of tropical intertidal reeds (Poon and Hyde, 1998b). The observed highest species richness on the lower parts of standing culms by Poon & Hyde (1998b) must have been due to the higher species richness on basal stem parts compared to leaf sheaths.

Our research suggests that the species rich fungal colonizers have a considerable impact on standing leaf sheath decay, which is dependant on the duration of leaf sheaths in a standing position and on the enzymatic capabilities of the microbial community.

The standing phase is abruptly ended for some culms when they fall into the litter layer (e.g. modelled by Soetaert et al., 2004). This is influenced by extreme weather conditions such as storms or extreme winds that snap the culms. The shift from standing decay to litter layer results in an almost complete change in species composition. A set of characteristic taxa appears with both high % abundance and % recurrence in the litter layer: *Phaeosphaeria pontiformis*, *Halosphaeria hamata*, *Phomatospora berkeleyi*, *Massarina arundinacea* and *Phoma* sp. III (Table 2). The best indicators for the litter layer leaf sheath are late successional species (Table 4). Most of the above taxa are uncommon in the canopy with the exception of *Massarina arundinacea* and *Phoma* sp. III, which are also common in the basal canopy indicating the resemblance of basal leaf sheath mycota to the litter layer mycota. This is not surprising due to the proximity and similar environmental conditions in both these microhabitats characterized by periodical flooding by brackish water. Several species such as *Halosphaeria hamata* and *Massarina arundinacea* are well adapted to sporulate in these saline conditions. *Halosphaeria hamata* was found to be adapted to brackish conditions both in the field and in culture experiments (Van Ryckegem & Verbeken, 2005). *Massarina arundinacea* is a eurytopic species, sporulating vigorously on *P. australis* litter from fresh water till mesohaline conditions and a culture experiment revealed tolerance for salinity (Van Ryckegem & Verbeken, 2005). The

overall low frequency of fungi sporulating in the litter layer (Fig. 2b) is mainly due to the lower sporulation of coelomycetes compared to ascomycetes in the litter layer (Figs 2b, 8b). This aspect has also been noticed in other reed habitats (Van Ryckegem and Verbeken, 2005).

Successional sequence

The successional sequence of fungal sporulating structures at different stages of heterotrophic succession has been observed by several authors and summarized in Frankland (1998) and Hyde and Jones (2002 and references herein). Several successional stages can also be discerned in fungal colonization of *P. australis* leaf sheaths. Canopy subcommunities (basal, middle and top leaf sheaths) show their own unique pattern comprising three different stages in fungal succession (Figs 3, 5, 8a, b and summarized in Table 3). This three-step-succession of fungal community development is comparable to the observations by Apinis et al. (1972) and the general patterns described by Dix and Webster (1995) and Cooke and Rayner (1984). We recognize an initial pioneer community (Phase I), which is relatively open for fungal invasion and usually characterized by low species diversity, with few stress tolerant or ruderal species having a high abundance. The second stage (Phase II) is a more closed, mature community with high diversity of more combative species. The third phase (Phase III) is characterized as an impoverished community dominated by few species with low sporulation frequency typified as stress tolerant and/or highly combative taxa.

In particular, during Phase I, *P. australis* leaf sheaths are characterized by weak pathogens, biotrophic species and opportunistic saprotrophs found on living, moribund or initially dead leaf sheaths. Some taxa, such as *Sporobolomyces* sp. could be considered as phyllosphere fungi, but this group of fungi are not easily observed through direct observation on the leaf surface. More phyllosphere fungi would have been found if leaf sheaths were incubated in moist conditions or other techniques were used as presented in Apinis et al. (1972). Some other initial colonizers seemed to be confined to damaged or necrotic parts, or the thin margins of leaf sheaths where browning occurs quickly. These fungi are *Alternaria alternata* and *Cladosporium* sp(p). (also on leaf blades; unpubl. data). These species are regarded to be opportunistic, non host specific saprotrophes found on nearly all plant species (Hudson, 1968, 1971). Although *Alternaria* and *Cladosporium* have also been found to be endophytic genera on *P. australis* (Wirsel et al., 2001), and may be host specific endophytes growing biotrophically. Other species were able to form sporulatation structures on the green, living tissues e.g. *Phaeosphaeria* sp. II, *Septoriella* sp(p). and *Phoma* sp. III. *Phaeosphaeria* spp., were shown to be systemic endophytes of *P. australis* that are also dispersed by seed (Ernst et al., 2003) and characterized by *Stagonospora* like anamorphs (Leuchtmann, 1984). Our *Septoriella* sp(p). and *Hendersonia* spp. show morphological affinities to these anamorphs. *Septoriella* sp(p). showed a disjunct appearance in space and time and this raises the suspicion that several species are lumped in this taxon with highly variable conidia, in size and septation.

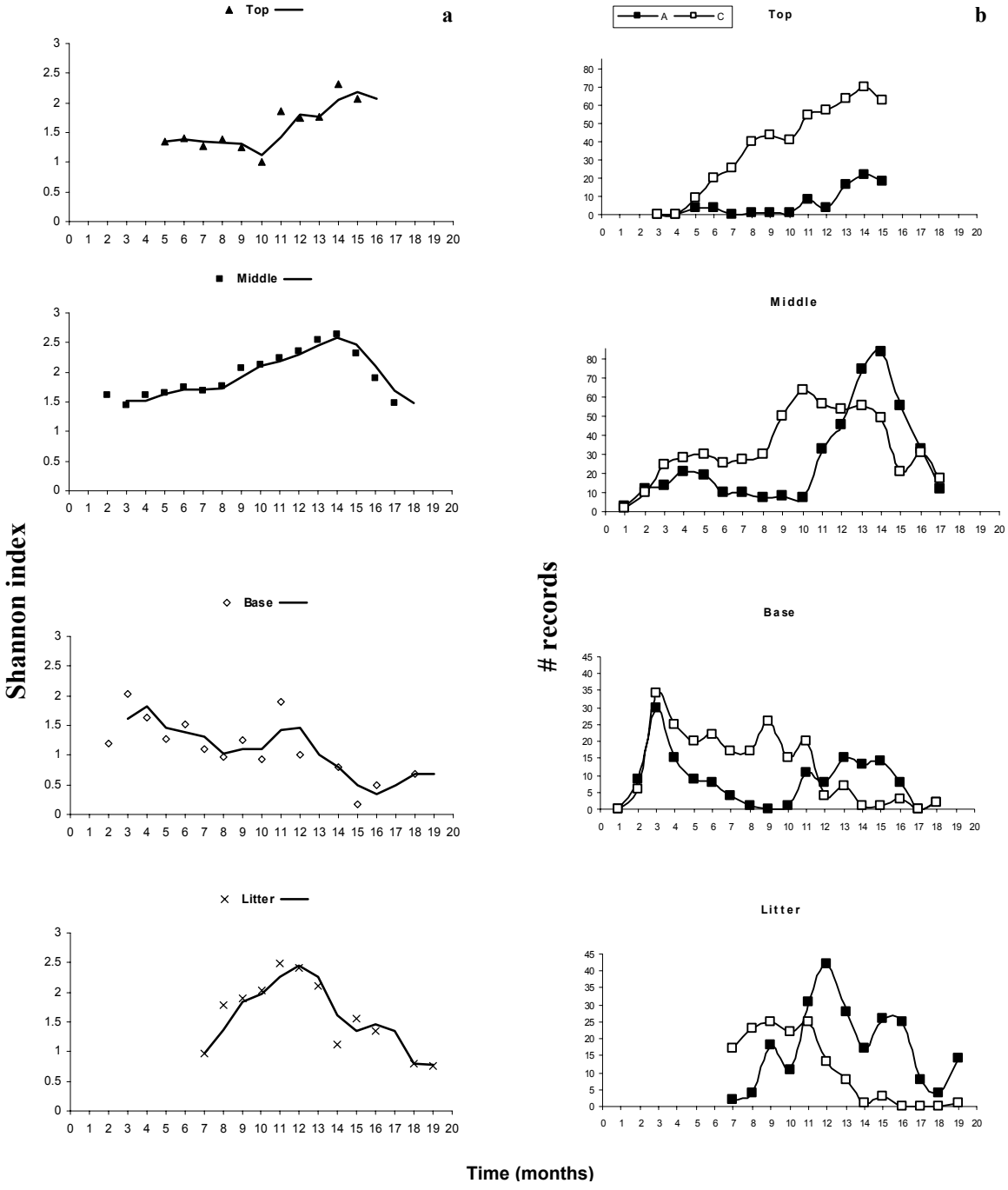


Figure 8a. Shannon diversity indices for the different microhabitats – subcommunities – sampled on leaf sheaths of *Phragmites australis* during the experimental period. The line is a moving average fit with a period of two. **b.** sporulation records in de different microhabitats on leaf sheaths of *Phragmites australis* for the two dominant pseudo-systematic groups: A = ascomycetes; C = coelomycetes.

The *Septoriella* sp(p). initially colonizing the basal and middle leaf sheaths is probably different from the taxon dominant on the dead upper leaf sheaths (Table 2).

Phase I is species poor for top and middle leaf sheaths but appears to be the most diverse stage for the basal leaf sheaths (Fig. 8a). The latter is probably due to the fact that most basal leaf sheaths soon lose their assimilation function and die. Furthermore, the tough bud scales surrounding the young emerging reed cones were screened in this study as if they were leaf sheaths. These bud scales are dead within the first month after shoot emergence and available for fungal decay. The peak sporulation (Fig. 8b) during the first months is mainly on these bud scales, which are characterized by some typical mycota (group 1, Figs 3, 4). Of those species, *Discostroma* sp. is most typical. The first phase of fungal community development in the litter layer (Phase X, Table 3) is not comparable with the initial pioneer community described above for standing shoots because it represents a transition phase of fungi. Species originating from various heights in the canopy, not adapted to the physicochemical stress present in the litter layer, are replaced by more typical estuarine fungi.

Phase II, the mature community, develops along the vertical axis of the shoots and is dependent on the moment of leaf sheaths senescence. The most basal leaf sheaths are already dead a few months before the middle and top leaf sheaths. The retarded senescence of the top leaf sheaths did not detectably slow the establishment of a mature community as compared with the middle height leaf sheaths. Considering the natural senescence pattern of leaf sheaths one would expect to see a gradual appearance of species first colonizing the initially dead middle leaf sheaths followed by colonization of senescent top leaf sheath. However, because of the dissimilarity between vertically separated fungal subcommunities (Table 5) and possibly the inappropriate time lag of one month between the subsequent collections, these temporal colonization patterns were observed for only a few species (e.g. *Phoma* sp. III, Table 2). Furthermore, colonization and fruiting patterns could also be inferred by interspecific competition more than by the lag in senescence. At the end of phase II fungal communities reach their peak diversity (Fig. 8a). This pattern is less clear for the basal subcommunity. Although, it appears that the mature subcommunity on basal leaf sheaths matures together with the litter layer assemblages (Fig. 8b). While all subcommunities show a peak in species richness and sporulation of asexual coelomycetes before the sexual ascomycetes, the top community is characterized by a common peak of sexual and asexual taxa richness and sporulation (Fig. 8b). Whether these asexual and sexual sporulation structures represent the sequential appearance of the different stages of the holomorphic fungus remains to be established for most of the observed taxa. Few corresponding ana- and teleomorphs are known.

Soon after maximal diversity the communities start to become impoverished (Fig. 8a) and Phase III in fungal succession commenced. This phase shows few stress tolerant species that persist to sporulate in the last stage of decay, characterized by increasing carbon and/or nutrient stress and a reduced resource size by fragmentation. The top leaf sheath community could not be sampled long enough to demonstrate clearly a third phase (shoot tips broken off),

although the last samples indicated an already lowered diversity. A final fourth phase in fungal succession is to be expected in the litter layer characterized by common soil fungi (e.g. Frankland, 1976), but these where not found sporulating during this study on the leaf sheath remains in a final state of decay.

Occurrence and indicator taxa

Several taxa recorded during this study appeared with only one or few records (Table 1). Besides being rare, these taxa could be common mycelial inhabitants or the recorded life stage (the sexual or asexual stage) is formed rarely on the leaf sheaths. Only four taxa had overall abundance higher than 5% (Table 1). How to evaluate species importance and whether to call taxa common or rare and where to draw the line for these terms is an ongoing point of discussion between different fungal diversity studies (Jones and Hyde, 2002). Based on sporulation data the most objective way to pinpoint a species as characteristic for a certain phase or microhabitat during fungal community development is thought to be an indicator algorithm such as TWINSPAN (two-way indicator species analysis) (Hill, 1979) or ISA (indicator species analysis) (Dufrêne and Legendre, 1997). The latter technique is preferred in ecological studies with several underlying gradients (McCune & Grace, 2002) and was used in this study. The hierarchical output in Fig. 3 contrasts the performance of taxa at different levels of the community (generalists – eurytopic versus specialists – stenotopic). Ideally, the best indicators should be abundant, present on all unit samples of a group and faithful to a group. Such a species would be easily detected during field sampling. Alternatively a less abundant species that is faithful to a group to the point that its mere presence in all group samples is also a reliable indicator with high IV. However, such a species will be more easily overlooked when the community is undersampled. The species shown to have a significant IV (Fig. 3) could have a relatively low IV. Those taxa are often confined to a single group but their presence remained undetected on several sample units (asymmetrical indicators *sensu* Dufrêne and Legendre, 1997). Such taxa should not be used alone to predict a subcommunity type or a temporal phase. Asymmetrical indicators are considered to be a common type of indicators for fungal communities because of the many rare, specialized taxa. Therefore, typifying fungal (sub)communities should be based on all indicators united in 'core' assemblages of taxa (Fig. 3).

The natural falling of whole colonized shoots on the sediment explains the absence of indicator taxa for reeds initially in the litter layer and lowers the overall indicative power of some taxa typical of canopy communities. Some terrestrial taxa could persist with fruit bodies on the reed in the litter layer for a few months. Therefore, the IV of taxa characteristic for the standing subcommunities (Table 4), and more specific for group 2a, 2b and group 5, are considered to be minimum values (Fig. 3).

Seasonality

While most taxa showed sporulation during a specific, continuous period, few taxa showed a seasonal pattern observed as a sporulation stop during colder winter months. Most obvious examples of species presumably not adapted to sporulate at colder temperatures are *Phaeosphaeria* sp. II and III and *Myrothecium cinctum* (Table 2; see Fig. 9 for seasonal weather dynamics). However, those taxa probably stay cryptic as mycelia during winter or they could be pleomorphic. Although seasonality has been mentioned before to have an important effect on fungal succession (e.g. Swift, 1976; Zhou and Hyde, 2002), most studies seem to be unable to demonstrate regular seasonal patterns directly linked to climate (Hawker, 1966; Widden, 1981). For microfungi on specific substrates, part of the difficulty is due to the close relationship between host physiology, life history and changing nutritional quality of the resource with seasons or time. Sporulation of taxa soon after the resource senescences is probably controlled by endogenous changes within the resource while sporulation on the dead resource is likely to be influenced more by environmental variables. Changes in resource quality could however have a substantial impact on fungal dynamics (Gessner et al., 1997; Unpublished data).

During our study, sporulation of many taxa started already during winter, with maximal species richness recorded in March-April which meant that temperatures were still below 11°C and night frost was not uncommon. However, diversity increased till the start of the summer (Fig. 8A). It would seem that coelomycetes can bear lower temperatures (Fig. 8B) (see Anonymous, 1979). Perhaps their fruit body formation is triggered by low temperatures causing sporulation sooner in the season, while ascomycetes were mainly found later in the season, suggesting that their ascomata formation (ie. fertilization) could be triggered by different factors such as increasing temperatures. The sequential occurrences of teleomorphs and anamorphs could be due to the fact that anamorphs are simpler and thus develop more rapidly (Müller, 1981). The adaptation of fungal phenology to the host's life history was demonstrated by comparing leaf blade and sheath communities. In our study site leaf blades had all but three of their 35 species in common with leaf sheaths (unpublished data). However, all these species predominantly sporulated on moribund and senescent hanging leaves in autumn, serving as potential inoculum resource for the leaf sheaths. While the same species sporulate on the standing leaf sheaths in spring, closing the circle by inoculating the newly formed leaf blades and sheaths.

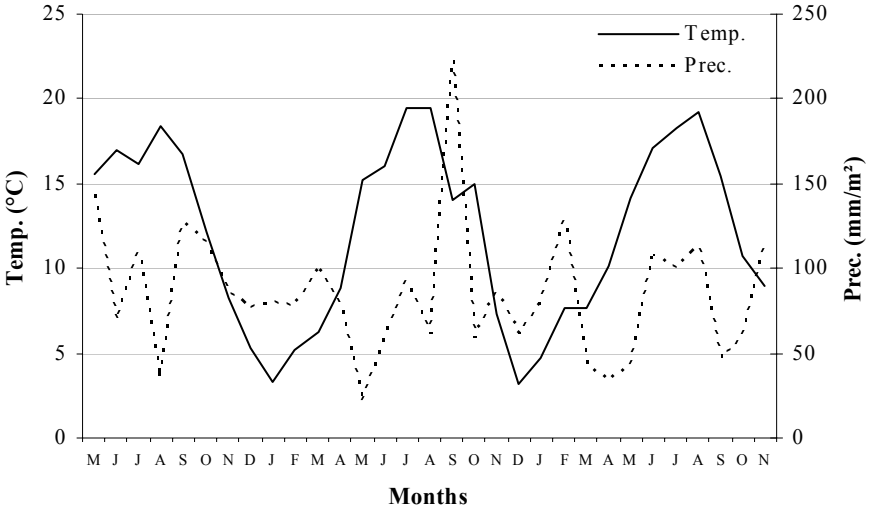


Fig. 9. Average daily air temperature and monthly precipitation during the study period. Both temperature and precipitation were measured in a nearby official weather station of the Royal Meteorological Institute of Belgium (Stabroek, Antwerp).

Acknowledgements

We are grateful for the funding by the Institute for the Promotion of Innovation by Science and Technology in Flanders, Belgium (to G.V.R.).

References

ANONYMOUS (1979). Dialogue on the ecology of anamorphs and teleomorphs. *In* The Whole Fungus. Vol. 2. (ED. B. KENDRICK). Proceedings of the Second international Mycological Conference, Alberta, Canada: 623-634.

APINIS, A.E., CHESTERS, C.G.C. & TALIGOOLA, H.K. (1972). Colonisation of *Phragmites communis* leaves by fungi. Nova Hedwigia 23: 113-124.

APINIS, A.E., CHESTERS, C.G.C. & TALIGOOLA, H.K. (1975). Microfungi colonizing nodes and internodes of aerial standing culms of *Phragmites communis* Trin. Nova Hedwigia 26: 495-507.

BENNER, R., MORAN, M.A. & HUDSON, R.E. (1986). Biogeochemical cycling of lignocellulosic carbon in marine and freshwater ecosystems: relative contributions of prokaryotes and eukaryotes. Limnol. Oceanogr. 31: 89-100.

BIONDINI, M.E., MIELKE JR., P.W. & BERRY, K.J. (1988). Data-dependent permutation techniques for the analysis of ecological data. Vegetatio 75: 161-168.

CAI, L., ZHANG, K.Q., MCKENZIE, E.H.C. & HYDE, K.D. (2003). Freshwater fungi from bamboo and wood submerged in the Liput River in the Philippines. Fungal Div. 13: 1-12.

COOKE, R.C. & RAYNER, A.D.M. (1984). Ecology of Saprotrophic Fungi. Longman Group Limited, London.

DIX, N.J. & WEBSTER, J. (1995). Fungal Ecology. Chapman & Hall, London.

DUFRÊNE, M. & LEGENDRE, P. (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67: 345-366.

ERNST, M., MENDGEN, K.W. & WIRSEL, S.G.R. (2003). Endophytic fungal mutualists: seed-borne *Stagonospora* spp. enhance reed biomass production in axenic microcosms. Molec. Pl.-Microbe Interact. 16: 580-587.

FINDLAY, S.E.G., DYE, S. & KUEHN, K.A. (2002). Microbial growth and nitrogen retention in litter of *Phragmites australis* compared to *Typha angustifolia*. Wetlands 22: 616-625.

FRANKLAND, J.C. (1976). Decomposition of bracken litter. Bot. J. Linn. Soc. 73: 133-143.

FRANKLAND, J.C. (1992). Mechanisms in fungal succession. *In* The Fungal Community. Its Organisation and Role in the Ecosystem. 2de ed. (EDS. G.C. CAROLL & D.T. WICKLOW). Marcel Dekker, New York: 383-401.

FRANKLAND, J.C. (1998). Fungal succession - unraveling the unpredictable. Mycol. Res. 102: 1-15.

FRYAR, S.C. (2002). Fungal succession or sequence of fruit bodies? Fungal Div. 10: 5-10.

GESSNER, M.O. (2000). Breakdown and nutrient dynamics of submerged *Phragmites* shoots in the littoral zone of a temperate hardwater lake. Aquatic Bot. 66: 9-20.

GESSNER, M.O. (2001). Mass loss, fungal colonisation and nutrient dynamics of *Phragmites australis* leaves during senescence and early decay. Aquatic Bot. 69: 325-339.

GESSNER, M.O., SUBERKROPP, K. & CHAUVET, E. (1997). Decomposition of plant litter by fungi in marine and freshwater ecosystems. *In* The Mycota IV: Environmental and Microbial Relationships (EDS. D.C. WICKLOW & B. SÖDERSTRÖM). Springer-Verlag, Berlin Heidelberg: 303-322.

GESSNER, M.O. & VAN RYCKEGEM, G. (2003). Water fungi as decomposers in freshwater ecosystems. *In* Encyclopedia of Environmental Microbiology (ED. G. BITTON). Wiley, New York (online edition: DOI: 10.1002/0471263397.env314).

GRANÉLI, W. (1990). Standing crop and mineral content of reed, *Phragmites australis* (Cav.) Trin. ex Steud., in Sweden - management of reed stands to maximize harvestable biomass. Folia Geobot. Phytotax. 25: 291-302.

HAWKER, L.E. (1966). Environmental influences on reproduction. *In* The Fungi. Vol. 2 (eds. G.C. Ainsworth & A.S. Sussman). Academic Press, New York: 435-469.

HAWKSWORTH, D.L. (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. 105: 1422-1432.

HILL, M.O. (1979). TWINSPAN - a FORTRAN Program for Arranging Multivariate Data in an Ordered Two-Way Table by Classification of the Individuals and Attributes. Ecology and Systematics. Cornell University, New York.

HUDSON, H.J. (1968). The ecology of fungi on plant remains above the soil. New Phytol. 67: 837-874.

HUDSON, H.J. (1971). The development of the saprophytic fungal flora as leaves senesce and fall. *In* Ecology of Leaf Surface Micro-organisms (EDS. T.F. PREECE & C.H. DICKINSON). Academic Press, London: 447-455.

HYDE, K.D. & JONES, E.B.G. (2002). Fungal Succession. Fungal Diversity volume 10. Fungal Diversity Press, Yunnan University, P.R. China.

JONES, E.B.G. & HYDE, K.D. (2002). Succession: where do we go from here? Fungal Div. 10: 241-253.

KOMÍNKOVÁ, D., KUEHN, K.A., BÜSING, N., STEINER, D. & GESSNER, M.O. (2000). Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a litteral reed stand of a large lake. Aquat. Microb. Ecol. 22: 271-282.

LEGENDRE, P. & LEGENDRE, L. (1998). Numerical Ecology. 2nd English edn. Elsevier, Amsterdam.

LEUCHTMANN, A. (1984). Über *Phaeosphaeria* Miyake und andere bitunicate Ascomyceten met mehrfach querseptierten ascosporen. Sydowia 37: 75-194.

LUO, J., YIN, J., CAI, L., ZHANG, K. & HYDE, K.D. (2004). Freshwater fungi in Lake Dianchi, a heavily polluted lake in Yunnan, China. Fungal Div. 16: 93-112.

MANLY, B.F.J. (1994). Multivariate Statistical Methods. 2nd edn. Chapman & Hall, Boca Raton.

MASON, C.F. (1976). Relative importance of fungi and bacteria in the decomposition of *Phragmites* leaves. Hydrobiologia 51: 65-69.

MCCUNE, B. & GRACE, J.B. (2002). Analysis of Ecological Communities. MjM Software design, Gleneden Beach, Oregon, U.S.A.

MCCUNE, B. & MEFFORD, M.J. (1999). PC-ORD for Windows. Multivariate Analysis of Ecological Data. Version 4.0. MjM Software, Gleneden Beach, Oregon, U.S.A.

MÜLLER, E. (1981). Relations between conidial anamorphs and their teleomorphs. *In* Biology of Conidial Fungi, Vol 1 (EDS. G.T. COLE & B. KENDRICK). Academic Press, New York: 145-169.

NAG RAJ, T.R. (1993). Coelomycetous Anamorphs with Appendage-bearing Conidia. Mycologue Publications, Ontario, Canada.

NEWELL, S.Y. (1993). Decomposition of shoots of a salt-marsh grass, methodology and dynamics of microbial assemblages. Adv. Microb. Ecol. 13: 301-326.

NEWELL, S.Y. (1996). Established and potential impacts of eukaryotic mycelial decomposers in marine/ terrestrial ecotones. J. Exper. Mar. Biol. Ecol. 200: 187-206.

POON, M.O.K. & HYDE, K.D. (1998a). Biodiversity of intertidal estuarine fungi on *Phragmites* at Mai Po marshes, Hong Kong. Bot. Mar. 41: 141-155.

POON, M.O.K. & HYDE, K.D. (1998b). Evidence for the vertical distribution of saprophytic fungi on senescent *Phragmites australis* culms at Mai Po marshes. Bot. Mar. 41: 285-292.

RAYNER, A.D.M. & TODD, D.K. (1979). Populations and community structure and dynamics of fungi in decaying wood. Adv. Bot. Res. 7: 333-420.

RICE, W.R. (1990). A consensus combined P-value test and the family-wide significance of component tests. Biometrics 46: 303-308.

SOETAERT, K., HOFFMANN, M., MEIRE, P., STARINK, M., VAN OEVELEN, D., VAN REGENMORTEL, S. & COX, T. (2004). Modeling growth and carbon allocation in two reed beds (*Phragmites australis*) in the Scheldt estuary. Aquatic Bot. 79: 211-234.

SUTTON, B.C. (1980). The Coelomycetes. Commonwealth Mycological Institute, England.

SWIFT, M.J. (1976). Species diversity and the structure of microbial communities in terrestrial habitats. *In* The role of Terrestrial and Aquatic Organisms in Decomposition Processes (eds. J.M. Anderson & A. Macfadyen). Blackwell Scientific, Oxford: 185-222.

TALIGOOLA, H.K., APINIS, A.E. & CHESTERS, C.G.C. (1972). Microfungi colonizing collapsed aerial parts of *Phragmites communis* Trin. in water. Nova Hedwigia 23: 465-472.

VAN RYCKEGEM, G. (2005). Fungi on common reed (*Phragmites australis*): fungal diversity, community structure and decomposition processes. Ph.D. thesis. Ghent University, Ghent.

VAN RYCKEGEM, G. & VERBEKEN, A. (2005). Fungal diversity and community structure on *Phragmites australis* (Poaceae) along a salinity gradient in the Scheldt estuary (Belgium). Nova Hedwigia 80 (in press).

WAKEFIELD, E.M. (1939). Nomina generica conservanda. Contributions from the nomenclature committee of the British Mycological Society. II. Trans. Brit. Mycol. Soc. 23: 281-292.

WIDDEN, P. (1981). Patterns of phenology among fungal populations. *In* The Fungal Community, its Organization and Role in the Ecosystem (EDS. D.T. WICKLOW & G.C. CAROLL). Marcel Dekker, New York: 387-401.

WIRSEL, S.G.R., LEIBINGER, W., ERNST, M. & MENDGEN, K. (2001). Genetic diversity of fungi closely associated with common reed. New Phytol. 149: 589-598.

WONG, M.K.M. & HYDE, K.D. (2001). Diversity of fungi on six species of Gramineae and one species of Cyperaceae in Hong Kong. Mycol. Res. 105: 1485-1491.

ZHOU, D. & HYDE, K.D. (2002). Fungal succession on bamboo in Hong Kong. Fungal Div. 10: 213-227.

Fungal ecology and succession on *Phragmites australis* in a brackish tidal marsh. II. Stems

Van Ryckegem, G. & Verbeken, A.
Submitted to Fungal Diversity (accepted)

Abstract Fungal succession and community development has been studied by direct observation for twenty-four months on stems of *Phragmites australis* in a brackish tidal marsh of the river Scheldt (Belgium). In total forty-nine taxa were found during the successional series of which twenty-six taxa (53%) were ascomycetes, sixteen taxa (33%) coelomycetes, four taxa (8%) hyphomycetes and only three taxa (6%) basidiomycetes. Fungal sporulation on standing stems started only after a minimum of three months in standing dead position and showed different fungal assemblages along the vertical axis of the shoots. Cluster analysis and detrended correspondence analysis showed that all investigated microhabitats (middle, basal and litter layer) showed a different species composition that changed the decay pattern. This successional sporulation was described by a three-staged pattern comparable for all reed plant parts and in each subcommunity. For all of the different microhabitats – subcommunities – and for the groups recognized by cluster analysis, indicator taxa were pointed.

Introduction

Tidal wetlands and other *Phragmites australis* (Cav.) Trin. ex Steud. dominated wetlands are among the most productive ecosystems in the world (e.g. Mitsch and Gosselink, 2000). Most of this produced biomass exists out of recalcitrant, woody stem tissue accounting for about 50% of the total aboveground mass (Granéli, 1990; Meganck, 1998; Gessner, 2000). This huge amount of dead plant matter enters the detritial system and becomes available for fungal saprotrophic colonization first in a standing decay phase of variable length and subsequently in the litter layer (Haslam, 1972; Pieczyńska, 1972; Granéli, 1990). Both of these microhabitats show a substantially different mycoflora (Apinis *et al.* 1972a, b, 1975; Van Ryckegem and Verbeken, 2005a, b) and a vertical distribution of taxa is noticed on the standing shoots (Apinis *et al.*, 1975; Poon and Hyde, 1998; Van Ryckegem and Verbeken, 2005a, b).

This paper is a second part of a study (see Van Ryckegem and Verbeken, 2005b) which aims to describe the fungal sporulation sequence (succession) on *P. australis* and focuses on the stems in different microhabitats naturally available in a reed stand. For each of the groups identified by cluster analysis and for the different subcommunities indicator taxa are provided.

Material and Methods

For a description of the study site, field and laboratorial procedures and data processing, the reader is referred to (Van Ryckegem and Verbeken, 2005b). Collecting of stems

was done as for the leaf sheaths at monthly base and was repeated the year after in an adjacent site to check for between year variations. Both plots were sampled monthly starting from emergence in May 2000 (Plot 1) or May 2001 (Plot 2) during 24 months of growth and decay. The mycota were followed during 19 and 21 months of standing colonization and decay in the middle and basal regions respectively. In July 2001 and 2002, after 15 months in a standing dead position, 70 still standing culms in each plot were marked at the base with red flagged cable ties, to be sure of the age of the dead sampled culms. Once the stems collapsed onto the sediment they were followed in the litter layer for 18 months (see also Table 3).

No top stem collections were included because the top section of stems is very thin as the upper part of the culm consists mainly out of tightly packed leaf sheaths. Moreover, the upper parts of standing shoots are soon entering the litter layer as they are fragile and more susceptible to snap by gusts of wind especially if they carry a panicle.

Ten stem pieces were collected at two heights in the canopy and fifteen replicates were taken in the litter layer (see Van Ryckegem and Verbeken, 2005b for a discussion on adequacy of sample size). This resulted in a relative occurrence of the fungal taxa on a scale of 10 for the standing stems while a relative abundance on a scale of 15 for the litter stems were obtained. Before data analysis the latter scores were transformed to a scale of 10 as a matter of conformation.

Results

In total forty-nine fungal taxa were found on stems during growth and decomposition of common reed. For analyses the two data sets were pooled, this seemed justified because of the high similarity between the two series based on species composition (Jaccard similarity: 61%) and considering species records (Bray-Curtis similarity: 78%). Both series proved to have the same distribution as tested with a Mann-Whitney U test ($P = 0.6296$). Furthermore, the results of the cluster analysis showed that 90% of all stem samples compared with one year interval, clustered in the same group identified (Fig. 2).

Fungal diversity

In general most of the taxa on reed were rare, which means observed only once or twice (Table 1). Stem mycoflora showed almost the same proportions of taxa within the major taxonomic groups as leaf sheaths (Van Ryckegem and Verbeken, 2005b) with 96% of the records belonging to either ascomycetes or coelomycetes, but on stems ascomycetes were better represented with 62% of the total fungal records (versus 37% on leaf sheaths). In total twenty-six taxa (53%) ascomycetes, sixteen taxa (33%) in the coelomycetes, four taxa (8%) belonging to the hyphomycetes and three (6%) basidiomycetes were discovered on the stems.

Table 1. Taxa list and frequency distribution of all taxa found on stems of *Phragmites australis* during growth and decomposition. Data for the two successive series pooled. Total % frequency of occurrence (% oc.) is the proportion of records of a taxon on the number of stem sections screened with fungal presence*. % abundance (% ab.) is the proportion of records of a taxon on the total number of records. Taxa acronyms code for taxa names in Fig. 2. The first letter of the acronym codes for the pseudo-systematic position of a taxon: A = ascomycetes; B = basidiomycetes; C = coelomycetes; H = hyphomycetes.

Taxa	Acronyms	# rec.	% oc.	% ab.
Massarina arundinacea (Sowerby: Fr.) Leuchtm.	AMASARU	241	25.4	30.1
Halosphaeria hamata (Höhnk) Kohlm.	AHALHAM	116	12.2	14.5
Septoriella phragmitis Oudem.	CSEPPHR	86	9.1	10.8
Stagonospora vexata Sacc.	CSTAVEX	85	9.0	10.6
Lophiostoma arundinis (Pers.) Ces. & De Not.	ALOPARU	61	6.4	7.6
Botryosphaeria festucae (Lib.) Arx & E. Müll.	ABOTFES	43	4.5	5.4
Phomatospora berkeleyi Sacc.	APHOBER	28	3.0	3.5
Stagonospora cylindrica Cunnell	CSTACYL	25	2.6	3.1
Lophiostoma semiliberum (Desm.) Ces. & De Not.	ALOPSEM	24	2.5	3.0
Stagonospora incertae sedis I		22	2.3	2.8
Phoma sp. II		21	2.2	2.6
Phoma sp. IIa		20	2.1	2.5
Phaeosphaeria pontiformis (Fuckel) Leuchtm.	APHAPON	18	1.9	2.3
Stagonospora sp. II		16	1.7	2.0
Phoma sp. III	CPHOMAC	13	1.4	1.6
Keissleriella linearis E. Müll.		12	1.3	1.5
Aposphaeria sp.		11	1.2	1.4
Arthrinium phaeospermum (Corda) M.B. Ellis		10	1.1	1.3
Tremella cf. *spicifera* Van Ryck., Van de Put & P. Roberts		10	1.1	1.3
Septoriella sp(p).		9	< 1	1.1
Stagonospora elegans (Berk.) Sacc. & Traverso		9		1.1
Dictyosporium oblongum (Fuckel) S. Hughes		6		< 1
Phoma sp. I		6		
Mollisia retincola (Rabenh.) P. Karst.		5		
Morenoina phragmitidis J.P. Ellis		5		
Bactrodesmium atrum M.B. Ellis		4		
Neottiosporina australiensis B. Sutton & Alcorn		4		
Maireina monacha (Speg.) W.B. Cooke		3		
Mycosphaerella lineolata (Roberge ex Desm.) J. Schröt.		3		
Phaeosphaeria luctuosa (Niessl) Otani & Mikawa		3		
Phaeosphaeria sp. III		3		
Phomatospora sp. III		3		
Asco sp. Dothideales incertae sedis II		2		
Cytoplacosphaeria rimosa (Oudem.) Petrak s.l.		2		
Didymella glacialis Rehm		2		
Massarina fluviatilis Aptroot & Van Ryck.		2		
Phialophorophoma sp.		2		
Asco sp. Dothideales incertae sedis I		1		
Basidiomycete, (sterile mycelium)		1		
Camarosporium sp.		1		
Cistella fugiens (Pholl. ex Bucknall) Matheis		1		
Fusarium sp. III		1		
Gibberella zeae (Schwein.) Petch		1		
Haligena spartinae E.B.G. Jones		1		
Lophodermium arundinaceum (Schrad.) Chevall.		1		
Massariosphaeria sp.		1		
Phaeosphaeria eustoma (Fuckel) L. Holm s.l.		1		
Phomatospora dinemasporium J. Webster		1		
Phomatospora sp. II		1		
Total # records		947		
Total # samples*			800	

* Total number of 30 cm long stem section screened for fungal presence was 1160, however up till February there was no fungal sporulation on stems in a standing position. Those 360 initial samples (see Table 3) were excluded to calculate total % of frequency of occurrence (% oc.).

Basidiomycetes and hyphomycetes are present with only few taxa and their occurrence, measured by sporulation, in the each microhabitat is very low (Fig. 1a,b). Ascomycetes and the

coelomycete asexual states were most common. Ascomycete taxa richness is highest in the litter layer (Fig. 1a). However, the few ascomycete taxa present on the basal standing stems were more common, this is clear from the higher average number of sporulating ascomycetes on the basal standing stems compared to the litter layer stems (Fig. 1b). Over the entire study period considered ascomycetes sporulate significantly less on the middle canopy stems compared to the other microhabitats ($P < 0.001$ for both comparisons, $N = 44$ for middle-top test and $N=58$ for middle-litter test, one-tailed, *t*-test, data square root transformed) (Fig. 1b). The occurrence of ascomycetes is significantly higher in the litter layer compared to the occurrence of coelomycetes ($P < 0.001$, $N = 35$, one-tailed, paired *t*-test, data square root transformed) (Fig. 1b). The asexual coelomycetes perform generally better on the aerial parts of the reed culms (Fig. 1b) (see also Van Ryckegem and Verbeken, 2005a, b).

Spatial and temporal characterization of the subcommunities

Before further analysis the dataset was reduced by removing some outliers in the given sequence. First species which were only found once or twice during the entire study were eliminated. Second, all samples with only one or two records were removed from the dataset. Thirdly, outlier samples were identified by means of detrended correspondence analysis and eliminated (108STX; 207STX; 111ST0; 111ST1). All those initially colonized samples showed a species poor assemblage with one or two taxa. Such outliers mask the potential information in ordination by compressing the remaining ordination output (McCune and Mefford, 1999). Eventually this resulted in a reduced dataset matrix of 66 samples and 31 taxa.

Cluster analysis (CA) (Fig. 2) of the samples shows several interpretable groups well separated from each other by spatial and temporal species characteristics. Group 1 – clusters all samples comprising initial species assemblages from the basal stem sections and from the litter layer. Group 1 can be divided in two subgroups 1a – all initial litter layer collections; 1b – all initial basal stem collections. Group 2 – is a cluster of the basal stem collections in a later phase of decay. Group 3 – comprises the litter layer stem samples in a further decay phase, the two final collections which are characterized by an impoverished community, are included in this group, a decision supported by ordination outcome. Group 4 – is the outgroup in the cluster analysis and gathers all stem collections from middle height canopy.

Detrended Correspondence analysis (DCA) (Figs. 3, 4) shows two interpretable axes with a high eigenvalue (axis 1: 0.70; axis 2: 0.32). The high eigenvalue and a large length of gradient (axis 1: 5.021; axis 2: 2.530) indicate a distinct β-diversity along both axes and a high explanatory power. An after-the-fact evaluation of the variation explained by the axes by relative Euclidean correlation (McCune and Mefford, 1999) showed 57% explanatory power for axis 1 and 5% by axis 2. DCA groupings are concurrent with the clusters identified by CA.

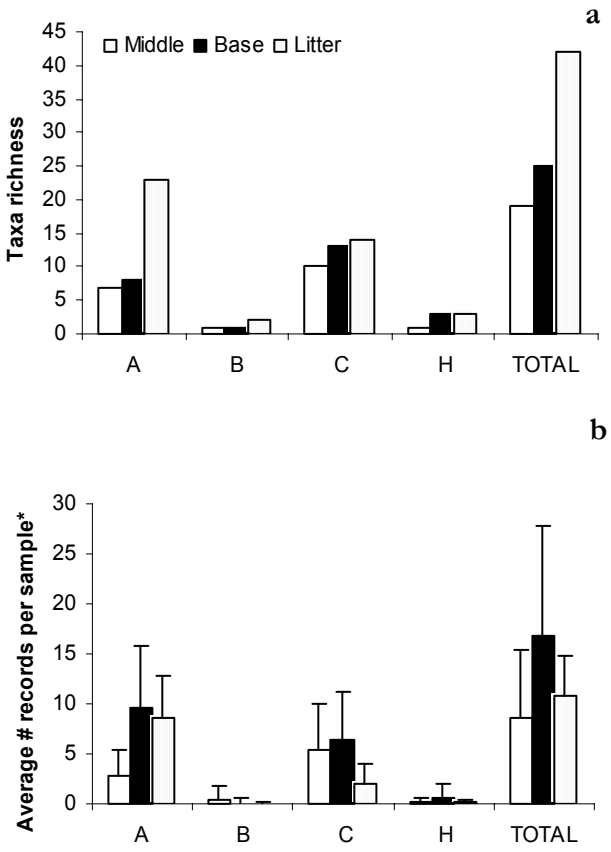


Figure 1a. Taxa richness and distribution of the major pseudo-systematic groups on stems of *Phragmites australis* in the different microhabitats investigated. **b.** Average number of records per sample occasion* in the different microhabitats investigated. The same column coding for both panels.

* The initial samples without fungal records for the basal and middle section of standing shoots were not included for the proportional calculation of the frequency of occurrence (see Table 3).

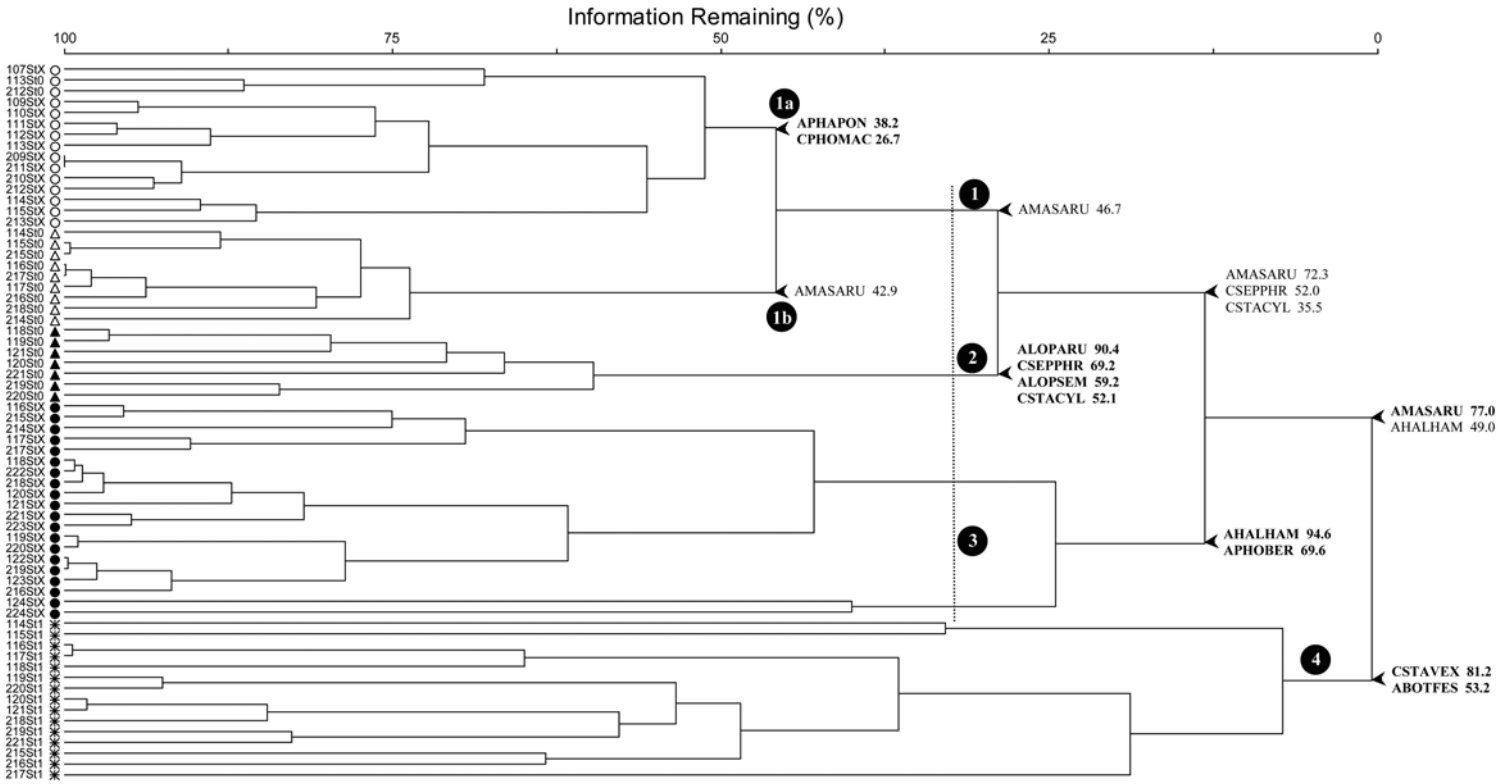


Figure 2. Dendrogram from cluster analysis of the pooled data from series 1 and 2 with 66 samples and 31 taxa included. Sample code exists of four character states. The first figure indicates the plot number being 1 or 2; the second number stands for month of collection (between 7-24), (see Table 3); the letter code 'st' stands for stem sample and the last figure indicates the microhabitat (0 = basal canopy, 1 = middle canopy and X = litter layer). Indicator species with indicator values are shown for the groups generated by cluster analysis. All species with indicator value > 25 and Monte-Carlo *P*-values < 0.01 are included. The maximum indicator values for a species are printed in bold. Acronym abbreviations are found in Table 1. Dendrogram is scaled with the percentage of information remaining in the branches.

The two samples split up in group 3 in CA, don't show extremely different in DCA and were included in group 3 for further analysis. Axis 1 represents a spatial pattern, separating the different microhabitats sampled and is negatively correlated (Kendall rank correlation) with a temporal factor in the litter layer samples (Fig. 4). Axis 2 shows a negative correlation with a temporal factor for the standing samples (Fig. 4).

Indicator species analysis (ISA) for the groups generated by CA shows 10 selected indicator species plotted on the CA-diagram (Fig. 2). Species selected at the highest hierarchical level of the community dendrogram show two specific colonizers for middle height stems (see also Table 5): *Stagonospora vexata* and *Botryosphaeria festucae*. The more eurytopic species, *Massarina arundinacea* and *Halosphaeria hamata* show indicator power for all basal and litter layer stem collections. *Halosphaeria hamata* and *Phomatospora berkeleyi* show the highest indicative power for the litter layer stems in a further state of decay. Characteristic species found on the basal stem part in a further stage of decay are *Lophiostoma arundinis*, *L. semiliberum*, *Septoriella phragmitis* and *Stagonospora cylindrical*. One of the first colonizers of *P. australis* stems is *M. arundinacea* which is also the indicator species for group 1 and more typically found on the initial basal stem parts. However *M. arundinacea* is more a generalist as shown by its higher indicator value (IV) on a higher hierarchical level (Fig. 2). Group 1b is more negatively characterized by the absence of taxa more typically found on litter layer stems freshly fallen on the sediment: *Phaeosphaeria pontiformis* and *Phoma* sp. III.

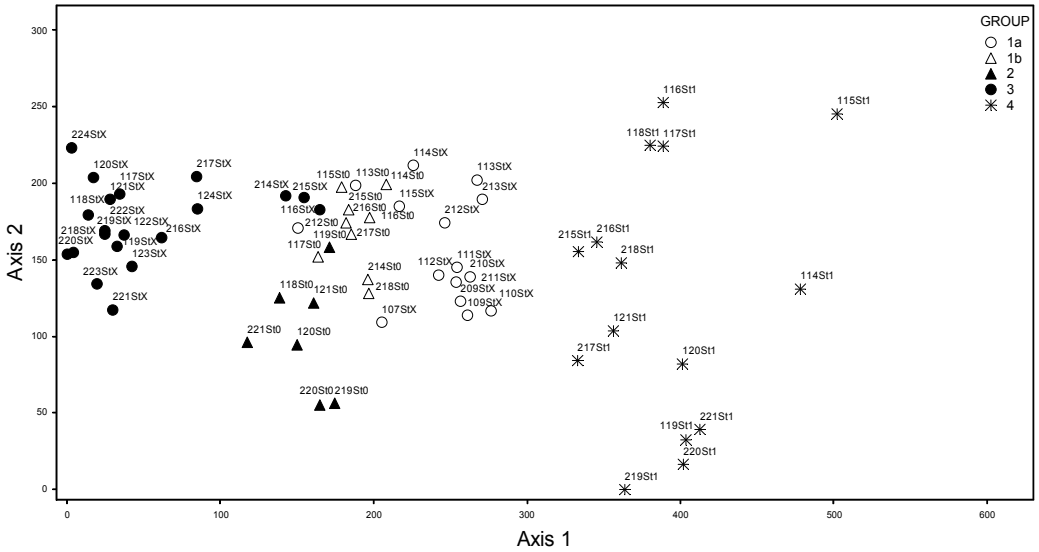


Figure 3. First two ordination axes of a DCA based on species occurrences on stems of *Phragmites australis* with 31 taxa (raw data) and 66 samples included. Sample grouping symbols and acronyms are the same as in the cluster analysis (Fig. 2). Axes are scaled in SD units (x 100).

Table 2. Sequence in fungal sporulation on stems of *Phragmites australis* during decomposition in three different microhabitats (middle and basal height of standing stems and litter layer samples). Only taxa with more than 3 records (27 taxa) during the entire study are shown with their number of records, percentages abundance (calculation base on all taxa records per microhabitat), percentage recurrence and percentages occurrence in each month sampled starting from November (see Table 3) (no sporulation during the first six months of monitoring). Data from the two series are pooled, 20 stem sections per month per microhabitat. 'Gr' codes for the pseudo-systematic group a taxon belongs: A = ascomycetes; B = basidiomycetes; C = coelomycetes; H = hyphomycetes.

Gr	Species	Micro-habitat	Σ records	% ab.	% rec.	% occurrence (months)																
						N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A
C	*Phoma* sp. III	middle	1	1	8					5													
		base	2	1	15					5		5											
		litter	10	3	77	20	10	10	5	5													
A	*Massarina arundinacea*	middle	8	5	3									10	20		5	5					
		base	131	35	53					15	15	40	85	80	95	100	75	45	30	75			
		litter	102	27	43	20	5	40	45	45	60	60	55	55	45	35	5	5			15	15	5
C	*Stagonospora vexata*	middle	68	38	80							10	15	20	55	35	40	45	55	65			
		litter	17	5	20	5	5	10	20	20	15	10											
C	*Septoriella phragmitis*	middle	11	6	13												10	15	15	15			
		base	61	17	71					5			15	5	25	35	60	60	50	50			
		litter	14	4	16	5	5	5	10	15	10	5	0	10	5								
C	*Stagonospora elegans*	base	8	2	89								10					20	10				
		litter	1	0	11	5																	
A	*Phaeosphaeria pontiformis*	middle	4	2	22									5	0	5			10				
		litter	14	4	78		5	20	20	10	5	5			5								
C	*Septoriella* sp(p).	middle	3	2	33					10						5							
		base	4	1	44								5							15			
		litter	2	1	22		5		5														
C	*Stagonospora* sp. II	middle	2	1	13									10									
		base	9	3	56					5			25	0	5	0	10						
		litter	5	1	31				5	5	5	5	0	5									
C	*Neottiosporina australiensis*	middle	1	1	25										5								
		litter	3	1	75					5	5	5											
A	*Phomatospora berkeleyi*	litter	28	7	100					5			15	10	10	25	20	10	25	0	10	0	10
C	*Stagonospora* incertae sedis I	base	9	3	41						5						5	10	0	25			
		litter	13	4	59								5	5	5	0	5	20	15	0	5	5	
A	*Botryosphaeria festucae*	middle	30	17	70									10	45	40	50	5					
		base	1	0	2												5						
		litter	12	3	28							25	10	15	10								
A	*Morenoina phragmitidis*	middle	5	3	100							25											
A	*Mollisia retincola*	litter	5	1	100							5	20										

Table 2 continued.

Gr	Species	Micro-habitat	Σ records	% ab.	% rec.	% occurrence (months)																	
						N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A
A	*Keissleriella linearis*	middle	8	5	67													20	10	10			
		base	1	0	8															5			
		litter	3	1	25							5	5	5									
A	*Halosphaeria hamata*	base	9	3	8										5	5	10	5	0	20			
		litter	107	28	92								15	10	45	40	85	50	80	85	60	55	10
C	*Stagonospora cylindrica*	base	25	7	100								5	10	15	40	20	25	5	5			
C	*Phoma* sp. IIa	middle	14	8	70										30	25	10			5			
		base	3	1	15										5			10					
		litter	3	1	15								5						5	5			
C	*Phoma* sp. II	middle	15	9	71										5	20	0	40	0	10			
		base	4	1	19													10	10				
		litter	2	1	10								5							5			
C	*Phoma* sp. I	middle	1	1	17											5							
		base	4	1	67												10			10			
		litter	1	0	17								5										
A	*Lophiostoma semiliberum*	base	16	5	67											5	15	15	15	30			
		litter	8	2	33									5	5	0	5	10	0	5	5	5	
H	*Bactrodesmium atrum*	base	4	1	100									5				5	5	5			
H	*Arthrinium phaeospermum*	middle	3	2	30												15						
		base	5	1	50										5	5	0	10	5				
		litter	2	1	20									5				5					
C	*Aposphaeria* sp.	base	10	3	91						15			15					20				
		litter	1	0	9										5								
A	*Lophiostoma arundinis*	base	52	15	85										5	15	40	45	75	80			
		litter	9	2	15										5	0	10			15	5	10	
H	*Dictyosporium oblongum*	base	5	1	83														10	15			
		litter	1	0	17										5								
B	*Tremella* cf. *spicifera*	middle	10	6	100												10	20	20				

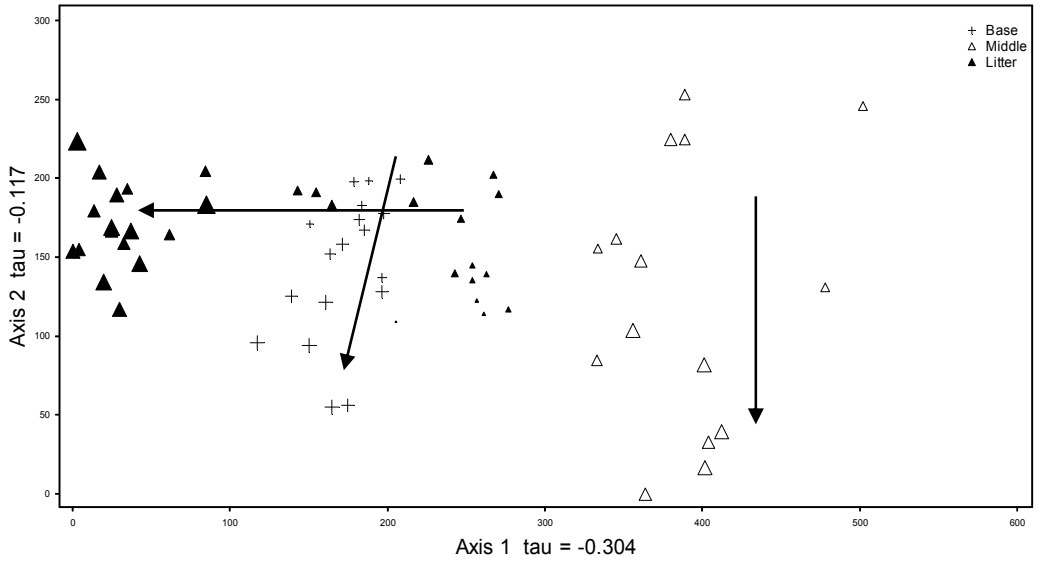


Figure 4. DCA plot (see Fig. 3) stem samples from *Phragmites australis* showing the temporal correlation of the first two ordination axes with Kendall rank correlation (tau) indicated. Smallest symbols indicate earliest samples in the successional community development. Arrows indicate the fungal successional pattern in each microhabitat. Base: basal canopy stem samples; middle: Middle canopy stem samples; litter: litter layer stem samples.

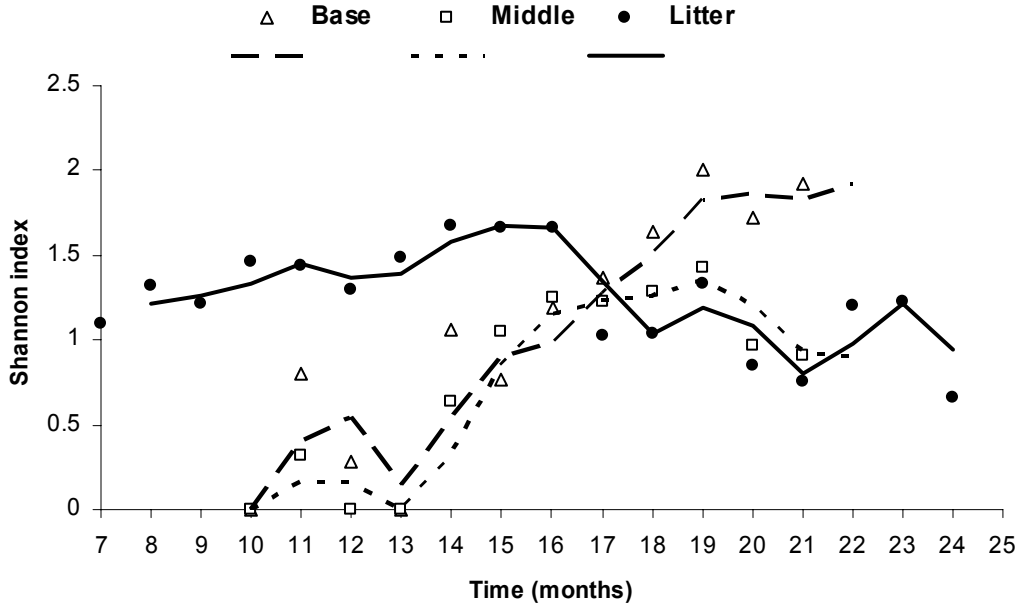


Figure 5. Shannon diversity indices for the different microhabitats – subcommunities – sampled during the experimental period on stems of *Phragmites australis*. The line is a moving average fit with a period of two. Base: basal canopy stem samples; middle: Middle canopy stem samples; litter: litter layer stem samples.

Discussion

Diversity

A frequency distribution with few abundant and a long tail of rare taxa is often observed during fungal succession sequences (e.g. Apinis *et al.*, 1972a, b; Zhou and Hyde, 2002). However, comparing species lists obtained for standing stems of *P. australis* (Apinis *et al.*, 1975; Poon and Hyde, 1998) with our list (Table 1), shows little similarity in species composition. The most remarkable difference is the higher number of hyphomycetes recorded by the above authors and the low number of ascomycetes, presumably due to the moist chamber incubation (see Van Ryckegem and Verbeken, 2005a). Similar to our results, Poon and Hyde (1998) found the basal parts of standing stems to be the most species rich. Ongoing research provides evidence for a very high world-wide diversity of phragmiticolous fungi a topic dealt with in a future paper (Van Ryckegem in prep.; see also Wong & Hyde, 2001).

Vertical distribution

Vertical distribution patterns of saprotrophic fungi have been observed on standing stems of *P. australis* (Apinis *et al.*, 1975; Poon and Hyde, 1998; Van Ryckegem and Verbeken, 2005a, b) and were observed on standing shoots in this study. The different microhabitats investigated (litter layer, base and middle canopy) showed a differential species composition (MRPP overall $P < 0.0001$; all three comparisons were highly significant $P < 0.0001$, Bonferroni corrected). And based on presence-absence data (Jaccard similarity) a low resemblance was noticed between the different microhabitats (Table 4). Although eleven taxa sporulated in all the three microhabitats on stems screened, each of those taxa (Table 2) showed a higher recurrence in one of the subcommunities (see also Poon and Hyde, 1998). In Table 5 indicator taxa for each of the investigated microhabitats are presented. The species pointed as the most typical taxon for the middle height stems is *Stagonospora vexata*, however considering the whole fungal community on reed this species will score lower as it is also common on leaf sheaths (Van Ryckegem and Verbeken, 2005b). At the contrary, *Botryosphaeria festucae* is a typical, although not abundant sporulator, on the middle height stems. The basal stem parts are characterized by four species of which the first is also common in the litter layer: *Massarina arundinacea*. The other taxa are less common and show a shorter fructification period on basal stem parts. These species are specialists on the lower parts of stems influenced by tidal exchange water or just above this zone. *Stagonospora elegans* was mentioned by Apinis *et al.* (1972b) as a typical colonizer of basal stem sections but although this species occurred in our study site in the lower parts of standing shoots (Table 2), it was also found on upper leaf sheaths (Van Ryckegem & Verbeken, 2005b). However, *Stagonospora cylindrica* a species resembling *S. elegans* (Sutton, 1980) appeared to be restricted to and rather frequent on the basal stem parts.

Most typical colonizers of litter stems were *Phomatospora berkeleyi* and *Halosphaeria hamata* two species pointed also as indicator taxa for leaf sheaths (Van Ryckegem & Verbeken,

2005b) and considered to be the dominant species in a later phase of reed decay in our study site.

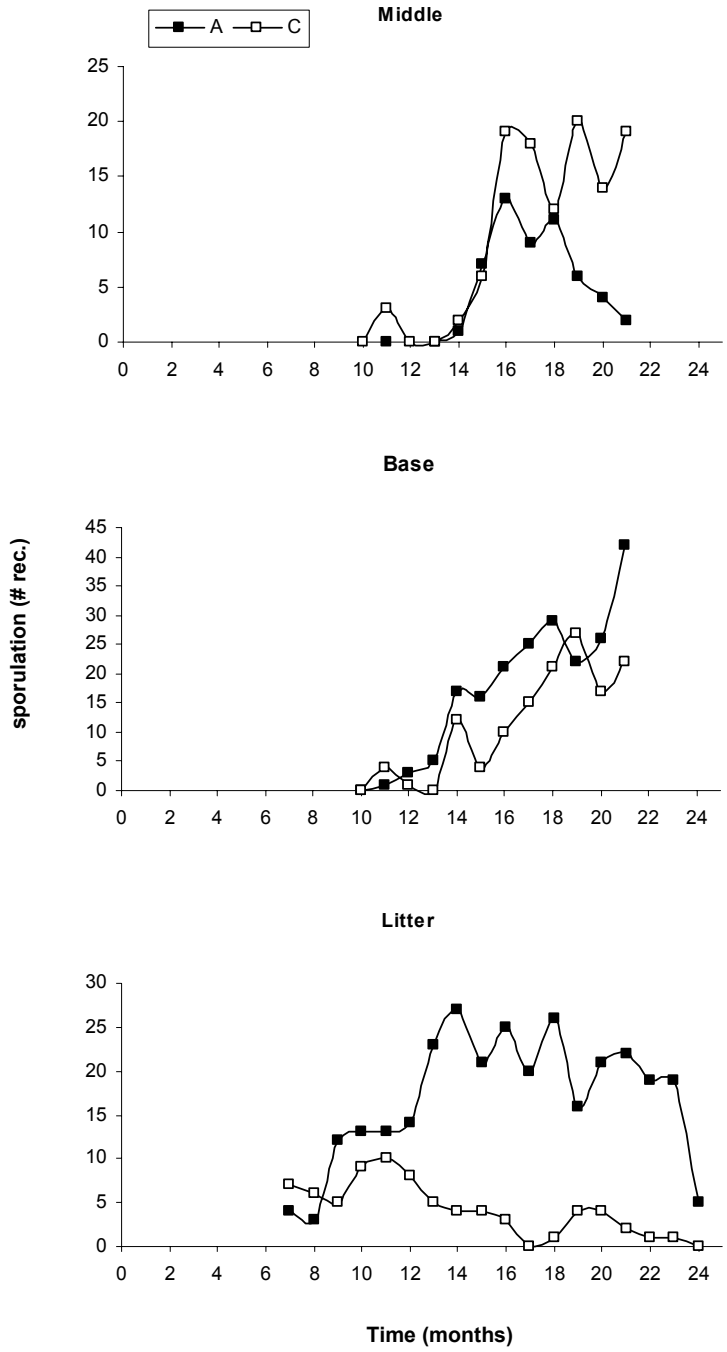


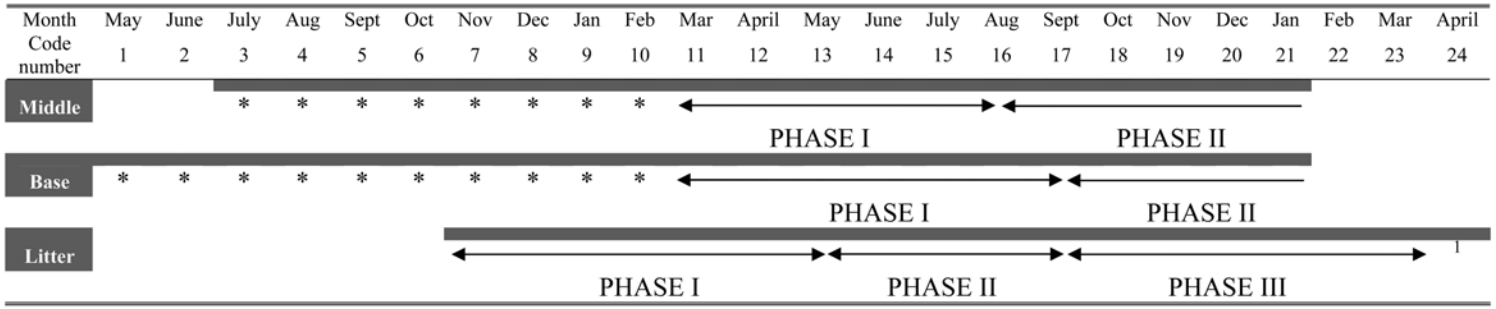
Figure 6. Sporulation records in de different microhabitats on stems of *Phragmites australis* for the two dominant pseudo-systematic groups: A = ascomycetes; C = coelomycetes.

Successional sequence

A consequent successional appearance of fungal sporulation structures with comparable abundance and taxa composition was observed between two successive years in the same tidal marsh habitat. Succession of the most dominant taxa is shown in Table 2 and phasing of species sporulation in the different subcommunities is further illustrated in Table 3 and Fig. 4 and shows comparable stages as delimitated in Van Ryckegem and Verbeken, (2005b) for reed leaf sheaths. No fungal species were found sporulating on the green, living stems (see also Apinis *et al.* 1972b) and the species colonizing dead stems are slowly developing. *M. arundinacea*, one of the first to develop on the standing bases of stems, has ascomata already visible during January and February but most fruit bodies are mature during late spring and in summer. The slowest appearance of fungal fruit bodies was noticed on the middle height sections of stems with only sporadic sporulation structures formed till July, six months after senescence of the shoots (Table 2). Overall sporulation on those middle stem sections was poor (Fig. 1) and between month variation in species community was rather large (Fig. 3) because of the many rare species, resulting in an unclear successional pattern for this subcommunity. However, similar to the basal stem section, diversity gradually increases (Phase I) and reaches a peak for the middle height section in the sixteenth month (Fig. 5; Table 3) and maintains high (Phase II), with only a slight decrease to the end of the study period. The basal subcommunity seems to mature later (Fig. 5; Table 3), which could be related to the natural top-down senescence of *P. australis* stems (Granéli, 1990) and no impoverished community was observed in the canopy, probably because the sampling time was too short. Additional observations (but not replicated sufficiently and only for one year) of fungal sporulation structures on the few standing stems remaining, showed that an impoverished community established on basal stem sections characterized by *Halosphaeria hamata* being hypersaprotrophical on *M. arundinacea* and *Lophiostoma* spp. In the middle canopy, *Keissleriella linearis* becomes the dominant species during the next summer, seemingly a characteristic species for the impoverished community as it was nearly the only species seen to form fruit bodies in the middle canopy during this period. The litter layer subcommunity which was an initially divers mixture of culms fallen from the canopy on the sediment, shows a slight increase in diversity with a short period characterized as a mature community (Table 3).

The three stages in litter layer sporulation are discerned in the ordination diagram (Figs. 3, 4) as separated groups. Phase I for the litter layer stems is considered as an initial fungal community establishment conform to the successional sequence described by Dix and Webster (1995) and not to the Phase X described for leaf sheaths entering the litter layer (Van Ryckegem and Verbeken, 2005b) characterized as a turn-over community with losing the aerial species and establishment of estuarine species.

Table 3. Sampling design and the different stages in fungal succession on stems of *Phragmites australis* recognized in our study. The complete dataset exists of two successional series with one year interval, with series 1 started in May 2000 and series 2 in May 2001.



[1] Collections from month 24 suggest that a fourth phase starts.

[*] No fungal records on the collections from these months.

In contrast to the prompt sporulation observed on standing leaf sheaths (Van Ryckegem and Verbeken, 2005b), the standing stems show a lag period in appearance of sporulation structures with the first fungal sporulation structures on standing stems noticed in March, about three months after senescence. However, it took about six months before sporulation started to become vigorously. Possibly the more recalcitrant stems are less susceptible in fungal colonization because of fewer stomata, more sclerenchymatous tissue and thicker cuticle compared to leaf sheaths (Rodewald and Rudescu, 1974). Furthermore, they are surrounded by the leaf sheaths which lower the inoculum potential by air-spora and probably create less favourable conditions for stem mycota because of the lower water availability. The latter point could be important as fungi colonizing plants in a standing dead position mainly depend on periodical (nightly) dew wetting for their activities (Newell *et al.*,1985, 1996; Kuehn *et al.* 1998, 2004; Kuehn and Suberkropp, 1998). Sporulation on stems surrounded by leaf sheaths started when nearly 50% of the leaf sheath tissue was decomposed (unpublished data). At this point probably better water condition prevails as leaf sheaths are softened and retain more water. Furthermore, at this stage, stems have proportionally more aerial contact making them more vulnerable to fungal colonization. This free aerial contact could also induce the development of fungal sporulation structures.

The observed sequential sporulation on litter layer culms is only one of the possible natural decay patterns present in a reed stand. We described the successional pattern for culms entering the litter layer mainly during late autumn and probably some of the culms had fallen in the litter layer after storms during the growth season (pers. obs.). The eventual impact of falling in the litter layer on the present community will depend on the duration of their standing decay (ie. extend a canopy community is already developed) and the season in which they snap. Especially the establishment and duration of phase I [which could become more a turnover phase as found for leaf sheaths (Van Ryckegem & Verbeken, 2005b)] and phase II of the succession could be altered, while phase III and the presumed phase IV will probably be alike for all stems entering the litter layer.

Consequences of fungal colonization on standing shoots

The results show an extensive sporulation on standing shoots both on leaf sheaths (Van Ryckegem and Verbeken, 2005b) and stems. The shoots could stay upright for longer periods than monitored here (e.g. 3-4 years, Haslam, 1972), meaning that a substantial decay could happen in an upright position possibly significantly altering the amount of necromass entering the litter layer (e.g. Kuehn et al., 2004), a point which should be incorporated in organic matter dynamics of wetland ecosystems (e.g. Pieczyńska, 1972; Gessner *et al.*, 1996). All dominant taxa found on standing dead stems have the potential to weaken the standing shoots and being involved in the collapse of the culms. Apinis *et al.* (1972b) identified '*Septoria arundinacea*', *Stagonospora elegans* and *Massarina arundinacea* as responsible species for the collapse of standing shoots. Although *S. arundinacea* Sacc. is also found on *P. australis* (but on leaves) (Jorstad, 1967),

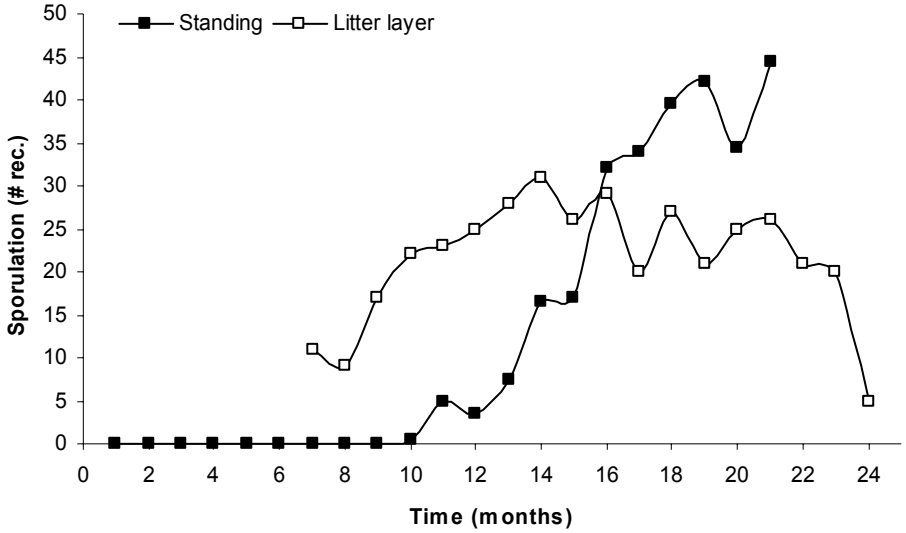


Figure 7. Comparing fungal sporulation on standing stems and litter layer stems of *Phragmites australis*. The standing sporulation records were divided by two as two microhabitats (basal and middle height section) were sampled during the study.

it is thought that Apinis *et al.* (l.c.) meant *Septoriella phragmitis* with it, a species also characterized by multiseptate, long conidia of comparable size and an indicator taxon frequently found on basal stem sections of *P. australis* (Table 5). Weeda *et al.* (1994) mention *Ustilago grandis* Fr. as potential actor in reducing culm strength. However, this species was not found during this study and seems only dominant within restricted patches in the Scheldt estuary. At these locations *U. grandis* could play a role in weakening the middle and upper parts of standing stems because of its vigorous growth and sporulation.

Table 4. The Jaccard similarity (%) matrix, based on presence-absence data of fungal taxa occurring on *Phragmites australis* stems used as a qualitative measurement for β-diversity among the microhabitats.

	Middle	Base	Litter
Middle	0		
Base	38	0	
Litter	37	54	0

Table 5. Indicator taxa with indicator value (IV) on *Phragmites australis* stems identified for the three different subcommunities sampled: middle and basal height stems and litter layer stems.

Middle		**Base**		**Litter**	
Taxa	IV	Taxa	IV	Taxa	IV
Stagonospora vexata	78.4	*Massarina arundinacea*	66.1	*Phomatospora berkeleyi*	54.5
Botryosphaeria festucae	49.6	*Septoriella phragmitis*	62.3	*Halosphaeria hamata*	52.5
		Stagonospora cylindrica	61.1		
		Lophiostoma arundinis	55.8		

Acknowledgements

We are grateful for the funding by the Institute for the Promotion of Innovation by Science and Technology in Flanders, Belgium (to G.V.R.).

References

APINIS, A.E., CHESTERS, C.G.C. & TALIGOOLA, H.K. (1972a). Colonisation of *Phragmites communis* leaves by fungi. Nova Hedwigia 23: 113-124.

APINIS, A.E., CHESTERS, C.G.C. & TALIGOOLA, H.K. (1972b). Microfungi colonizing submerged standing culms of *Phragmites communis* Trin. Nova Hedwigia 23: 473-480.

APINIS, A.E., CHESTERS, C.G.C. & TALIGOOLA, H.K. (1975). Microfungi colonizing nodes and internodes of aerial standing culms of *Phragmites communis* Trin. Nova Hedwigia 26: 495-507.

DIX, N.J. & WEBSTER, J. (1995). Fungal Ecology. Chapman & Hall, London.

GESSNER, M.O. (2000). Breakdown and nutrient dynamics of submerged *Phragmites* shoots in the littoral zone of a temperate hardwater lake. Aquatic Bot. 66: 9-20.

GESSNER, M.O., SCHIEFERSTEIN, B., MÜLLER, U., BARKMANN, S. & LENFERS, U.A. (1996). A partial budget of primary organic carbon flows in the littoral zone of a hardwater lake. Aquatic Bot. 55: 93-105.

GRANÉLI, W. (1990). Standing crop and mineral content of reed, *Phragmites australis* (Cav.) Trin. ex Steud., in Sweden - management of reed stands to maximize harvestable biomass. Folia Geobot. Phytotax. 25: 291-302.

HASLAM, S.M. (1972). Biological flora of the British Isles no. 128, *Phragmites communis* Trin. J. Ecol. 60: 585-610.

JORSTAD, I. (1967). *Septoria* and septorioid fungi on Gramineae in Norway. Skr.Norske Vidensk.-Akad. Oslo, Mat.-Naturvidensk. Kl. Serie 24: 1-63.

KUEHN, K.A. & SUBERKROPP, K. (1998). Diel fluctuations in rates of CO_2 evolution from standing dead leaf litter of the emergent macrophyte *Juncus effusus*. Aquatic Microb. Ecol. 14: 171-182.

KUEHN, K.A., CHURCHILL, P.F. & SUBERKROPP, K. (1998). Osmoregulatory responses of fungi inhibiting standing litter of the freshwater ermergent macrophyte *Juncus efffusus*. Appl. Environm. Microbiol. 64: 607-612.

KUEHN, K.A., STEINER, D. & GESSNER, M.O. (2004). Diel mineralization patterns of standing-dead plant litter: implications for CO_2 flux from wetlands. Ecology 85: 2504-2518.

MCCUNE, B. & MEFFORD, M.J. (1999). PC-ORD for Windows. Multivariate Analysis of Ecological Data. Version 4.0. MjM Software, Gleneden Beach, Oregon, U.S.A.

MEGANCK, B. (1998). The Morphology of *Phragmites australis* (Cav.) Steud. in Relation to Hydrodynamics, Management and Salinity in the Scheldt Estuary. Unpublished master thesis. Ghent University, Ghent, Belgium. [in Dutch]

MITSCH, W.J. & GOSSELINK, J.G. (2000). Wetlands. 3rd edn. Van Nostrand Reinhold, New York, U.S.A.

NEWELL, S.Y., ARSUFFI, T.L. & PALM, L.A. (1996). Misting and nitrogen fertilization of shoots of a saltmarsh grass: effects upon fungal decay of leaf blades. Oecologia 108: 495-502.

NEWELL, S.Y., FALLON, R.D., RODRIGUEZ, R.M.C. & GROENE, L.C. (1985). Influence of rain, tidal wetting and relative humidity on release of carbon dioxide by standing-dead salt-marsh plants. Oecologia 68: 73-79.

PIECZYŃSKA, E. (1972). Ecology of the eulittoral zone of lakes. Ecol. Polska 20: 637-732.

POON, M.O.K. & HYDE, K.D. (1998). Evidence for the vertical distribution of saprophytic fungi on senescent *Phragmites australis* culms at Mai Po marshes. Bot. Mar. 41: 285-292.

RODEWALD, L. & RUDESCU, L. (1974). Das Schilfrohr. Die Binnengewässer Band 27: 1-302.

SUTTON, B.C. (1980). The Coelomycetes. Commonwealth Mycological Institute, England.

VAN RYCKEGEM, G. & VERBEKEN, A. (2005a). Fungal diversity and community structure on *Phragmites australis* (Poaceae) along a salinity gradient in the Scheldt estuary (Belgium). Nova Hedwigia 80 (In press).

VAN RYCKEGEM, G. & VERBEKEN, A. (2005b). Fungal ecology and succession on *Phragmites australis* in a brackish tidal marsh. I. Leaf sheaths. Fungal Div. 19 (in print).

WEEDA, E.J., WESTRA, R., WESTRA, CH. & WESTRA, T. (Eds.) (1994). Dutch Ecological Flora, Plants and their Relations. Vol. 5. CIP-gegevens Koninklijke Bibliotheek, Den Haag, The Netherlands. [in Dutch]

WONG, M.K.M. & HYDE, K.D. (2001). Diversity of fungi on six species of Gramineae and one species of Cyperaceae in Hong Kong. Mycol. Res. 105: 1485-1491.

ZHOU, D. & HYDE, K.D. (2002). Fungal succession on bamboo in Hong Kong. Fungal Div. 10: 213-227.

Fungi on leaf blades of *Phragmites australis* in a brackish tidal marsh: diversity, succession and leaf decomposition

Van Ryckegem, G., Gessner, M.O*. & Verbeken, A.

Manuscript in preparation, target Mycologia

Abstract Fungi are well known to colonize and decompose plant tissues in various environments, but information on fungal communities on wetland plants, their relation to microhabitat conditions and link to decomposition processes is scant. We examined fungal diversity and succession on leaf blades of *Phragmites australis* naturally attached to standing shoots and on leaf blades decaying in the litter layer of a brackish tidal marsh. Additionally, we determined fungal biomass (ergosterol content), leaf nitrogen dynamics and litter mass loss on the sediment surface of the marsh. Thirty-five fungal taxa were recorded by direct observation of sporulation structures. Cluster analysis revealed distinct species compositions in the three different microhabitats examined (middle canopy, top canopy and litter layer), and Indicator Species Analysis identified a total of seven taxa characteristic of the identified subcommunities. High fungal biomass developed in decaying leaf blades attached to standing shoots, with a maximum ergosterol concentration of 548 ± 83 μg g^{-1} ash-free dry mass (AFDM; mean ± SD). However, when dead leaves were incorporated in the litter layer on the sediment surface, fungi experienced a sharp decline in fungal biomass (to 191 ± 60 μg ergosterol g^{-1} AFDM) and other species sporulated. Probably there was a mass extinction that involved species replacement. Leaves placed in litter bags on the sediment surface lost 50% of their initial AFDM within 7 months (k = -0.0035 day^{-1}) and only 21% of the original AFDM was left after 11 months. Fungal biomass accounted for up to 34 ± 7% of the total nitrogen in dead leaf blades attached to shoots but to only 12% in the litter layer. These data suggest that fungi are instrumental in N retention and leaf mass loss during leaf senescence and early aerial decay but that their importance particularly in N retention diminishes during decomposition on the marsh surface.

Introduction

Emergent macrophytes such as *Phragmites australis* (Cav.) Trin. ex Steud. are often quoted for their high production, the resultant large standing-dead shoot biomass, and their critical role in wetland carbon and nutrient dynamics (Mitsch & Gosselink, 2000). In addition, these plants can be hotspots of biodiversity as they serve as shelter, food or substrate for a wide range of organisms (Tscharntke, 1992; Gessner & Van Ryckegem, 2003). Increasing evidence suggests that fungi are the primary microbial colonizers of standing-dead emergent macrophytes,

* This co-author did not see the last version of this manuscript

including *P. australis* (Kuehn et al., 2000; Newell & Porter, 2000; Gessner, 2001; Findlay et al., 2002), and may show a remarkable diversity (Apinis et al., 1972; Gessner, 1977; Kohlmeyer & Volkmann-Kohlmeyer, 2001; Wirsel et al., 2001; Buchan et al., 2002; Van Ryckegem & Verbeken, 2005). However, information on the relations between fungal communities on emergent macrophytes and ecosystem processes in wetlands is remarkably scarce.

Anatomical and chemical characteristics of *P. australis* leaves change with shoot height, resulting in habitat conditions for fungi that vary with vertical position in the canopy. In upper, sun-exposed leaves, the number of stomata is greater (Willmer & Fricker, 1996) and the cuticle and epidermal cell walls are generally thicker (Webster, 1956). Phytoliths are also more numerous in upper leaves (Deleebeeck, 2000) where they may hamper fungal penetration of leaf surfaces (Samuels et al., 1991). Likewise, microclimatological conditions such as temperature and humidity vary with shoot height and thus may be important in influencing fungal colonization and growth in different layers of the plant canopy (e.g. Cooke & Rayner, 1984; Kuehn et al., 2004). The lower and middle canopy are typically more sheltered, resulting in higher temperature and humidity favourable to fungal development (Rodewald & Rudescu, 1974). Dewset – a key wetting phenomenon in standing decay (Newell, 2001b; Kuehn et al., 2004) – would appear to be highest at the upper, other edges of the canopy. However, this dew filter is probably soon evaporated when sun or wind exposed and favourable dew deposition could stay longer in the middle or lower half of the canopy. This difference would appear more important in warmer climate compared to more temperate regions. Therefore, the leaves lower in the canopy would appear to provide a more benign microhabitat for fungi and this could be reflected in greater fungal diversity, biomass and reproduction than in upper canopy layers.

The natural decay pattern of *P. australis* leaves and other emergent macrophytes is characterized by an initial decay phase with leaves remaining attached to standing shoots and subsequent decomposition on the sediment or soil surface (e.g. Gessner, 2000, 2001; Kuehn et al., 2000). Initial decay of such attached leaves proceeds sequentially from the shoot base to its tip, following the gradual senescence and death of leaves at different vertical canopy positions. This process may take several weeks to months (Pieczyńska, 1972; Gessner, 2001). During this period, leaves are colonized by microbial decomposers, most notably fungi (Apinis et al., 1972; Kuehn & Suberkropp, 1998a; Van Ryckegem & Verbeken, unpublished data), and may experience a significant mass loss (up to 28%) that is partly mediated by microbial decomposers (Gessner, 2001; Kuehn et al., 2004). Those canopy fungi could possibly alter elemental content of *P. australis* plant parts (Engloner et al., 2000). Fungi in this habitat are highly adapted to the standing decay environment characterized by periodical wetting and drying (Newell et al., 1985; Kuehn & Suberkropp, 1998b; Kuehn et al., 2004). However, important changes in fungal species composition have been noticed when leaves are eventually dropped, exposing the decomposers to radically different environmental conditions (Tanaka, 1991; Van Ryckegem & Verbeken, 2005; Van Ryckegem, unpublished data).

Litter decomposition of emergent wetland plants and nutrient dynamics of decomposing litter have been studied intensively (e.g. Polunin, 1982, 1984; Hietz, 1992,

Gessner, 2000; for *P. australis*), with some data available also on fungal biomass dynamics (Komínková et al., 2000; Findlay et al., 2002). Information is scarce, however, on species composition and dynamics of fungal communities in the litter layer of marshes, although such data may provide clues to the striking changes in fungal biomass as observed in the rush, *Juncus effusus*, when leaves moved from the standing decay phase to the litter layer on the sediment (Kuehn et al., 2000). Similar observations have been made for *P. australis* (M.O. Gessner, unpublished data).

Table 1. Selected characteristics of the investigated tidal marsh.

Variable	Mean ± SD	*N*
Tidal marsh		
Flooding frequency (%)[$]	15	
Average flood height (cm)[£]	17	
Sedimentation rate ($mm.yr^{-1}$)[!]	34	
Water chemistry[°]		
pH	7.59 ± 0.23	11
Cl^- (mg/l)	2455 ± 1510	19
NH_4^+-N (mg/l)	0.46 ± 0.35	21
NO_2^-N (mg/l)	0.06 ± 0.03	21
NO_3^-N (mg/l)	4.9 ± 0.65	21
Total P (mg/l)	0.73 ± 039	21
Reed stand		
Shoot height (cm)	196 ± 21	20
Stem diameter at first internode (mm)[£]	4.0 ± 0.2	60
Density of living shoots (m^{-2})[*]	191 ± 65	6
Above-ground biomass ($g\ m^{-2}$)[*]	927 ± 293	6
Leaf biomass ($g\ m^{-2}$)[*]	320 ± 131	6

[$] Flooding frequency is the relative number of times high tides are higher than the marsh surface and are thus supposed to flood the marsh (Meganck, 1998).

[£] From Meganck (1998).

[!] Calculated as: 157 x (flooding frequency) + 10, $r^2 = 0.52$, $P < 0.001$ (Van Damme et al. 1999).

[°] Annual average in 2002 (Flemish Environment Agency, Belgium, site code 154100, http://www2.vmm.be).

[*] Estimated at end of the growing season (10 September 1997) by harvesting all above-ground living reed matter in six 0.25 m^2 quadrats; biomass ≈ annual above-ground net production (Meganck, 1998).

This study aimed to elucidate fungal community structure and succession both on leaf blades naturally attached to standing shoots and on fallen leaves decaying in the litter layer of a brackish tidal marsh. In addition, mass loss and nitrogen dynamics of decaying leaf blades in the litter layer were determined and the involvement of fungi assessed by estimating fungal biomass and N content contained in fungal biomass.

Study site, materials and methods

The study was carried out in a brackish tidal marsh of the Scheldt Estuary located 53.9 km inland near Doel, The Netherlands (51° 21' N, 4° 14' E) (Table 1). Vegetation of the 50 ha marsh mainly consists of monospecific stands of *P. australis* with fringes of *Scirpus maritimus* L. near the river. In our site, *Phragmites* growth starts at the end of April and ends in August. As shoots grow taller and form new leaves at the tip, the leaves lower in the canopy start to senesce and to be dropped in late June or early July. Most leaves, however, do not senesce until September; they typically fall 4-6 weeks later, with some of the uppermost leaves remaining attached to shoots even during winter.

Three adjacent plots (3 x 2 m) were established in an area of the tidal marsh about 50 m from the landward margin and 60 m from the river (Table 1). Two plots (P1 and P2) were fenced (1.4 m high, 1 cm mesh) to prevent input to the litter layer of reed leaves of unknown age and origin. To follow fungal sporulation over time, plant litter was collected every 4 weeks for 15 months, from May 2000 till July 2001 in P1 (hereafter referred to as Series 1) and from May 2001 till July 2002 in P2 (Series 2) (Table 2). Ten leaves were randomly collected from both the middle and top canopy (30 cm sections) of standing shoots as long as attached leaves remained. In addition, leaves were collected from the litter layer starting in the last week of November 2000 (P1) and 2001 (P2) (Table 2). To ensure that leaves periodically collected in the litter layer were from the same growing season, leaf blades were also exposed on the marsh surface in litter bags. Ten leaf blades collected from the litter layer were each enclosed in a total of 12 litter bags (35 x 20 cm, 4 mm mesh) and later periodically retrieved for 9 months in each plot. All samples were placed in clean plastic bags in the field and brought immediately to the laboratory, where they were kept at 4 °C and screened within 2 weeks under a dissecting microscope (180x) for mature fungal sporulation structures. All fungi were microscopically identified and original descriptions and illustrations of all taxa are available at http://biology.ugent.be/reedfungi. This procedure resulted in a relative frequency of occurrence of fungal taxa on a scale of 10 for each sample (= 10 leaf blades).

On 4 October 2001, fully brown leaves were collected from standing reed shoots in the third, unfenced plot (P3). Leaves were removed from middle height in the plant canopy, about 1 m above ground. Only leaves easy to detach from shoots were taken. Samples were placed in a cool box, transported to the laboratory and used to fill 38 additional litter bags with 5.0 g fresh mass. The next morning litter bags were placed in the marsh in P3, which had been mown and from which all litter had been removed. Litter bags were positioned flat on the sediment surface and secured with hooked bars to prevent bags from being lifted by growing reed shoots in the next season and from being washed away during high tides. Ten litterbags were immediately retrieved, returned to the laboratory and the leaves dried for 3 days at 40°C to determine initial leaf dry mass (4.7 ± 0.09 g; mean ± SD). Two litter bags were retrieved from the marsh at monthly intervals over a period of 12 months until leaves were very fragile and almost completely decomposed. Samples were immediately transported to the laboratory

in a cool box and processed the same day. They were rinsed with distilled water as thoroughly as possible without fragmenting leaves, to remove adhering clay and macro-invertebrates. One leaf or a few fragments from each litter bag were randomly chosen, blotted dry, weighed wet, freeze-dried, ground (2 mm mesh) and kept in methanol-KOH (8 g l^{-1}) for less than 1 month. These samples were used to measure fungal biomass. The remaining leaves were dried at 40°C for 72 h to determine dry mass remaining; the estimated dry mass of the material used to measure fungal biomass was added to determine total dry mass remaining.

All remaining leaf material was ground to pass a 2-mm mesh and 5-8 mg was used to determine carbon and nitrogen content with a Carlo-Erba NA-1500 elemental analyser. Nitrogen concentration in decomposing litter was corrected for the associated sediment, which had a lower nitrogen concentration than the leaf material (average N = 0.5%; Van Damme et al., 1999; Flemish Environment Agency (http: www2.vmm.be)), based on an assumed 10% ash content of plant origin, as in the leaf blades in the canopy (Blair, 1988; Hunt et al., 1999). Ash content was determined by combustion of 250 mg samples for 4 hrs at 550 °C.

Fungal biomass in leaf material was estimated by determining ergosterol concentration in freeze-dried samples by means of solid-phase extraction and high performance liquid chromatography (HPLC) with detection of ergosterol by UV absorbance at 282 nm (Gessner & Schmitt, 1996). The chromatographic systems consisted of a Kratos Analytical Instruments Spectroflow 400 with a Spectroflow 757 UV detector. The system was run isocratically with 100% methanol at a flow rate of 1.5 ml/min and an RP18 LiChrosphere column (Gessner & Schmitt, 1996). Column temperature was maintained at 30°C. Peak area was quantified with an Applied Biosystems Model 610A data analysis system for protein sequencing, version 1.2.2. Conversion factors for calculating fungal biomass and % fungal N were 5.8 mg ergosterol per g fungal dry mass (Findlay et al., 2002), and 65 mg N per g fungal dry mass, as determined for fungal strains isolated from *P. australis* (Findlay et al., 2002).

Litter mass loss data were fitted to the simple exponential model $m_t = m_o . e^{-kt}$, where m_t is litter ash- free dry mass (AFDM) remaining after time t, m_o is the original AFDM and k the decay coefficient. Data were not transformed and non-linear regression analysis was used to estimate parameters using the default settings in SYSTAT, version 10.2. Pearson correlation analysis was used to relate fungal biomass data with relative occurrence of sporulation records and with nitrogen concentrations during decomposition.

The following indices were calculated to assess the importance of fungal taxa in subcommunities (Table 3): (1) *relative abundance* of taxon X_m compared to other taxa on middle and upper canopy leaves and in the litter layer during the entire study = $\frac{\sum records\ of\ taxon\ X_m}{\sum records\ of\ all\ taxa_m} \cdot 100$; (2) *recurrence* of taxon X_m as a measure of the 'preferred' microhabitat during the entire study = $\frac{\sum records\ of\ taxon\ X_m}{\sum records\ of\ taxon\ X} \cdot 100$; and (3) *frequency of occurrence*

at a given sampling date t of taxon $X_{m,t} = \frac{\sum \textit{records of taxon } X_{m,t}}{\textit{number of leaves examined at } t} \cdot 100$. The subscript m = the microhabitat (i.e. top or middle height of standing shoots or litter layer) where a taxon was collected and t = time in months. All indices are expressed in per cent.

Species diversity was calculated as Shannon's diversity index: $H' = -\sum_{i}^{S} p_i \ln p_i$, where S = number of species in the community, $p_i = n_i/N$ with n_i = number of records of species i and N = total number of records. Similarities between communities were assessed with Jaccard's index: $S_j = a/(a+b+c)$, where a = number of species shared between two groups, b = number of species restricted to the first group, and c = number of species restricted to the second group. Sørensen's (Bray-Curtis') quantitative index was used for comparisons based on species' relative occurrences: $S_s = 2w/(x + y)$, where w is the sum of shared abundances and $x + y$ is the sum of abundances in the individual groups. Diversity and similarity indices were calculated using PC-ORD version 4.26 (McCune & Mefford, 1999).

Cluster analysis (CA) was used to identify groupings of samples based on similarity in fungal species composition. The data matrix was untransformed and consisted of 37 samples. Four samples from May and three samples without fungal records were excluded (P2-02 and P2-03 from the upper canopy and P2-15 from the late litter layer). The Bray-Curtis distance measure and group average linkage method were used. The presented dendrogram was scaled by the percentage of information remaining (McCune & Mefford, 1999; McCune & Grace, 2002). Clusters were tested for significance with the multi-response permutation procedure (MRPP), which tests the null hypothesis that there is no difference among identified groups (Biondini et al., 1988). Euclidean distance was used and groups were weighted by $n \cdot [\text{sum}(n)]^{-1}$. P-values were Bonferroni corrected (Rice, 1990).

Indicator Species Analysis (ISA) was used to discern which species were driving differences among groups identified by cluster analysis (Dufrêne & Legendre, 1997). The Indicator Value (IV) for a species was determined by combining relative frequency and relative abundance in a given group; it can range from 0 (no indication) to 100 (perfect indication, meaning the species was present in all samples in a given group and absent from all samples in all other groups). Following Dufrêne & Legendre (1997), a IV threshold level of 25% was arbitrarily chosen, and a Monte Carlo simulation (1000 runs) was used to determine significance ($P \leq 0.01$) of species IVs. CA, MRPP and ISA were performed with PC-ORD version 4.26 (McCune & Mefford, 1999).

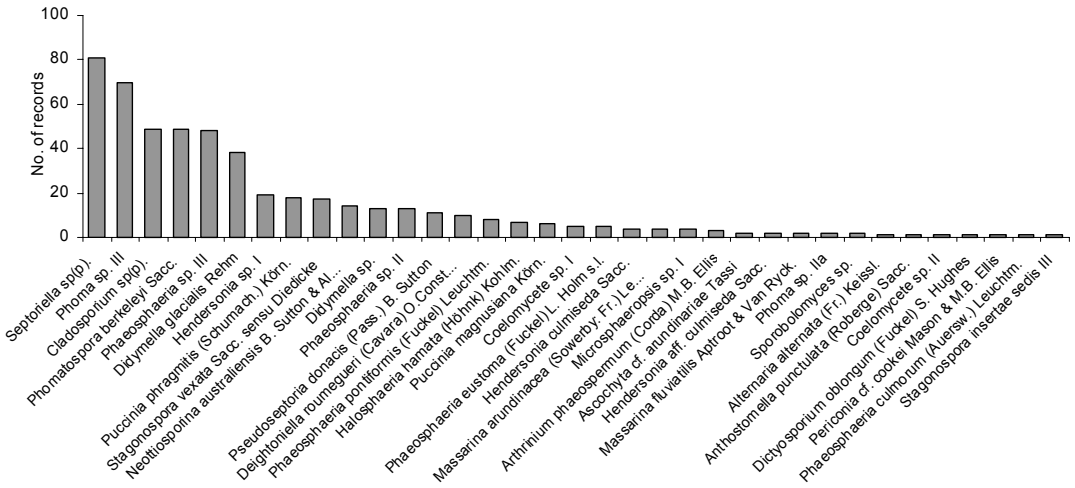


Figure 1. Species frequency distribution of the 35 fungal taxa recorded on living and decomposing leaves of *P. australis* attached to shoots and in the litter layer of a brackish tidal marsh.

Results

Fungal diversity and succession

A total of 35 fungal taxa was recorded on living green and decaying leaf blades in Series 1 (P1) and Series 2 (P2) (Fig. 1). Fourteen taxa (40%) were coelomycetes, twelve (34%) ascomycetes, six (17%) hyphomycetes and only three (9%) were basidiomycetes. Of all 513 fungal records, 45% (233) were coelomycetes, 37% (189) ascomycetes, 13% (65) hyphomycetes and only 5% (26) basidiomycetes. Based on species abundance data, fungal communities were highly similar in the two plots (Bray-Curtis similarity of 76.1%), as was the total number of records (268 versus 245), species richness (29 versus 26) and Shannon diversity (2.72 versus 2.74). Only two parasitic species showed strongly different frequencies between Series 1 and 2: *Puccinia phragmitis* was found only in Series 1 (18 records in P1) and *Deightoniella roumegueri* was only scored in Series 2 (9 records in P2).

The three microhabitats examined (top canopy, middle canopy and litter layer) showed distinct species richness and a different pattern in the number of sporulation structures calculated per sample (Fig. 2). The highest number of taxa was recorded from the litter layer while the highest number of sporulation structures was recorded in the middle canopy.

Cluster analysis revealed different species compositions in the three microhabitats. A total of four subcommunities were distinguished: a top and middle canopy community on leaves attached to standing shoots and an early and late successional fungal community in the litter layer (Fig. 3).

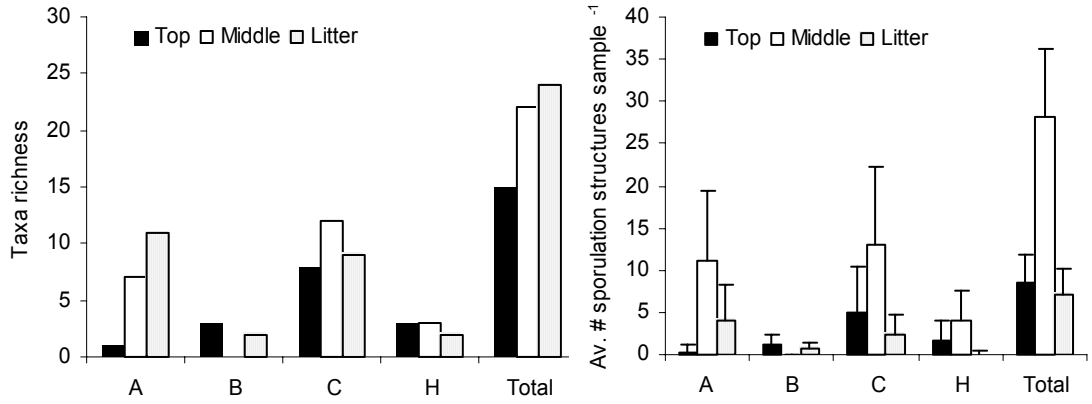


Figure 2. Fungal species richness and average number of sporulation structures within the main pseudo-systematic groups (A = ascomycetes, B = basidiomycetes, C = coelomycetes and H = hyphomycetes) on *P. australis* leaf blades in three microhabitats of a brackish tidal marsh. Error bars indicate mean ± STDEV.

Leaf blade samples categorized as early litter layer leaf blades (EL) were less than 3 months in the litter layer and this period is characterized as a transition phase (see below). Pairwise comparisons (MRPP) of these subcommunities showed highly significant differences ($P < 0.05$ after Bonferroni correction). One sample from the upper canopy (P1-06) and two initial samples from the litter layer (P1-07 and P2-07) were recognized as outliers; P1-06 mainly due to its low species richness (only three species recorded) and the early litter samples due to a high richness with low frequencies of individual taxa. These samples were excluded from the MRPP. Five taxa were identified by Indicator Species Analysis as indicative of communities on middle-canopy leaves and one taxon each of the upper canopy and late litter communities (Table 4). No specific indicators were found for the early litter samples, even if tolerance for significance was increased to $P < 0.05$.

The successional sequence of fungal sporulation structures differed among microhabitats both in composition and timing. However, communities representing different successional phases were distinguishable in all three microhabitats (Table 2). In the canopy, Phase I is characterized by an initial pioneer community with low diversity, followed by a gradually developing mature community (Phase II) with higher diversity (Fig. 4). In the litter layer, the initial stage is a transition phase (Phase X, Table 2) in which terrestrial taxa are being lost and estuarine species become established (Fig. 3). A mature community (Phase II) with maximum diversity was not well defined in the litter layer, although sporulation frequency and taxa richness transiently increased in this microhabitat (Fig. 5) before an impoverished community with few dominant species was established (Phase III) (Fig. 4; Tables 2 and 3).

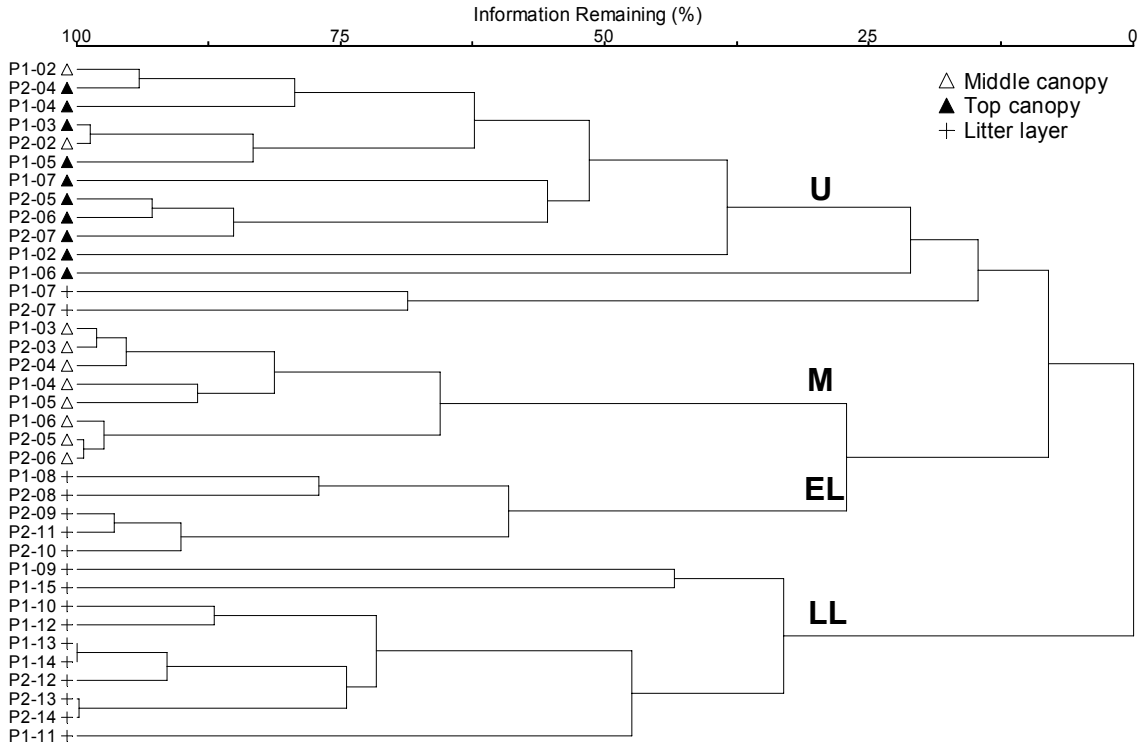


Figure 3. Cluster analysis of fungal communities in 37 leaf blade samples from a brackish tidal marsh dominated by the emergent macrophyte, *P. australis*. Bold letters U (upper canopy samples), M (middle canopy samples), EL (early litter samples) and LL (late litter samples) designate the major fungal subcommunities recognized. Symbols indicate the microhabitat from where samples originated. The first part of the sample code indicates the sampling plot: P1 or P2. Leaf blades were screened for fungal taxa starting from May 2000 (P1) and from May 2001 (P2). The number stands for the collection month (1-15) with May = 1 (see Table 2).

Litterbag experiment

Leaves in the litter layer were fully covered by mud after spring tides (pers. obs.), and although part of the deposits were washed off during normal tides or rain, ash content of the decomposing leaves varied markedly (9-51 % of dry mass) over time (data not shown). Leaves lost about 50 % of their initial ash-free dry mass (AFDM) in 7 months (Fig. 6), corresponding to an exponential decay coefficient, k, of -0.0035 ± 0.0002 per day (mean ± asymptotic standard error (ASE); corrected r^2 = 0.93, N = 27, estimated m_o = 96.8 ± 2.4 (mean % ± ASE)). Only 21 % of the original leaf AFDM remained after 11 months.

The C:N ratio of leaf litter decreased slightly from initially 24.8 to 16.6 in July 2002 (Fig. 7), due to slight changes in both C and N concentration (Oct. 2001: 46.19 ± 0.22 C, 1.86 ± 0.08 N; July 2002: 33.70 ± 0.69 C, 2.03 ± 0.16 N (mean % ± STDEV). The lower than initial N contents per leaf pack (N stocks), implies there may have been net N mineralization within the last three months.

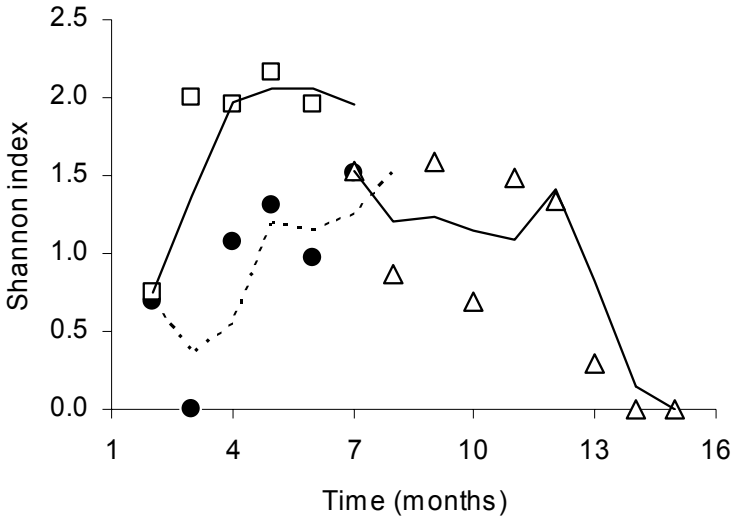
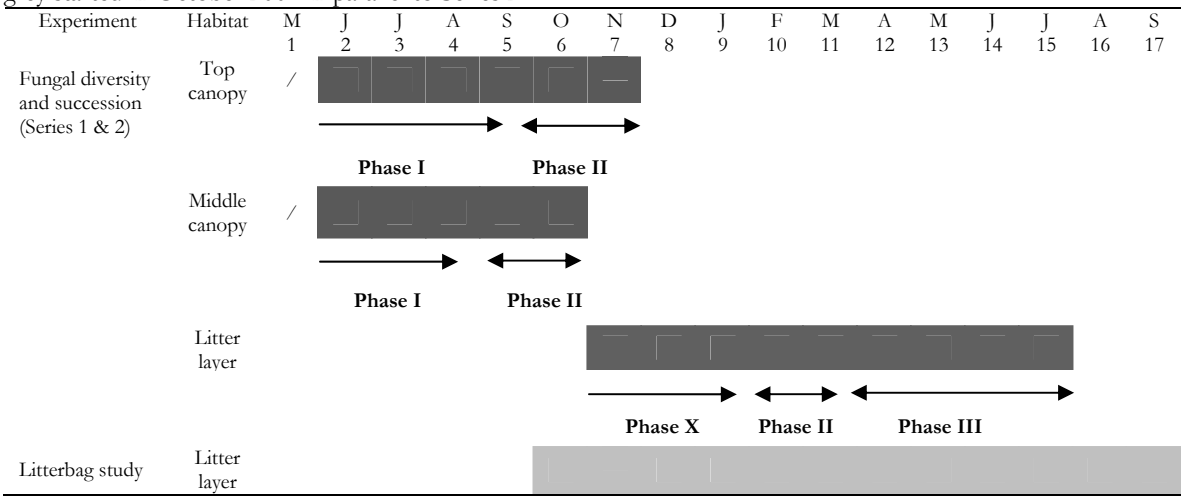


Figure 4. Shannon diversity indices of fungal subcommunities on leaf blades of *P. australis* in different microhabitats: middle canopy leaf blades (□), top canopy leaf blades (●), and leaf blades in the litter layer (△). Lines are moving averages with a period of two. Month 1 refers to May, when no fungi where recorded during either survey year (2000/2001 and 2001/2002).

Fungal biomass was high in fully brown leaves collected at middle height from standing *P. australis* shoots just before leaves were naturally dropped. The ergosterol concentration of 548 ± 83 μg g^{-1} AFDM (mean ± SD, N = 10) in those leaves corresponds to a fungal biomass of 9.8 ± 1.6% and accounts for 34.4 ± 6.6% of the total N in the leaf material (mean ± SD, N = 4). Following placement of this leaf material on the marsh surface, fungal biomass declined by more than 50% within two months; it recovered slightly during the following months before declining and later increasing again (Fig. 8). During the first eight months of litter decomposition on the marsh surface, fungal biomass in litter was positively correlated (Pearson correlation, r = 0.80, P < 0.01, N = 8) with the sum of the relative occurrence of sporulation structures (Fig. 8). However, at the end of the study when only 21% of the initial AFDM remained, fungal biomass was still sizeable (127 ± 8 μg ergosterol per g AFDM, N = 3) but no sporulation structures were detected.

Table 2. Dataset structure for two fungal succession series and a decomposition study in litter bags. A total of 44 samples was investigated in Series 1 (started in May 2000) and Series 2 (started in May 2001), both indicated in dark grey. Samples taken in May revealed no fungal sporulation in either year. The litterbag study indicated in light grey started in October 2001 in parallel to Series 2.



Discussion

Fungal diversity and community composition on leaf blades

The number of fungal taxa we observed on *P. australis* leaf blades on leaf blades attached to standing *P. australis* shoots (29 taxa) is comparable with the richness noted by Apinis et al. (1972) at their best studied site, Attenborough in southern England, where 27 taxa were recorded on aerial leaf blades. Although the two sites are geographically close, they had less than one third of the taxa in common. This rather low degree of similarity may to some extent reflect methodological differences between studies. Part of the records by Apinis et al. (1972) was from leaf discs placed on agar plates, an indirect cultivation method favouring the development of hyphomycetes that grow rapidly on laboratory media (Hudson & Webster, 1958; Fell & Hunter, 1979). In contrast, our direct observation of sporulation structures was likely to reveal a greater proportion of slowly developing (teleomorphic) taxa, which may have been more active in the field. Storage of our samples at 4°C for up two weeks did not bias our results, as suggested by very similar community structure on ten leaves screened both immediately after collection and again two weeks later.

In addition to possible methodological differences, the dissimilarity in fungal community composition between studies is likely to be related to differences in environmental conditions and/or plant characteristics. For example, even in a single study, large differences in community composition have been observed at 4 sites along a salinity gradient: Only 13% of the taxa in the litter layer were in common among all sites in that study and a Jaccard similarity index between 0-40% was calculated for any two sites in that study, (Van Ryckegem & Verbeken, 2005; their Table 3).

Table 3. Number of records, relative abundance (ab.), percentage recurrence (rec.) and percentage occurrence of fungal taxa recorded in different months on leaf blades of *P. australis* in different microhabitats (top and middle canopy and litter layer). Data from 2000 and 2001 are pooled, which resulted in 20 subsamples (leaf blades) per calendar month per microhabitat. Only the 23 taxa with more than 2 records during the entire study are shown. Fungal sporulation started in June. Samples were taken in May but no sporulation was observed. G = major fungal group: A = ascomycetes, B = basidiomycetes, C = coelomycetes, H = hyphomycetes.

Taxa	G	Micro-habitat	Σ records	% ab.	% rec.	% occurrence (months)													
						J	J	A	S	O	N	D	J	F	M	A	M	J	J
Septoriella sp(p).	C	top	38	40	47	5	15	35	35	25	75								
		middle	39	14	48	55	75	35	15	15									
		litter	4	3	5						10	0	5	0	5				
Cladosporium sp(p).	H	top	12	13	24	5	0	45	5	0	5								
		middle	37	13	76	25	80	30	30	20									
Phaeosphaeria sp. III	A	middle	47	17	98	10	85	75	45	20									
		litter	1	1	2										5				
Phoma sp. III	C	middle	41	15	59		45	35	65	60									
		litter	29	23	41						5	20	25	40	45	10			
Didymella sp.	A	middle	13	5	100		25	25	10	5									
Neottiosporina australiensis	C	top	1	1	7						5								
		middle	11	4	79		20	5	20	10									
		litter	2	2	14						10								
Hendersonia sp. I	C	top	7	7	37				15	0	20								
		middle	11	4	58		15	5	30	5									
		litter	1	1	5							5							
Arthrinium phaeospermum	H	middle	3	1	100		15												
Phaeosphaeria sp. II	A	middle	11	4	85		10	15	30										
		litter	2	2	15						10								
Hendersonia culmiseda	C	middle	2	1	50		10												
		litter	2	2	50								5	0	5				
Phaeosphaeria pontiformis	A	middle	6	2	75		5	20	5										
		litter	2	2	25										10				
Pseudoseptoria donacis	C	top	10	10	91					10	40								
		middle	1	0	9		5												
Didymella glacialis	A	top	4	4	11				10	10									
		middle	31	11	82			25	50	80									
		litter	3	2	8										5	10			
Puccinia phragmitis	B	top	11	11	61			10	5	25	15								
		litter	7	6	39							10	5	5	0	10	5		
Stagonospora vexata	C	top	2	2	12						10								
		middle	13	5	76				20	45									
		litter	2	2	12						5	5							
Deightoniella roumegueri	H	top	9	9	90				10	10	25								
		litter	1	1	10						5								
Coelomycete sp. I	C	middle	4	1	80				10	10									
		litter	1	1	20								5						
Massarina arundinacea	A	middle	2	1	50				10										
		litter	2	2	50										5	5			
Microsphaeropsis sp. I	C	middle	4	1	100				5	15									
Puccinia magnusiana	B	top	2	2	33						10								
		litter	4	3	67						20								
Phomatospora berkeleyi	A	middle	0	0	0														
		litter	49	40	100						5	0	10	20	15	60	65	65	5
Phaeosphaeria eustoma	A	litter	5	4	100									5	0	20			
Halosphaeria hamata	A	litter	7	6	100										10	20	5		

Significantly, only two of the dominant genera involved in the decay of *P. australis* leaf blades in the canopy at our study site (*Cladosporium* and *Phaeosphaeria*) were also recorded by Apinis et al. (1972).

Vertical variation

Results of the present study support the contention that middle-canopy leaves are a more benign habitat for fungi than the more exposed, tougher leaves in the upper shoot regions. As hypothesised, both species diversity and relative frequency of sporulation were indeed higher

Table 4. Indicator Values and Monte Carlo *P*-values for species with Indicator Values > 25 and Monte Carlo *P*-values < 0.01, for three of the four distinct fungal subcommunities identified by cluster analysis (Fig. 3). No particular taxa were associated with the early litter layer community.

Indicator Taxon	Fungal Subcommunity	Indicator Value	*P*
Didymella sp.	Middle height canopy	87.5	0.001
Phaeosphaeria sp. III	Middle height canopy	82.8	0.001
Cladosporium sp(p).	Middle height canopy	65.7	0.002
Neottiosporina australiensis B. Sutton & Alcorn	Middle height canopy	64.7	0.001
Didymella glacialis Rehm	Middle height canopy	55.3	0.005
Phomatospora berkeleyi Sacc.	Late litter layer	94.5	0.001
Septoriella sp(p).	Top height canopy	48.3	0.008

at middle-canopy height (Fig. 1) and sexual stages of ascomycetes were virtually restricted to this region. The same pattern has also been found on leaf sheaths (Van Ryckegem & Verbeken, unpublished data) and is consistent with observations on both fungal communities (Van Ryckegem & Verbeken, 2005; unpublished data) and fungal biomass (Gessner, 2001; Kuehn et al., 2004) in other marshes.

Most fungal taxa appear to be restricted to either the middle or upper canopy (Table 3, Fig. 3). This tendency, which was also noted by Apinis et al. (1972) and Van Ryckegem & Verbeken (2005), results in two distinct fungal subcommunities within the plant canopy. Such distinct vertical distribution patterns of fungi may be explained by vertical differences in habitat conditions (Petrini et al., 1992; Van Ryckegem & Verbeken, unpublished data), since both leaf tissue quality and environmental conditions change from the shoot base to its tip (see Introduction). The time lag in leaf senescence of the upper shoot region (Gessner, 2001), which was also noted in the present study, was apparently unimportant in structuring fungal communities, because only a few taxa (e.g. *Septoriella*) were found to first colonize leaves in the middle and later in the upper canopy as leaf senescence and death proceeded from the base toward the tips of shoots.

Fungal succession

Growth, senescence and decomposition of leaves on *P. australis* shoots is a continuous process (Granéli, 1990), which is accompanied by changes in fungal taxa composition (Fig. 5, Table 2, 3). In the tidal marsh we studied, these temporal changes are reflected in both species diversity and relative frequency of occurrence of the colonizing taxa in leaves attached to shoots at all stages of growth, senescence and decomposition (Figs. 4, 5, Table 3). However, the temporal sequence of fungi on attached leaves suggests only gradual changes in the relative importance of different taxa rather than a true succession involving species replacement (cf. Gessner et al., 1993; Frankland, 1998).

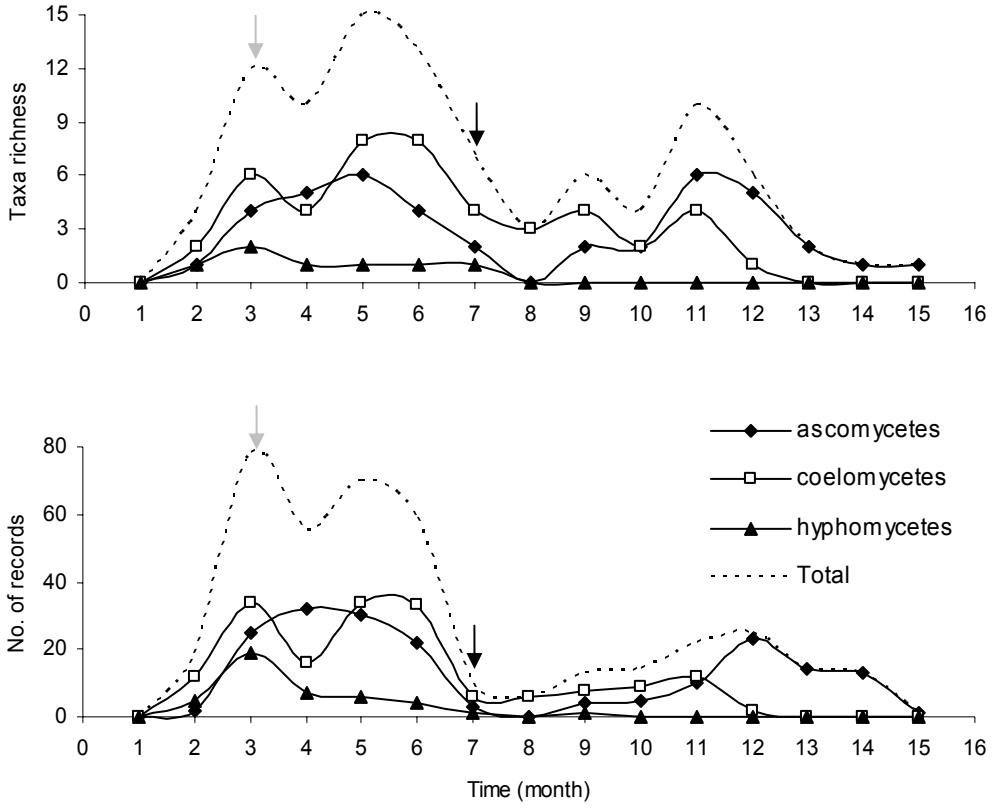


Figure 5. Fungal species richness and relative occurrence of sporulation structures during growth, senescence and decay of *P. australis* leaf blades at middle canopy height and in the litter layer. Single records of taxa were excluded. Grey arrows indicate leaf senescence and black arrows the first collection of leaf blades after one month of decomposition in the litter layer.

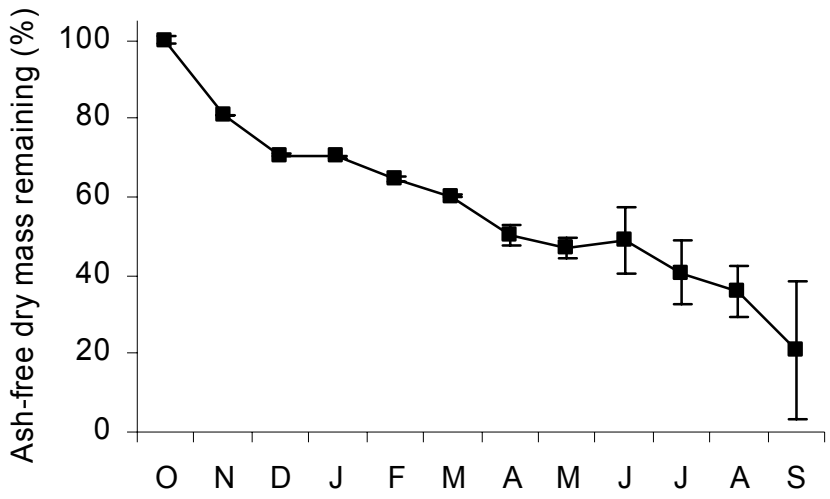


Figure 6. Mass loss of *P. australis* leaf blades in the litter layer of a brackish tidal marsh.

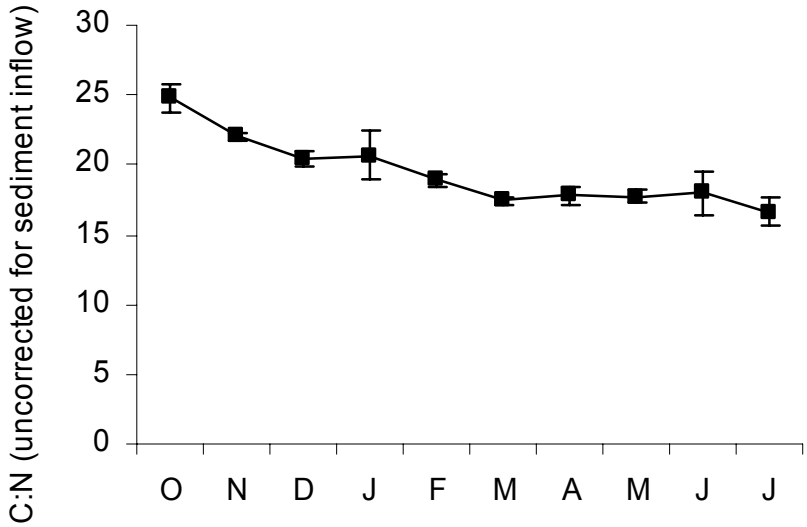


Figure 7. Changes in the proportion of C to N in *P. australis* leaf blades during decomposition in litter bags placed on the sediment surface of a brackish tidal marsh. Error bars indicate standard deviations ($N = 4$).

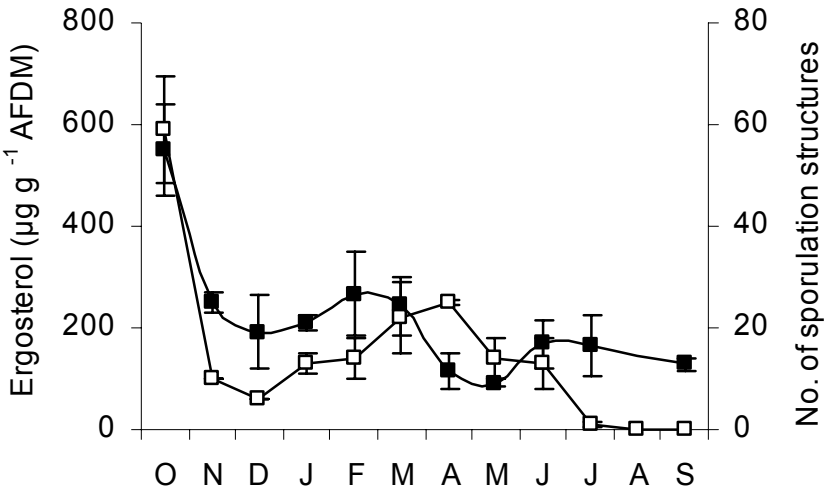


Figure 8. Dynamics of fungal biomass as ergosterol and of fungal sporulation structures recorded on leaf blades of *P. australis* from litter bags placed on the sediment of a brackish tidal marsh. Error bars indicate ±1 SD with $N = 4$, except for June, July and September where $N = 3$ for ergosterol and $N = 2$ for sporulation structures. Ergosterol (■); number of sporulation structures recorded (□).

Once leaves fall on the sediment, however, true successional events with species replacements occur (Fig. 5, Table 3), as observed previously by Apinis et al. (1972). Although some terrestrial taxa persisted on the leaf blades for a few months after entering the litter layer, this shift was associated with a rapid mass extinction of taxa in the present study, accompanied by a large decrease in the abundance of sporulation structures and in fungal biomass, the latter by as much as 65% (Figs. 5, 8, Table 3). Similar deleterious effects have been reported for fungi on *P. australis* leaf blades (Tanaka, 1991, 1993) and other emergent macrophytes such as *Spartina alterniflora* (Newell et al., 1989) and *Juncus effusus* (Kuehn et al., 2000). In a study by Komínková et al. (2000), biomass decreased only after an initial increase, but fungal growth rate declined sharply immediately after submergence of brown leaves that were collected from standing shoots. Thus, fungal communities clearly undergo drastic changes as leaves colonized during attachment to standing shoots move to a periodically or permanently aquatic environment on the sediment surface of marshes.

Such large changes in fungal community structure, biomass, growth and reproduction are most likely related to the markedly altered environmental conditions in the litter layer compared to the plant canopy. In our study area, fungi in the litter layer experience periodic flooding (Table 1), which causes sudden salinity shifts and repeated smothering of leaf surfaces by a fine mud layer during inundation by tidal water with often high loads of suspended clay particles, resulting in sediment deposition at the margin of the marsh at an estimated rate of 34 mm yr^{-1} (Van Damme et al., 1999). Shear stress by tidal flood water aggravates these unfavourable habitat conditions for fungi in the litter layer, particularly for terrestrial

hyphomycetes with their fragile conidiophores. Tidal wetting appears to be too infrequent, however, for truly aquatic fungi to colonize the litter layer (Van Ryckegem & Verbeken, 2005).

Increases and subsequent decreases following the initial strong declines in fungal biomass and sporulation structures occurred in a largely synchronized fashion during at least the following six months (Fig. 8). As a result, fungal sporulation in the litter layer was highly correlated with biomass during this time, with the highest relative occurrence observed in April one month after the second biomass maximum was reached. Overall correlations could have been higher as the successional sequence of sporulation records from leaves in P1 and P2 were supposed to reflect also fungal species dynamics in litter bags (P3). This procedure was followed because direct observation on rinsed litter from P3 was impossible because fungal sporulation structures could have been washed off. A significant positive relationship between rates of ascospore release from ascomata and fungal biomass within leaf blades was also observed by Newell (2001a) in a salt marsh, suggesting that growth and reproduction of fungi can be tightly coupled on marsh plants both in leaves attached to standing shoots (Newell, 2001a) and on the sediment surface (this study). In advanced stages of leaf decomposition, notable ergosterol concentrations suggested continued fungal presence in the present study, although no sporulation was observed on the fragile remaining leaf pieces (Fig. 8). Therefore, it is possible that the fungi present at this stage were not very active, or that ergosterol persisted after fungal death (Mille-Lindblom et al., 2004), or that more typical soil fungi became established but did not yet sporulate (Frankland, 1998).

Litter decomposition

The decay rate of *P. australis* leaves in recorded during this study is within the upper range of values reported from both brackish (Lee, 1990; Tanaka, 1991) and freshwater (Larsen & Schierup, 1981; Kufel & Kufel, 1988; Cowie et al., 1992; Komínková et al., 2000; Windham, 2001; Gessner, 2000 and references herein) marshes. The net fungal biomass increase that occurred between December and February corresponds to a fungal dry mass production of at least 11.9 mg per g leaf dry mass present in litter bags in December. Leaf mass loss during that period was 9% and if fungal growth efficiency was 35% (Suberkropp, 1991), then 37% of this litter mass loss could be accounted for by fungal activity. Although the evidence presented above indicates that fungal importance for leaf litter decomposition may vary with decomposition stage, and possibly with season, the conservative estimate of 37% illustrates that fungi can contribute notably to leaf decomposition in the litter layer of marshes at least during some decomposition stages.

Feeding traces and direct observations in the field on decomposing plant matter in litter bags indicated that *P. australis* litter was heavily grazed by marsh gastropods (cf. Fig. 8 in Polunin, 1982). The main snail species is *Assiminea grayana*, which occurs at an estimated density of 12,000 animals per m^2 in the marsh (Bruyndonck et al., 2000). If this snail consumes fungi preferentially as has been shown for salt-marsh periwinkles (Graça et al., 2000; Newell & Porter, 2000) and/or its feeding stimulates fungal decomposers (Graça et al., 2000), then snail

feeding activity would have a significant impact on leaf decay at our study site, channelling a major fraction of fungal production into the large-metazoan food web.

Nutrient dynamics of decomposing litter

The poor correlation between fungal biomass and nitrogen concentration of leaves decomposing in the litter layer ($r = -0.45$) indicates that fungal nutrient immobilization in litter was small. Given a nitrogen concentration of 6.5% in fungal biomass (Findlay et al., 2002), fungi could account for as much as 34% of the total N in leaf blades from the middle canopy just before shedding, but this percentage was reduced to 12 %, when leaves entered the litter layer. This suggests that (i) a significant fraction of N was bound to dead fungal mass (e.g. as glucosamine in the cell walls of dead hyphae), or that (ii) a fraction was released from fungi but retained by other chemical or microbial mechanisms, or that (iii) N losses were compensated by fresh inputs. One possible source of such fresh inputs was the fine mud transported with the tides and regularly deposited on leaves. As described by Newell et al. (1989), part of the N associated with these fine particles may have infiltrated the increasingly porous leaf tissue as decomposition proceeded.

Conclusion

In conclusion, our results show that fungi are a rich component of the microflora on leaves attached to *P. australis* in a temperate tidal marsh, with strikingly different communities establishing in different vertical layers of the plant canopy. As dead brown leaves eventually fall to the sediment surface, fungal communities experience dramatic qualitative and quantitative changes. These include mass extinction, species replacements, and drastic reductions in fungal biomass and reproduction. Subsequently, after a transition phase, a characteristic species-poor community develops in the litter layer. These litter fungi, particularly *Phomatospora berkeleyi* and *Phoma* sp. III, are likely to contribute significantly to litter decay over an extended period even under the harsh conditions in the litter layer of tidal marshes. Fungi may also be instrumental in N retention during leaf senescence and early aerial decay, but appear not to play a major role in litter nitrogen dynamics during decomposition on the marsh surface.

Acknowledgements

We would like to thank R. Heynderickx and A. Van Heuverswijn for assistance with making litter bags and A. Van Kenhove for assistance with nitrogen analyses. We are also indebted to Dr. G. Vandriessche for technical assistance and help with analysing HPLC chromatograms. Prof. Dr. J. Van Beeumen kindly provided the HPLC-system. This research was funded by the Institute for the Promotion of Innovation by Science and Technology in Flanders, Belgium, through a grant to G.V.R.

References

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1972). Colonisation of *Phragmites communis* leaves by fungi. Nova Hedwigia 23: 113-124.

BLAIR, J.M. (1988). Nitrogen, sulfur and phosphorus dynamics in decomposing deciduous leaf litter in the southern Appalachians. Soil Biol. Biochem. 20: 693-701.

BIONDINI, M.E., MIELKE, JR.P.W., BERRY, K.J. (1988). Data-dependent permutation techniques for the analysis of ecological data. Vegetatio 75: 161-168.

BRUYNDONCK, L., JORDAENS, K., DE WOLF, H., MEIRE, P. & BACKELJAU, T. (2000). New records of *Assiminea grayana* Fleming, 1828, *Myosotella myosotis* (Draparnaud, 1801) and *Pisidium subtruncatum* Malm, 1855 (Mollusca: Gastropoda, Bivalvia) in the Scheldt estuary. Bull. Inst. Roy. Sci. Nat. Belgique Biol. 70: 103-106.

BUCHAN, A., NEWELL, S.Y., MORETA, J.I.L. & MORAN, M.A. (2002). Analysis of internal transcribed spacer (ITS) regions of rRNA genes in fungal communities in a southeastern U.S. Salt Marsh. Microb. Ecol. 43: 329-340.

COOKE, R.C. & RAYNER, A.D.M. (1984). Ecology of saprotrophic fungi. Longman Group Limited, London, 415 pp.

COWIE, N.R, SUTHERLAND, W.J., DITLHOGO, K.M. & JAMES, R. (1992). The effects of conservation management of reed beds. II. The flora and litter disappearance. J. Appl. Ecol. 29: 277-284.

DELEEBEECK, N. (2000). Phytolyts in common reed (*Phragmites australis* (Cav.) Steudel (Poaceae)) along the Scheldt and Durme (Belgium). Unpublished master thesis (in Dutch). Ghent University, Gent, 73 pp.

DUFRÊNE, M. & LEGENDRE, P. (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67: 345-366.

ENGLONER, A.I., BÁN, R., SUGAR, E. & VIRANYI, F. (2000). Effects of fungal pathogens on the element content of read leaf blades. Z. Pflanzenkrankh. Pflanzenschutz. 107: 627-636.

FELL, J.W. & HUNTER, I.L. (1979). Fungi associated with the decomposition of *Juncus roemerianus* in south Florida. Mycologia 71: 322-342.

FINDLAY, S.E.G., DYE, S. & KUEHN, K.A. (2002). Microbial growth and nitrogen retention in litter of *Phragmites australis* compared to *Typha angustifolia*. Wetlands 22: 616-625.

FRANKLAND, J.C. (1998). Fungal succession - Unraveling the unpredictable. Mycol. Res. 102: 1-15.

GESSNER, M.O. (2000). Breakdown and nutrient dynamics of submerged *Phragmites* shoots in the littoral zone of a temperate hardwater lake. Aquatic Bot. 66: 9-20.

GESSNER, M.O. (2001). Mass loss, fungal colonisation and nutrient dynamics of *Phragmites australis* leaves during senescence and early decay. Aquatic Bot. 69: 325-339.

GESSNER, M.O. & SCHMITT A.L. (1996). Use of solid-phas extraction to determine ergosterol concentrations in plant tissue colonized by fungi. Appl. Environ. Microbiol. 62: 415-419.

GESSNER, M.O. & VAN RYCKEGEM, G. (2003). Water fungi as decomposers in freshwater ecosystems. In: Bitton G (ed) Encyclopedia of Environmental Microbiology, Wiley, New York (online edition: DOI: 10.1002/0471263397.env314).

GESSNER, M.O., THOMAS, M., JEAN-LOUIS, A.M. & CHAUVET, E. (1993). Stable successional patterns of aquatic hyphomycetes on leaves decaying in a summer cool stream. Mycol. Res. 97: 163-172.

GESSNER, R.V. (1977). Seasonal occurrence and distribution of fungi associated with *Spartina alterniflora* from a Rhode Island estuary. Mycologia 69: 477-491.

GRAÇA, M.A., NEWELL, S.Y., KNEIB, R.T. (2000). Grazing rates of organic matter and living fungal biomass of decaying *Spartina alterniflora* by three species of salt-marsh invertebrates. Mar. Biol. 136: 281-289.

GRANÉLI, W. (1990). Standing crop and mineral content of reed, *Phragmites australis* (Cav.) Trin. ex Steud., in Sweden - management of reed stands to maximize harvestable biomass. Folia Geobot. Phytotax. 25: 291-302.

HIETZ, P. (1992). Decomposition and nutrient dynamics of reed (*Phragmites australis* (Cav.) Trin. ex Steud.) litter in Lake Neusiedl, Austria. Aquatic Bot. 43: 211-230.

HUDSON, H.J. & WEBSTER, J. (1958). Succession of fungi on decaying stems of *Argopyron repens*. Trans. Brit. Mycol. Soc. 41: 165-177.

HUNT, H.W., REUSS, D.E. & ELLIOTT, E.T. (1999). Correcting estimates of root chemical composition for soil contamination. Ecology 80: 702-707.

KOHLMEYER, J. & VOLKMANN-KOHLMEYER, B. (2001). The biodiversity of fungi on *Juncus roemerianus*. Mycol. Res. 105: 1411-1412.

KOMÍNKOVÁ, D., KUEHN, K.A., BÜSING, N., STEINER, D. & GESSNER, M.O. (2000). Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a litteral reed stand of a large lake. Aquat. Microb. Ecol. 22: 271-282.

KUEHN, K.A. & SUBERKROPP, K. (1998a). Decomposition of standing litter of the freshwater emergent macrophyte *Juncus effusus*. Freshwater Biology 40: 717-727.

KUEHN, K.A. & SUBERKROPP, K. (1998b). Diel fluctuations in rates of CO_2 evolution from standing dead leaf litter of the emergent macrophyte *Juncus effusus*. Aquat. Microb. Ecol. 14: 171-182.

KUEHN, K.A., LEMKE, M.J., SUBERKROPP, K. & WETZEL, R.G. (2000). Microbial biomass and production associated with decaying leaf litter of the emergent macrophyte *Juncus effesus*. Limnol. Oceanogr. 45: 862-870.

KUEHN, K.A., STEINER, D. & GESSNER, M.O. (2004). Diel mineralization patterns of standing-dead plant litter: implications for CO_2 flux from wetlands. Ecology 85: 2504-2518.

KUFEL, I. & KUFEL, L (1988). In situ decomposition of *Phragmites australis* Trin. ex Steudel and *Typha angustifolia* L. Ekol. Polska 36: 459-470.

LARSEN, V.J. & SCHIERUP, H.-H. (1981). Macrophyte cycling of zinc, copper, lead and cadmium in the littoral zone of a polluted and a non-polluted lake. II. Seasonal changes in heavy metal content of above-ground biomass and decopmosing leaves of *Phragmites australis* (Cav.) Trin. Aquatic Bot. 11: 211-230.

LEE, S.Y. (1990). Net aerial primary productivity, litter production and decomposition of the reed *Phragmites communis* in a nature reserve in Hong Kong: management implications. Mar. Ecol. Prog. Ser. 66: 161-173.

MCCUNE, B. & GRACE, J.B. (2002). Analysis of ecological communities. MjM Software design, Gleneden Beach, Oregon, U.S.A, 304 pp.

MCCUNE, B. & MEFFORD, M.J. (1999). PC-ORD for Windows. Multivariate Analysis of Ecological Data, Version 4.0, MjM Software, Gleneden Beach, Oregon, U.S.A.

MEGANCK, B. (1998). The morphology of *Phragmites australis* (Cav.) Steud. in relation to hydrodynamics, management and salinity in the Scheldt estuary. Unpublished MSc thesis (in Dutch). Ghent University, Ghent, 97 pp.

MILLE-LINDBLOM, C., VON WACHENFELDT, E. & TRANVIK, L.J. (2004). Ergosterol as a measure of living fungal biomass: persistence in environmental samples after fungal death. J. Microbiol. Meth. 59: 253-262.

MITSCH, W.J. & GOSSELINK, J.G. (2000). Wetlands (3rd edition) Van Nostrand Reinhold, New York, 920 pp.

NEWELL, S.Y. (2001a). Spore-expulsion rates and extents of blade occupation by Ascomycetes of the smooth-cordgrass standing-decay system. Bot. Mar. 44: 277-285.

NEWELL, S.Y. (2001b). Multiyear patterns of fungal biomass dynamics and productivity within naturally decaying smooth cordgrass shoots. Limnol. Oceanogr. 46: 573-583.

NEWELL, S.Y. (2003). Fungal content and activities in standing-decaying leaf blades of plants of the Georgia Coastal Ecosystems research area. Aquatic Microb. Ecol. 32: 95-103.

NEWELL, S.Y. & PORTER, D. (2000). Microbial secondary production from saltmarsh-grass shoots and its known and potential fates. In: Weinstein, M.P. & Kreeger, D.A. (eds), Concepts and controversion in tidal marsh ecology, Kluwer Academic Publishers, Dordrecht, 159-186.

NEWELL, S.Y., FALLON, R.D. & MILLER, J.D. (1989). Decomposition and microbial dynamics for standing, naturally positioned leaves of the salt-marsh grass *Spartina alterniflora*. Mar. Biol. 101: 471-481.

NEWELL, S.Y., FALLON, R.D., RODRIGUEZ, R.M.C. & GROENE, L.C. (1985). Influence of rain, tidal wetting and relative humidity on release of carbon dioxide by standing-dead salt-marsh plants. Oecologia 68: 73-79.

PETRINI, O., SIEBER, T.N., TOTI, L. & VIVET, O. (1992). Ecology, metabolite production, and substrate utilisation in endophytic fungi. Nat. Toxins 1: 185-196.

PIECZYŃSKA, E. (1972). Ecology of the eulittoral zone of lakes. Ekol. Polska 20: 637-732.

POLUNIN, N.V.C. (1982). Processes contributing to the decay of reed (*Phragmites australis*) litter in freshwater. Arch. Hydrobiol. 94: 182-209.

POLUNIN, N.V.C. (1984). The decomposition of emergent macrophytes in fresh water. Adv. Ecol. Res. 14: 115-166.

RICE, W.R. (1990). A consensus combined P-value test and the family-wide significance of component tests. Biometrics 46: 303-308.

RODEWALD, L. & RUDESCU, L. (1974). Das Schilfrohr. Die Binnengewässer, Band XXVII: 1-302.

SAMUELS, A.L., GLASS, A.D.M., EHRET, D.L. & MENZIES, J.G. (1991). Distribution of silicon in cucumber leaves during infection by powdery mildew fungus (*Sphaerotheca fuliginea*). Canad. J. Bot. 69: 140-149.

SUBERKROPP, K. (1991). Relationships between growth and sporulation of aquatic hyphomycetes on decomposing leaf litter. Mycol. Res. 95: 843-850.

TANAKA, Y. (1991). Microbial decomposition of reed (*Phragmites communis*) leaves in a saline lake. Hydrobiologia 220: 119-129.

TANAKA, Y. (1993). Aerobic cellulolytic bacterial-flora associated with decomposing *Phragmites* leaf-litter in a seawater lake. Hydrobiologia 263: 145-154.

TSCHARNTKE, T. (1992). Fragmentation of *Phragmites* habitats, minimum viable population size, habitat suitability, and local extinction of moths, midges, flies, aphids and birds. Conserv. Biol. 6: 530-536.

VAN DAMME, S., YSEBAERT, T., MEIRE, P. & VAN DEN BERGH, E. (1999). Habitatstructuren, waterkwaliteit en leefgemeenschappen in het Schelde-estuarium. Rapport IN 99/24, Instituut voor Natuurbehoud, Brussels, 119 pp.

VAN RYCKEGEM, G. & VERBEKEN, A. (2005). Fungal diversity and community structure on *Phragmites australis* (Poaceae) along a salinity gradient in the Scheldt estuary (Belgium). Nova Hedwigia 80: 173-197.

WEBSTER, J. (1956). Succession of fungi on decaying cocksfoot culms. I. J. Ecol. 44: 517-544.

WILLMER, C. & FRICKER, M. (1996) Stomata. Second edition, Chapman & Hall, London, 375 pp.

WINDHAM, L. (2001). Comparison of biomass production and decomposition between *Phragmites australis* (common reed) and *Spartina patens* (salt hay grass) in brackish tidal marshes of New Jersey, USA. Wetlands 21: 179-188.

WIRSEL, S.G.R., LEIBINGER, W., ERNST, M. & MENDGEN, K. (2001). Genetic diversity of fungi closely associated with common reed. New Phytol. 149: 589-598.

Fungal dynamics during decomposition of *Phragmites australis* leaf sheaths and stems in a brackish tidal marsh

Van Ryckegem, G., Van Driessche, G., Van Beeumen, J.J. & Verbeken, A.

Target Microbial Ecology

Abstract Decomposition of culms (sheaths and stems) of the emergent macrophyte *Phragmites australis* (common reed) was quantified in the litter layer of a brackish tidal marsh along the river Scheldt (The Netherlands). Standing dead culms were collected, placed in litter bags and retrieved monthly for a period of 16 months. Stems and leaf sheaths were separately analysed for mass loss, litter-associated fungal biomass (ergosterol), nutrient (N and P) and cell wall polymer concentrations (cellulose and lignin). The role of fungal biomass in litter nutrient dynamics was evaluated by estimating nutrient incorporation within the living fungal mass. Multivariate analysis was used to investigate which environmental variables had most power in explaining fungal succession on the decaying culms. This set of environmental factors was investigated by two pathways using either indirect or direct ordination techniques. After one year of standing decay fungal colonization and biomass pattern along the vertical axis of standing dead stems was assessed. Substantial fungal colonization was found, corresponding to 291 ± 108 μg ergosterol g^{-1} dry mass, and a vertical pattern in ergosterol concentration was noticed with minimal fungal colonization in the lowest internode but increased steeply a few centimetres higher up the stem. Fungal biomass decreased again towards the middle height of the standing stems and subsequently increased to reach maximal fungal biomass near the apex. The litter bag experiment showed that mass loss of stems was negligible during the first six months, whereas leaf sheaths lost almost 50% of their initial mass during that time. Exponential breakdown rates were -0.00395 ± 0.0004 d^{-1} and -0.0026 ± 0.0003 d^{-1} for leaf sheaths and stems respectively (excluding the initial lag period). In contrast to the stem tissue – which had no fungal colonization – leaf sheaths were heavily colonized by fungi (93 ± 10 mg fungal biomass g^{-1} dry mass) prior to placement in the litter layer. Once being on the sediment surface, 30% of leaf sheath's associated fungal biomass was lost, but ergosterol concentrations recovered the following months. In the stems, fungal biomass increased steadily after an initial lag period to reach a maximal biomass of about 120 mg fungal biomass g^{-1} dry mass for both plant parts at the end of the experiment. Fungal colonizers are considered to contain an important fraction of nutrients within the decaying plant matter. Fungal N incorporation was estimated to be 64 ± 13% and 102 ± 15% of total available N pool during decomposition for leaf sheaths and stems respectively. Fungal P incorporation was estimated to be 37 ± 9% and 52 ± 15% of total available P during decomposition for leaf sheaths and stems respectively. Furthermore, within the stem tissue, fungi are suggested to be active immobilizers of nutrients from the external environment because fungi were often estimated to contain more than 100% of the original nutrient stock. Multivariate analysis of the taxa dataset points to cellulose concentrations of plant litter being the best explanatory variable for the fungal succession observed on both plant parts.

Introduction

Decomposition of plant litter is an important component of nutrient cycling in wetlands (Mitsch & Gosselink, 2000). After an initial phase of releasing soluble nutrients by leaching, litter often immobilizes nutrients (e.g. Polunin, 1982, 1984; Hietz, 1992; Gessner, 2000). Nutrient immobilisation refers to an increase in concentrations or even a net increase in nutrient amount compared to the original stock present at the onset of decomposition. The process of nutrient immobilisation is realized by external nutrient incorporation and by the conservation of nutrients within the resource. These processes could be mediated by microbial decomposers or by adsorption (Webster & Benfield, 1986; Gessner, 2001). In a later phase of decomposition, mineralization predominates and nutrients are released in the environment. By immobilizing nutrients, litter may be a significant sink for nutrients and may increase the nutrient retention in wetlands (Webster & Benfield, 1986; Mitsch & Gosselink, 2000).

Common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) is the dominant macrophyte showing a vigorous growth, with an annual above ground production often exceeding 1 kg of dry mass per square meter (e.g. Meganck, 1998). The above ground parts of *P. australis* yearly die and naturally enter the detrital pool by means of two major pathways. Those pathways comprise a variable time of standing decay and eventually decay in the litter layer (Gessner, 2000, 2001; Komínková et al., 2000).

In both of those habitats, the standing dead shoots and the litter layer, fungi constitute the major fraction of microbial mass and productivity at least until reeds are fragmented or buried in the sediment (Komínková et al., 2000; Findlay et al., 2002; Kuehn et al. 2004). Furthermore, fungi show a huge diversity of species involved in the decay process (Van Ryckegem & Verbeken 2005b,c; Apinis et al., 1972, 1975). Distinct subcommunities are recognized on different plant 'organs' (leaf blades, leaf sheaths and stems). Furthermore, subcommunities differ along the vertical axes of standing shoots and change in time (Van Ryckegem & Verbeken, 2005d). Species composition is influenced by environmental variables such as salinity and flooding (height and frequency) along the longitudinal gradient of an estuary (Van Ryckegem & Verbeken, 2005a).

Little is known about the factors causing changes in microbial species composition or dominance during decomposition (Gessner et al., 1997). Studies attempting to establish relationships between breakdown rates, litter characteristics and fungal performance generally conclude that concentrations of nutrients, lignin, phenolic compounds or a combination of these constituents are critical in determining litter decomposability (Kjøller & Struwe, 1982; Melillo et al., 1982, 1984; Enriquez et al., 1993; Gessner & Chauvet, 1994; Gessner et al. 1997). However, different ecosystems seem to vary broadly according to the relative impact and interactions of a range of controlling factors, including both external ones related to specific environment inclusive site-specific invertebrate mycophagy and factors intrinsic to the decomposing plant material (Enriquez et al., 1993; Gessner et al., 1997; Graça et al., 2000; Gessner & Van Ryckegem, 2003).

This study reports on the decomposition of *P. australis* leaf sheaths and stems in the litter layer of a brackish tidal marsh by determining mass loss, nitrogen, phosphor and plant polymer dynamics. Involvement of fungi was assessed by estimating fungal biomass and containment of N and P in fungal biomass. Gradient analysis was used to elucidate possible factors influencing fungal taxa succession during decay of both plant parts.

Methods

Study site

The study was conducted in a brackish tidal reed stand in the Scheldt estuary on the Dutch-Belgian border in the Netherlands at the edge of Saeftinghe Marsh (51° 21' N, 4° 14' E). Common reed is the dominant emergent macrophyte on the marsh, forming nearly monospecific stands. Reed biomass and carbon allocation in this reed bed has been modelled by Soetaert et al. (2004), and selected site and reed characteristics are presented in Table 1. Meteorological data during the study period are presented in Fig. 1.

Field procedures

Dead standing culm sections from last growing season were collected on 3 December 2001 one metre above the sediment. Culm sections comprised two nodes and one internode, with the leaf sheath still surrounding the stem. This approach allowed studying the decay of stem and sheathing material in a state close to their natural association. It avoided quick penetration of water in the hollow internodium similar to natural conditions (cf. Gessner, 2000). The samples were placed in a cool box, transported to the laboratory and used to fill 45 plastic litter bags (35x20 cm, 4 mm mesh) with 50.0 g fresh mass of cut culm sections. 35 bags were placed in the tidal marsh reed stand the next morning. Litter bags were positioned flat on the sediment surface and anchored by hooked bars, and were left to be flooded regularly at high tides, about 15% of the river high tides. The remaining ten litter bags were immediately dried during 3 days at 40°C to determine the initial dry mass of the litterbag contents. After drying, stems and sheaths were separated and processed separately. The initial dry mass (g) was 21.74 ± 0.85 and 3.10 ± 0.34 (means ± STDEV) for stems and leaf sheaths respectively. The maximum difference between the summed mass of sheaths and stems and the total culm mass was < 0.2% of the initial dry mass. Initial ash was determined for five stems and sheaths. Two litter bags were retrieved at monthly intervals the next 16 months.

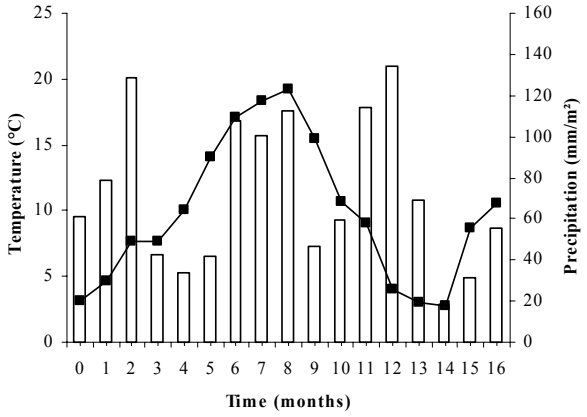


Figure 1. Average daily air temperature (line) and monthly precipitation (bars) during the study period. Both temperature and precipitation were measured in a nearby official weather station of the Royal Meteorological Institute of Belgium (Stabroek, Antwerp).

After collection, samples were immediately transported in a cool box to the laboratory and processed the same day. They were rinsed with distilled water as thoroughly as possible without fragmenting to remove adhering clay and macro-invertebrates. Sheath and stem material was separated, making it possible to follow the decomposition process of both plant 'organs' (Gessner, 2000). Subsamples were taken for fungal biomass quantification and the remaining material was dried at 40°C during 72 h, ground to pass a 2-mm mesh, and used for analyses as detailed below.

Mass loss, nutrient concentration and lignocellulose

The ash-free dry mass (AFDM) was determined following combustion (4 hrs at 550°C) of 250 mg subsamples and dry mass was corrected for the mass of subsamples removed for ergosterol analysis. Ash content varied little: 13.5 ± 2.3% and 4.6 ± 1.2% respectively for leaf sheaths and stems. Higher ash content was noticed during the last two experimental months both for leaf sheaths (ash 16-18%) and stems (ash 6-9%). Mass loss data were fitted to a simple exponential model, $m_t = m_o . e^{-kt}$, where m_t is litter ash- free dry mass (AFDM) remaining after time t, m_o is the original AFDM and k the breakdown coefficient (Boulton & Boon, 1991). Data were not transformed and non-linear regression analysis was used to estimate parameters using the default settings in SYSTAT, version 10.2.

Table 1. Selected characteristics of the investigated tidal marsh.

Variable	Mean ± STDEV	*N*
Tidal marsh		
Flooding frequency (%)[$]	15	
Average flood height (cm)[£]	17	
Sedimentation rate ($mm.yr^{-1}$)[!]	34	
Water chemistry[°]		
pH	7.59 ± 0.23	11
Cl^- (mg/l)	2455 ± 1510	19
NH_4^+-N (mg/l)	0.46 ± 0.35	21
NO_2^--N (mg/l)	0.06 ± 0.03	21
NO_3^--N (mg/l)	4.9 ± 0.65	21
Total P (mg/l)	0.73 ± 039	21
PO_4^{3-} (mg/l)	0.16 ± 0.05	21
Reed stand		
Shoot height (cm)	196 ± 21	20
Stem diameter at first internode (mm)[£]	4.0 ± 0.2	60
Density of living shoots (m^{-2})[*]	191 ± 65	6
Above-ground biomass ($g\ m^{-2}$)[*]	927 ± 293	6
Culm biomass ($g\ m^{-2}$)[*]	570 ± 216	6
of which stems ($g\ m^{-2}$)[§]	445 ± 16	5
of which leaf sheaths ($g\ m^{-2}$)[§]	125 ± 16	5

[$] Pererccentage of flooding is the relative number of times the total high tides are higher than the marsh surface and are supposed to flood the marsh (from Meganck, 1998).
[£] From Meganck (1998).
[!] Calculated as: 157 x (flooding frequency) + 10, $r^2 = 0.52$, $P < 0.001$ (Van Damme et al., 1999).
[°] Annual average in 2002 (Flemish Environment Agency, Belgium, site code 154100, http://www2.vmm.be).
[*] Estimated at end of the growing season (10 September 1997) by harvesting all above-ground living reed matter in six 0.25 m^2 quadrats; biomass ≈ annual above net above-ground production. Note: after mowing density of living shoots was found to increase the next year (~× 2.5 in our study site) (Hoffmann, pers. comm.).
[§] Estimate based on average of 22% (19-26%) leaf sheath dry mass and 78% stem dry mass (5 whole culms without inflorescence).

Total N and P concentrations were measured using an automatic Skalar chain (segmented flowanalyser (SAN PLUS Analyser) SKALAR)) after digestion of approximately 100 mg plant material with sulphuric acid and potassium sulphate at 360°C. Initial C concentration, C concentration after 7 months (leaf sheaths) and 14 months of breakdown (stems) was determined with a Carlo-Erba elemental analyser, type NA-1500.

Leaf sheaths and stems were analysed for content of structural plant polymers (cellulose and lignin) according to the acid-detergent method (Van Soest & Wine, 1967; Van Soest & Robertson, 1980; Kirk & Obst, 1988). This method adds an additional step, preceding the Klason method by pre-treatment of samples with an acid detergent to remove protein, hemicellulose and other components associated with lignocellulose, and is favoured for plant

quality characterization during decomposition (Palm & Rowland, 1997; but see Hatfield et al., 1994; Jung et al. 1999 for discussion on possible inaccuracies). All plant cell-wall constituents were corrected for ash content.

A Generalized Linear Model (GLM) procedure (SAS Statistical Package version 8.2, SAS 1999) was used to compare trends between plant parts for measured elements (N, P, cellulose and lignin) with plant parts, time and the two-way interaction of plant part and time of decomposition in the model. Significance of 'plantpart*time' interaction was tested by F-tests.

Fungal standing crop

Fungal biomass was estimated by measuring ergosterol concentration as adapted from the work of Gessner & Schmitt (1996). Freeze-dried stem and leaf sheath samples were kept in a KOH 8 $g.l^{-1}$ MeOH solution for maximally 1 month prior to solid-phase extraction, followed by injection into a high performance liquid chromatography (HPLC) system (Kratos Analytical Instruments Spectroflow 400) at a flow rate of 1.5 ml/min, and with a constant column temperature of 30°C, ergosterol eluted at ~ 8 min. Ergosterol concentration was estimated with a UV Spectroflow 757 detector at 282 nm. The software used to quantify peak area was Applied Biosystems Model 610A, a data analysis system for protein characterization, version 1.2.2. Conversion factors for calculation fungal biomass, % fungal N and % fungal C were 5.8 mg ergosterol/g fungal dry mass, 65 mg N/g fungal biomass and 431 mg C/g fungal biomass (Findlay et al., 2002).

Fungal taxa involved in decay

Species data sets for leaf sheaths and stems originated from two adjacent plots investigated at the same dates for fungal succession on *P. australis* (see Van Ryckegem & Verbeken, 2005b,c). Direct observation of sporulation structures in this study confirmed the comparable species composition and succession with those in the litter layer from the adjacent plots. The former data sets were reduced by including only those taxa found more than three times during the period of study. This criterion was met by 20 taxa on leaf sheaths and 13 taxa on stems (Table 2).

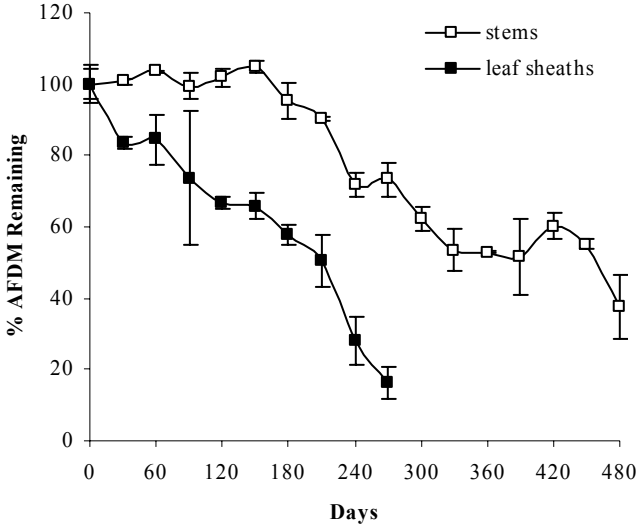


Figure 2. Ash-free dry mass remaining of leaf sheaths and stems of *Phragmites australis* in the litter layer of a brackish tidal marsh during decomposition. Error bars indicate ± STDEV (*N* = 2; except for the start of the experiment *N* = 10).

Gradient analysis

Multivariate ordination techniques were applied to analyse species data sets in combination with measured environmental data from this study. This way we investigated dominant parameters characterizing the fungal sporulation sequence. Because gradient length determined by detrended correspondence analysis (DCA), an indirect or unconstrained gradient analysis, exceeded two units of standard deviation, unimodal species response curves could be expected (Table 3) (McCune & Grace, 2002). Consequently, a direct ordination technique, canonical correspondence analysis (CCA), was applied (Palmer, 1993). In this technique the axes are constrained by linear combinations of environmental variables. The ordination was used in combination with multiple regression to investigate the relationship between potentially explanatory variables and the observed succession of fungal taxa during substrate decay.

The environmental matrix initially contained all measured internal substrate quality characteristics (N, P, cellulose and lignin), external variables (mean monthly temperature and precipitation, flooding water NO_3^-, PO_4^{-3} and salinity) and one variable (Shannon's diversity index) as a measure for fungal diversity. Each variable was tested separately in a CCA for individual explanatory power (marginal effects). The forward selection procedure in CANOCO (version 4.5, ter Braak & Smilauer, 2002) was followed to reduce the constraining matrix to a minimal set of environmental variables. They independently (avoiding multicollinearity; conditional effects) and significantly explain variation in the species data just as well as the full set of environmental variables. Significance was tested by a Monte Carlo

Table 2. Dominant fungal taxa found on *P. australis* leaf sheaths and stem litter. Acronyms refer to Figure 7. The first letter of the acronym refers to the systematic position of the taxon being A: ascomycetes; C: coelomycetes; H: hyphomycetes. Percentage abundance (% ab.) is the proportion of the number of records of a taxon to the total number of records of the taxon during the period the litter bag experiment was conducted. Data extracted from Van Ryckegem & Verbeken (2005b,c).

Taxa	Acronym	% ab.
Leaf sheath		
Asco sp. Dothideales incertae sedis II	AASCII	0.6
Didymella glacialis Rehm	ADIDGLA	2.0
Halosphaeria hamata (Höhnk) Kohlm.	AHALHAM	8.8
Lophodermium arundinaceum (Schrad.) Chevall.	ALOPHAR	1.4
Massarina arundinacea (Sowerby: Fr.) Leuchtm.	AMASARU	15.0
Phaeosphaeria culmorum (Auersw.) Leuchtm.	APHACUL	2.5
Phaeosphaeria pontiformis (Fuckel) Leuchtm.	APHAPON	11.3
Phaeosphaeria sp. II	APHASP2	1.1
Phaeosphaeria sp. III	APHASP3	1.4
Phomatospora berkeleyi Sacc.	APHOBER	11.6
Stictis sp.	ASTICTI	4.5
Coelomycete sp. I	CCOELI	2.3
Hendersonia culmiseda Sacc.	CHENCUL	2.0
Phialophorophoma sp.	CPHIALO	1.4
Phoma sp. III	CPHOMAC	16.4
Phoma sp. IIa	CPHOMBA	1.4
Septoriella sp(p).	CSEPTOR	4.8
Stagonospora vexata Sacc. sensu Diedicke	CSTAVEX	6.8
Hyphomycete sp. III	HHYPSP3	2.5
Myrothecium cinctum (Corda) Sacc.	HMYRCIN	2.0
Stem		
Botryosphaeria festucae (Lib.) Arx & E. Müll.	ABOTFES	3.5
Halosphaeria hamata (Höhnk) Kohlm.	AHALHAM	29.7
Lophiostoma arundinis (Pers.) Ces. & De Not.	ALOPARU	2.6
Lophiostoma semiliberum (Desm.) Ces. & De Not.	ALOPSEM	2.3
Massarina arundinacea (Sowerby: Fr.) Leuchtm.	AMASARU	31.1
Mollisia retincola (Rabenh.) P. Karst.	AMOLRET	1.5
Phaeosphaeria pontiformis (Fuckel) Leuchtm.	APHAPON	4.1
Phomatospora berkeleyi Sacc.	APHOBER	8.1
Phoma sp. III	CPHOMAC	2.9
Septoriella phragmitis Oudem.	CSEPPHR	4.1
Stagonospora incertae sedis I	CSTAINA	3.8
Stagonospora sp. II	CSTAINB	1.5
Stagonospora vexata Sacc. sensu Diedicke	CSTAVEX	4.9

permutation test (1000 permutations) with P set at 0.05 and use of a Bonferroni correction (Rice, 1990). Additionally, the first DCA-axis was interpreted indirectly in environmental terms. This technique was used to verify direct ordination results. If ordination diagrams of direct and indirect ordination look similar and identical environmental variables are selected in both procedures we have additional indications that important environmental variables are included in CCA (Jongman et al., 1987). This was evaluated by determining the Pearson correlation of environmental variables with the DCA-axes. Subsequently, multiple regression was applied to evaluate the complex structures of ecological gradients expressed along the DCA-axis. Backward elimination (Zar, 1999) was used to evaluate which variables should be selected at $P < 0.05$ for the multiple regression (SYSTAT, version 10.2). Ordination graphs were prepared in PC-ORD version 4.26 (McCune & Mefford, 1999).

Results

Decay and nutrient dynamics

Decomposition of *P. australis* leaf sheaths proceeded quickly after placement of litter bags on the sediment surface. Leaf sheaths lost 50% of their initial ash-free dry mass in 7 months (Fig. 2), corresponding to an exponential decay coefficient k of -0.00395 ± 0.0004 d^{-1} [mean ± asymptotic standard error (ASE); corrected $r^2 = 0.86$, $N = 23$; estimated $m_0 = 102.1$ ± 4% (± ASE)]. In contrast to the surrounding leaf sheaths, the mass loss from stems was non-detectable during the first six months of the experiment. Subsequently, breakdown of the stems followed a smooth exponential pattern (Fig. 2) with a loss of 50% ash-free dry mass after an exposure of about 15 months in the litter layer. This corresponds to an exponential decay coefficient, k of -0.0026 ± 0.0003 d^{-1} when the initial lag period was eliminated for nonlinear regression analysis [mean ± ASE; $r^2 = 0.81$; $N = 22$; estimated $m_0 = 96$ ± 3.7% (± ASE)].

Nitrogen and phosphorus concentrations (% of dry mass) increased over the study period for both leaf sheaths (Fig. 3a) and stems (Fig. 3b). Initial N and P concentrations in leaf sheaths exceeded those in stems by a factor > 2. Initial differences in nutrient concentrations were roughly maintained among both litter types during decay. This is demonstrated by a 2.5 versus 2.75 fold increase in N concentration for leaf sheaths and stems respectively and a 4 versus 6 fold increase in P concentration for leaf sheaths and stems respectively at the end of the experiment. However, patterns were different for both plant parts, with a different increase rate, even when the initial lag period of stems was neglected (for N, $F_{1, 32} = 7.42$, $P = 0.0104$; for P, $F_{1\ 32} = 17.82$, $P = 0.0002$, plant part*time, GLM-ANCOVA) (Fig. 3a, b). Nutrient concentrations in leaf sheaths fitted best an exponentially function for N ($r^2 = 0.89$) and linearly for P ($r^2 = 0.94$) up to the end of the study. Similar to the mass loss, nutrients in stems showed no changes in concentrations during the first six months of the experiment. This lag period was followed by a sharp rise and then levelled off (Fig. 3b).

Increases in N concentrations followed losses in dry mass, resulting in a moderate accumulation of N amounts in the litter bags during the study (Fig. 3c, d). The leaf sheaths showed a tendency for net N mineralization towards the end of the nine month study period. Stems showed a net immobilisation during decay, with a significant rise in N stock if we compare the period with mass loss with the initial lag period ($P < 0.01$, ANOVA) (Fig. 3d). This resulted in an increased N stock of about 155% of the original stock (Fig. 3d). The C:N ratio of leaf sheaths decreased from 71.5 ± 7.3 to 42.8 ± 16.6 in July after seven months of decay, and from 175.4 ± 24 to 86 ± 2.5 for stems after fourteen months of decay (mean ± STDEV, $N = 4$).

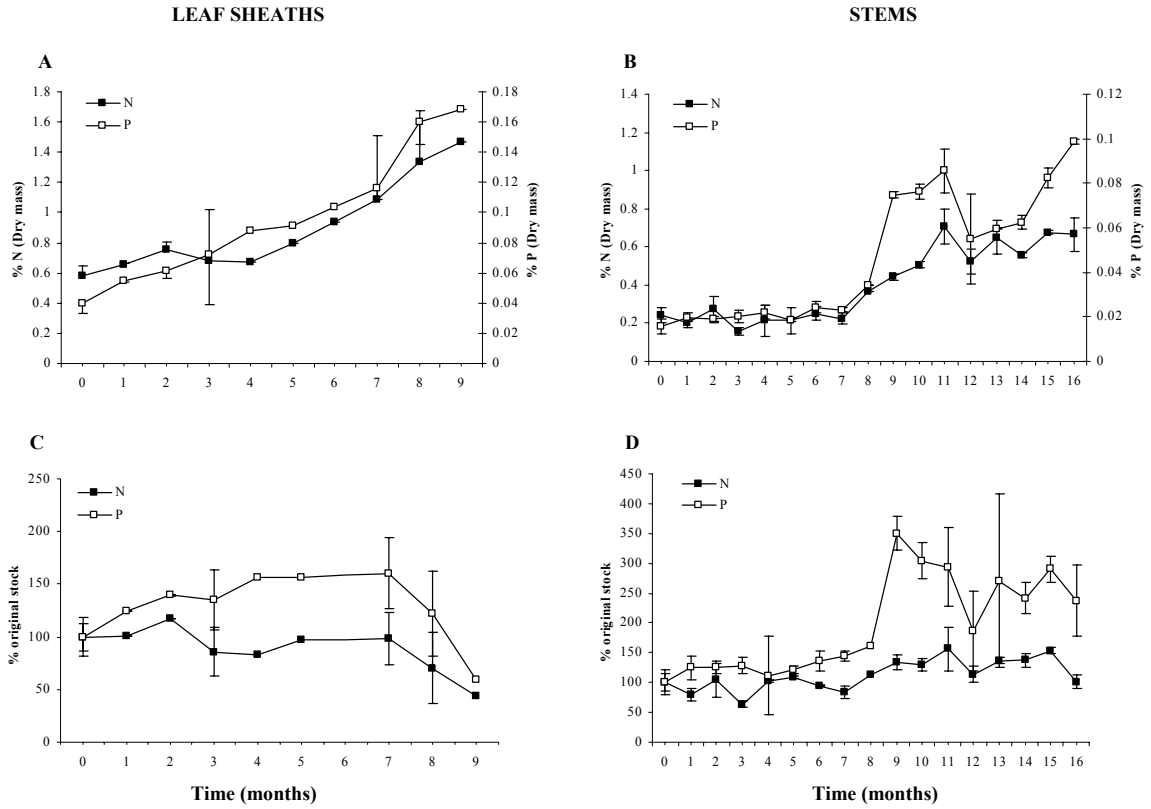


Figure 3. Dynamics of nitrogen and phosphorus concentrations of leaf sheaths (A) and stems (B) and changes in amounts of nitrogen and phosphorus per litter bag of leaf sheaths (C) and stems (D) of *Phragmites australis* litter enclosed in litter bags on the sediment surface of a tidal brackish marsh. Error bars indicate mean ± STDEV (N = 1-2 for leaf sheaths data; N = 2 for stem data; except for initial measurements N = 3).

Within the stems, the P stock increased significantly after the initial lag period ($P < 0.01$, ANOVA). Net immobilization of P was higher for stems than for leaf sheaths, resulting in a reduced difference in C:P proportion between litter types. The C:P ratio of leaf sheaths decreased from 1063.1 ± 192.8 to 370.5 ± 25.3 after seven months of decay and for stems from 2676.2 ± 429.3 to 763 ± 42.4 after fourteen months of decay (mean ± STDEV, $N = 4$). Only in the last month of the decomposition study a net P mineralization was noticed for leaf sheaths.

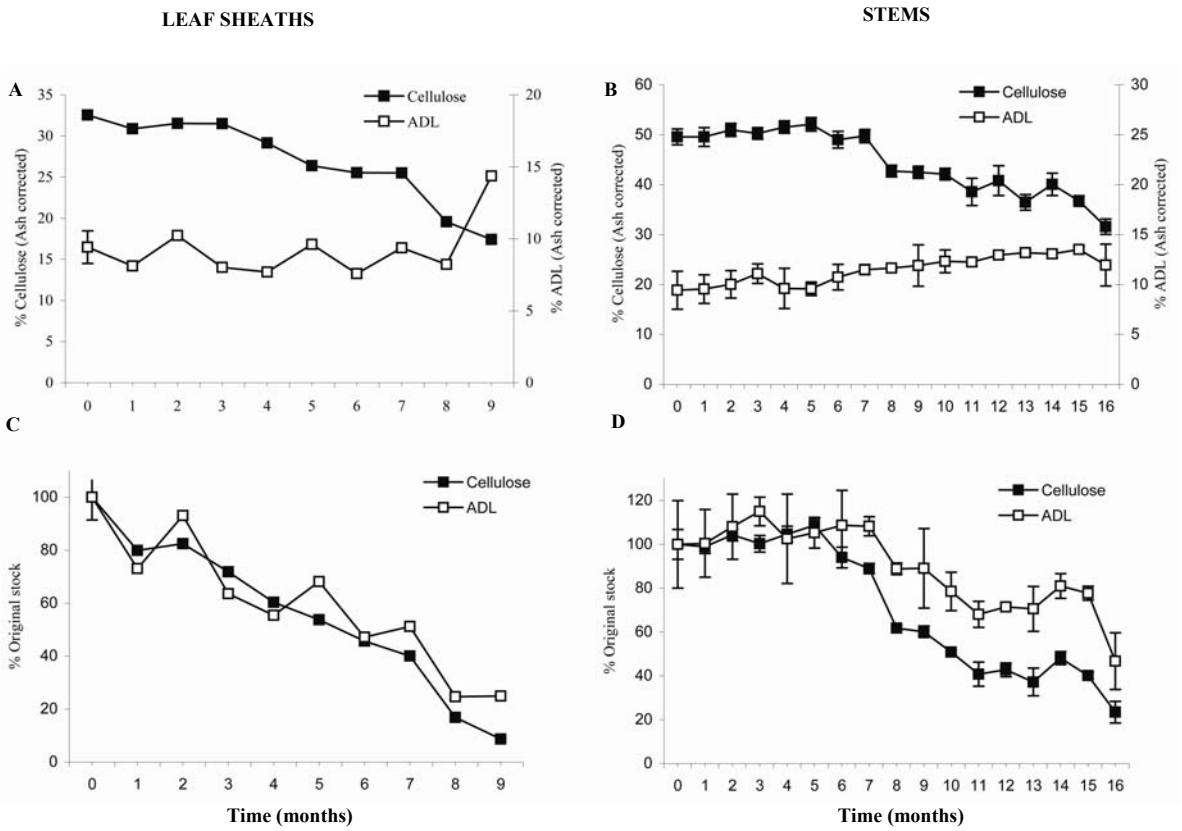


Figure 4. Dynamics in cellulose and acid detergent lignin concentrations of leaf sheaths (A) and stems (B) and changes in amounts of cellulose and acid detergent lignin per litter bag of leaf sheaths (C) and stems (D) of *Phragmites australis* litter enclosed in litter bags on the sediment surface of a tidal brackish marsh. Error bars indicate mean ± STDEV (N = 1-2 for leaf sheaths data; N = 4 for stem data; except for initial measurements N = 5).

Cellulose concentrations in both leaf sheaths and stems (once decay started) (Fig. 4a, b) tended to show a gradual and comparable decrease pattern ($F_{1, 60}$ = 0.06, P = 0.81, plantpart*time, GLM-ANCOVA). Lignin concentrations tended to increase in stems (Fig. 4b) while this was not observed for leaf sheath (except for a sharp rise in the last month). However, statistical testing showed that lignin concentrations were similar in both plant parts during decomposition ($F_{1, 60}$ = 0.61, P = 0.44, plantpart*time, GLM-ANCOVA). Parallel to the pattern of mass loss, standing stocks of both cellulose and lignin decreased, with a higher loss of the original stock of cellulose compared to lignin for both plant parts (Fig 4c, d).

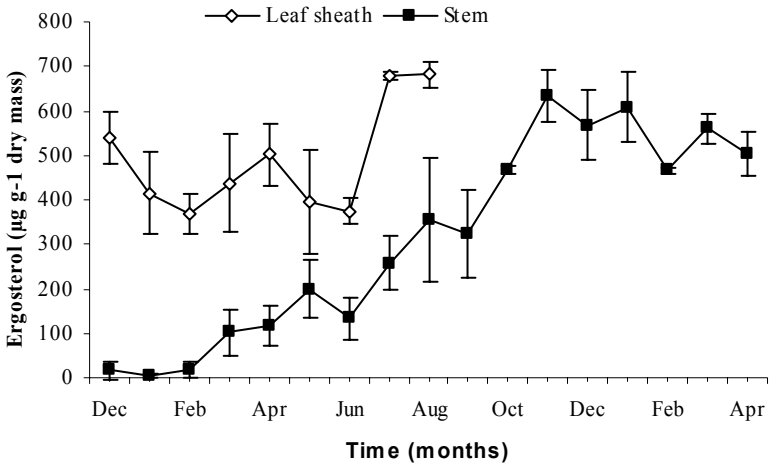


Figure 5. Dynamics of fungal biomass (ergosterol) associated with leaf sheaths and stems of *Phragmites australis* placed on the sediment surface in a brackish tidal marsh. Error bars indicate ± STDEV (N = 2 to 4; except for initial values N = 5).

Fungal biomass and taxa

In contrast to the leaf sheaths, which showed high fungal biomass present (93 ± 10 mg fungal biomass g^{-1} dry mass), middle height stem sections showed little detectable ergosterol at the start of the experiment (Fig. 5). After placement of the litter bags on the sediment, ergosterol concentrations in the leaf sheaths decreased by about 30%, but this difference was not significant ($P > 0.05$, $t = 1.42$, $N = 3$, paired t-test) (Fig. 5) and subsequently increased again to initial values. Maximal ergosterol values were obtained for leaf sheaths largely decomposed (117 ± 5 mg fungal biomass g^{-1} dry mass). Ergosterol concentrations in stems gradually increased over time to reach a maximum (118 ± 10 mg fungal biomass g^{-1} dry mass) and then levelled off after about eleven months of decay.

To check whether fungi are also important in the standing decay phase of stems, fungal biomass was measured for two comparable standing dead stems still carrying their panicle in November 2002, after one year standing decay. Stem tissue showed appreciable fungal colonization with an overall ergosterol recovery of 291 ± 108 µg ergosterol g^{-1} dry mass, corresponding to 20 ± 8 mg fungal C g^{-1} dry mass. A vertical pattern in ergosterol concentration was consistent between both selected stems, using the paired t-test ($P = 0.90$, $t = -0.13$, $N = 32$) (Fig. 6). Fungal colonization was minimal for the lowest internode, but increased steeply a few centimetres higher up the stem. Fungal biomass decreased again towards the middle height of the standing stems, and subsequently increased to reach maximal fungal biomass near the apex.

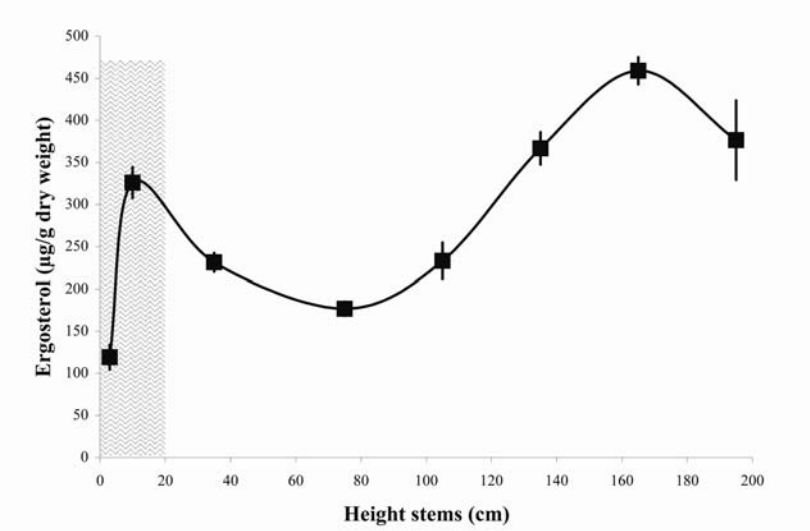
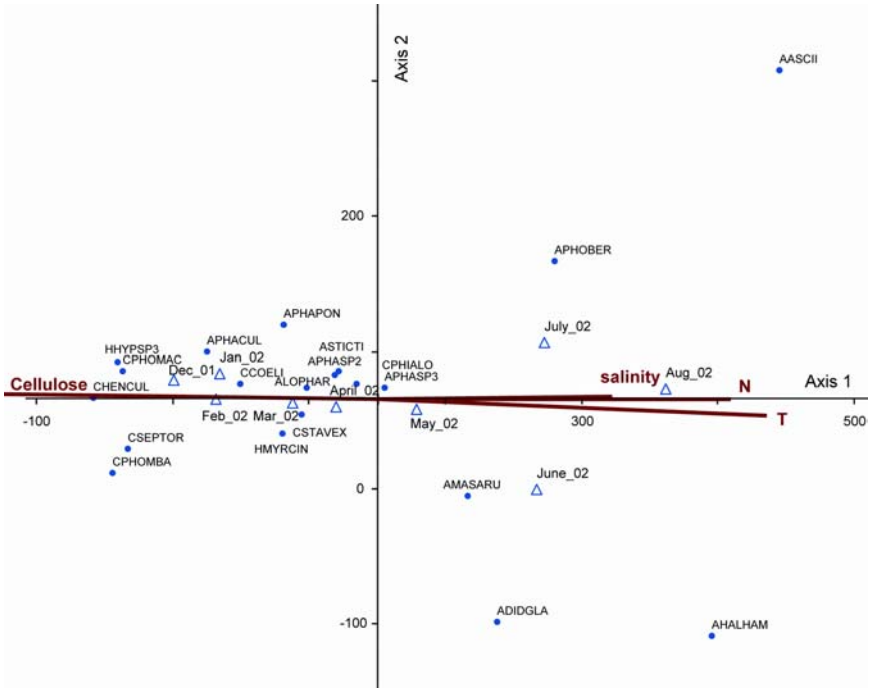


Figure 6. Vertical pattern in fungal biomass (ergosterol) after one year standing dead decay along the vertical axis of stems of *Phragmites australis* in a brackish tidal marsh. Error bars indicate ± STDEV (*N* = 2). The waved graph section indicates the part of the stems regularly inundated by tidal flooding water.

Gradient analysis

Eigenvalue reductions between the indirect and direct ordination technique were small, indicating that relatively little species information was lost by constraining the analysis by environmental variables (Table 3). Tables 4 and 5 show correlation coefficients between the investigated variables. Internal variables describing substrate quality show the highest significant correlation. Pearson correlations between the first DCA axis and the litter variables are described in Table 6. Except for mean temperature (TEMP) related to the leaf sheath data, all

A



B

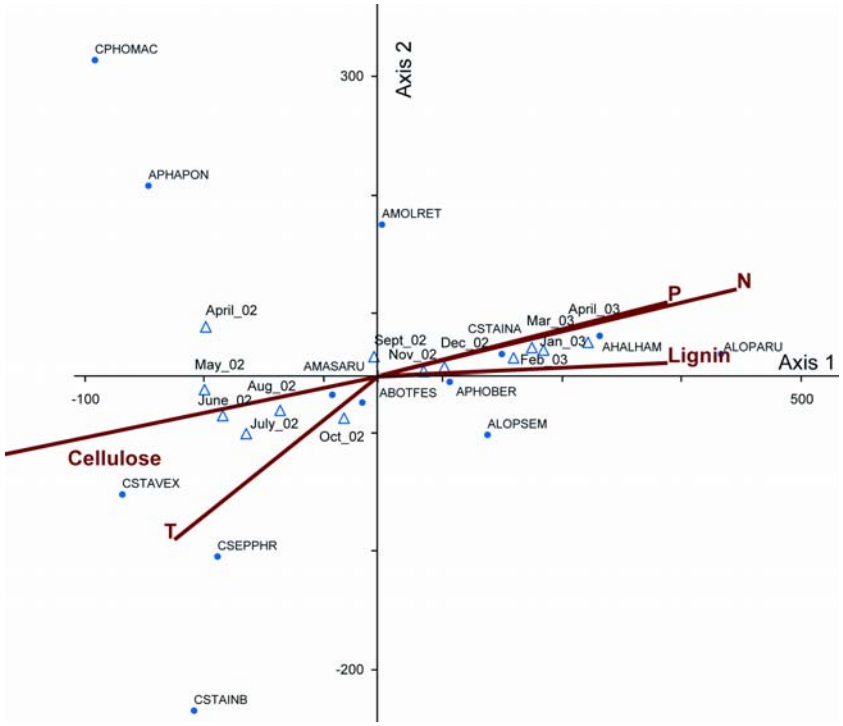


Figure 7. Detrended correspondence analysis (DCA) joint plots (Axis 1 and 2) of leaf sheath fungal data (A) and stem fungal data (B) with the most important marginal variables (cut off value $r^2 > 0.5$) (Tables 7, 8) as passive variables included on the plots. △ indicates the position of monthly samples in ordination space screened for fungal sporulation structures (see Van Ryckegem & Verbeken, 2005a,b); ● indicates the distribution of fungal taxa in ordination space. Acronyms of species are according to Table 2. Axes are scaled in SD units x 100.

Table 3. Summary of DCA on fungal sporulation records, and summary of CCA on fungal data and environmental data during decay for both leaf sheaths and stems of *Phragmites australis* in the litter layer of a tidal brackish marsh.

	Axis 1	Axis 2	Axis 3	Total inertia
Leaf sheaths				Total inertia
DCA gradient length	3.610	1.074	0.656	1.20
DCA eigenvalues (unconstrained)	0.62	0.056	0.015	
CCA eigenvalues (constrained)* (*P*- value)$	0.58	0.273 (NS)	0.103 (NS)	1.20
Cumulative % of species variance explained by CCA	47.9	--	--	
Pearson correlation between spp. and environment (*P*-value)$	1 (0.005)	--	--	
Stems				
DCA gradient length	3.211	0.892	0.857	1.32
DCA eigenvalues (unconstrained)	0.70	0.04	0.01	
CCA eigenvalues (constrained)* (*P*- value)$	0.610 (0.005)	0.289 (NS)	0.159 (NS)	1.32
Cumulative % of species variance explained by CCA	46.1	--	--	
Pearson correlation between spp. and environment (*P*-value)$	1 (0.005)	--	--	

* Constrained by 'Cellulose', the environmental variable selected after forward selection
$ Determined by Monte Carlo test (1000 permutations)

significant correlations with the first DCA axis are variables describing the internal substrate quality mainly cellulose, nitrogen and less phosphorus (Fig. 7).

Testing for marginal effects in the CCA identified four significant variables measured for leaf sheath data: cellulose, mean temperature, N and salinity of the flooding water (Table 7); three significant variables for stem data: cellulose, N and lignin concentration (Table 8). However, only cellulose was retained after testing for conditional effects in both models (Table 7, 8). In the final CCA models (Table 3), constrained by one significant (*P* set at 0.05) variable, only the first axis was significant and explained 48% and 46% of the taxa variance, for leaf sheath and stem respectively.

Multiple linear regression of the first DCA-axis sample scores against the selected significant environmental factors leads to a similar conclusion for stems (cellulose retained in model, corrected $r^2 = 0.88$, $P < 0.001$), but an additional significant environmental factor (i.e. temperature) was included (corrected $r^2 = 0.97$, $P < 0.001$) for the leaf sheaths.

For stem and leaf sheath data sets this first axis (both for DCA and CCA) represents a clear decay pattern, characterized by a sequence in fungal sporulation. This is best determined by the several variables describing the substrate quality, of which cellulose is the most explanatory, but which are strongly correlated with the other internal variables.

Table 4. Pearson correlation between variables included in the leaf sheath environmental data set

	N	P	CELLUL	TEMP	SHANNON	N WATER
N	1.00000					
P	0.90926*	1.00000				
CELLUL	-0.93934**	-0.91838**	1.00000			
TEMP	0.89167**	0.84311	-0.91786*	1.00000		
SHANNON	-0.74126	-0.44444	0.66807	-0.60589	1.00000	
NWATER	-0.69382	-0.60558	0.59267	-0.44213	0.61841	1.00000
SAL	0.67721	0.58500	-0.75280	0.74969	-0.73388	-0.42390

***$P < 0.001$; ** $P < 0.01$; * $P < 0.05$. With Bonferroni correction.

Table 5. Pearson correlation between variables included in the stem environmental data set

	N	P	LIGNIN	CELLUL	PREC	TEMP	SHANNON	N WATER
N	1.00000							
P	0.90977***	1.00000						
LIGNIN	0.82917*	0.69737	1.00000					
CELLUL	-0.94855***	-0.89279***	-0.80257*	1.00000				
PREC	-0.05705	-0.17581	0.06140	0.03843	1.00000			
TEMP	-0.64161	-0.44463	-0.57435	0.51981	0.24996	1.00000		
SHANNON	-0.36272	-0.31823	-0.36646	0.52345	0.18232	0.28955	1.00000	
N WATER	-0.21177	-0.25567	-0.46068	0.23330	-0.27599	-0.14722	-0.34633	1.00000
SAL	-0.19090	0.09621	-0.22634	0.18283	0.31021	0.66110	0.41120	-0.41743

***$P < 0.001$; ** $P < 0.01$; * $P < 0.05$. With Bonferroni correction.

Table 6. Pearson correlation between variables and first DCA axis; stem data set and leaf sheath data set.

Stems	DCA1	**Leaf sheaths**	DCA1
N	-0.92629***	N	0.92803**
P	-0.83377*	P	0.88602*
LIGNIN	-0.83205*	CELLUL	-0.96412***
CELLUL	0.94525***	TEMP	0.97416***
PREC	0.19545	SHANNON	-0.66117
TEMP	0.69833	NWATER	-0.60017
SHANNON	0.61043	SAL	0.75657
NWATER	0.08211		
SAL	0.39340		

***$P < 0.001$; ** $P < 0.01$; * $P < 0.05$. With Bonferroni correction.

Table 7. Results of CCA with Monte Carlo simulation (1000 permutations) testing for marginal and conditional effects of environmental variables for the leaf sheath fungal community data set (see methods)

Leaf sheath data	Marginal effects		Conditional effects	
Environmental variable	Eigenvalue	*P*	Eigenvalue	*P*
Cellulose	0.58	0.001*	0.58	0.001*
Temperature	0.57	0.001*	0.15	0.091 (NS)
Total N	0.56	0.001*	0.15	0.046 (NS)
Salinity	0.37	0.023*	0.09	0.144 (NS)
Shannon index	0.34	0.040	--	
Total P	0.32	0.082	--	
NO_3^- water	0.24	0.135	--	
PO_4^{-3} water	not enough variation			

* Significant at $P < 0.05$ after Bonferroni correction

Table 8. Results of CCA with Monte Carlo simulation (1000 permutations) testing for marginal and conditional effects of environmental variables for the stem fungal community data set (see methods)

Stem data	Marginal effects		Conditional effects	
Environmental variable	Eigenvalue	*P*	Eigenvalue	*P*
Cellulose	0.61	0.001*	0.61	0.001*
Total N	0.57	0.001*	0.04	0.538 (NS)
Total P	0.53	0.004	--	
Lignin	0.53	0.001*	0.11	0.072 (NS)
Temperature	0.42	0.005	--	
Salinity	0.24	0.076	--	
NO_3^- water	0.20	0.128	--	
Shannon index	0.14	0.230	--	
Precipitation	0.10	0.410	--	
PO_4^{-3} water	not enough variation			

* Significant at $P < 0.05$ after Bonferroni correction

Discussion

Fungal dynamics during natural decay

In natural circumstances culms from the same growing season enter the litter layer gradually and may stay in a standing position for up to several years (Haslam, 1972; Granéli, 1990). Because of this variable time of aerial breakdown, the plant parts that continuously enter the litter layer have a different chemical composition, differ in microbial colonization (Van Ryckegem & Verbeken, 2005b, c), and are confronted with different environmental (seasonal) conditions. Hence the experimental setup, following litter layer decay starting from December, describes only one of the multiple patterns present in a reed stand. Within the studied site, the estimated proportion of stems following the studied decay pattern in the litter layer is about 25%, while another 35-40% of the culms fall gradually on the sediment surface during the following year (Soetaert et al., 2004, see their Fig. 4). However, these general figures are strongly influenced by episodic events such as storms and heavy winds. Those extreme weather conditions snap the more fragile upper parts and leave the more robust lower culm parts in a standing position. Those upper culm parts consist mostly of leaf sheaths surrounding thin stems.

Analysis of the standing dead reed culms prior to placing the litter bags on the marsh surface indicates appreciable fungal colonization of the leaf sheaths (40 ± 4 mg C g^{-1} DM) while fungal presence was nearly undetectable in the stem tissue. This high fungal biomass and the observed high species richness on standing dead leaf sheaths (Van Ryckegem & Verbeken, 2005b) suggest that fungi are an important metabolic component within standing reed litter as was recently indicated by Kuehn et al. (2004). Leaf sheaths are the plant parts being proportionally the most decomposed in a standing position, as evidenced by the fact that a large proportion of culms stays in an upright position for at least several months (see above) combined with a relatively fast decay of leaf sheaths compared to stems (see above, Fig. 2; Gessner, 2000). Moreover, a complete breakdown of leaf sheaths was noticed in the standing position (Van Ryckegem & Verbeken, 2005b). Consequently, if standing decay is incorporated in carbon and nutrient budgets (Pieczyńska, 1972; Gessner et al. 1996; Gessner, 2001), leaf sheaths, accounting for 14% of total above ground biomass (Table 1), have an appreciable impact on the organic matter dynamics within *P. australis* stands in a relatively short term.

The unequal distribution of leaf sheath mass along the vertical axis of a standing reed shoot and the delay in fungal stem colonization is probably the reason why significantly higher fungal biomass was observed in the upper portions of *P. australis* reed shoots compared to the lower parts (Findlay et al., 2002). The proportionally higher stem content in basal culm samples might lower the overall ergosterol concentration compared to the upper culm parts. Furthermore, the vertical ergosterol patterns described during the first six months of standing dead decay of both *P. australis* (Findlay et al., 2002) and *Erianthus giganteus* culms (Kuehn et al., 1999) are possibly due to high fungal biomass in leaf sheaths and nearly fungal absence within the stem tissue.

Although (macroscopically) visible fungal colonization on standing stems is slower than fungal appearance on stems in the litter layer (Van Ryckegem & Verbeken, 2005c), there is an appreciable fungal biomass measured after one year in a standing dead position (Fig. 6). This indicates microbial mineralization for those plant parts in an upright position (see also Komínková et al., 2000; Kuehn et al. 2004). The observed fungal biomass pattern (as ergosterol) along the axis of standing stems (Fig. 6) could be caused by a combination of external conditions and physical and chemical characteristics of the resource. The first internode (3 cm above sediment surface) showed significant lower ergosterol concentration compared to the second internode (about 10 cm above sediment surface) (*t*-test, $P < 0.05$, $N = 7$). This pattern is consistent with the observation that the lowest internode, just above the sediment surface, stays viable for several more months (as being greenish in colour). This delayed senescence probably delays fungal colonization and could serve as a physiological barrier for fungal growth towards belowground rhizomes. The zone above the first internode shows higher fungal biomass compared to the region above which is not flooded by the tidal marsh water. It appears that fungal growth is promoted through the periodical wetting of the basal stem parts (except for the lowest part). Higher up the stems ergosterol concentrations gradually increased as stems become thinner and less refractory. This may be related to the absence or thin sclerenchymatous layers and thinner cuticle under the leaf sheaths (Rodewald & Rudescu, 1974; cf. Webster, 1956), resulting in a less restrained growth and/or a higher level of dew set at the top of the shoot canopy (cf. Newell et al., 1996) and thus in more benign conditions for fungal colonization.

Upper stem parts are completely isolated from the surroundings by leaf sheaths. Therefore initial fungal colonization seems to be through systemic endophytes becoming primary saprophytes (Parbery, 1996; Bills, 1996) or saprotrophic fungi growing from the leaf sheaths towards the stems.

Movement of the *P. australis* leaf sheath litter from a standing dead position to the sediment surface results in a decrease in fungal biomass (Fig. 5). This decrease in living fungal mass was previously observed for *P. australis* leaf blades (Van Ryckegem et al., 2005; Tanaka 1991, 1993). Those distinct changes in biomass are generally accompanied by a lower productivity rate (Komínková et al., 2000; Kuehn et al., 2000; Newell et al., 1989) and a shift in dominant taxa associated with the standing culms prior to falling on the marsh surface (Van Ryckegem and Verbeken, 2005b,c). Consequently, the change in environmental conditions leads to a shift in the dominance towards estuarine taxa adapted to tidal brackish water conditions (Van Ryckegem & Verbeken, 2005b) (Fig. 7a, b).

However, following to a decrease in fungal biomass after placement of the leaf sheaths on the sediment surface, fungal mass recovered and even surpassed initial standing dead amounts. Together with the steady increase in fungal biomass in stems this suggests an ongoing dominance of fungal decomposers in the litter layer. Although not measured during the present study, bacterial biomass and productivity during initial submergence or decay in the litter layer is generally estimated to be less than 10% of fungal biomass and productivity for *P.*

australis litter in freshwater tidal and submerged conditions (Findlay et al., 2002; Komínková et al., 2000). Furthermore, similar findings are reported for other emergent macrophytes both in freshwater and marine conditions (Findlay et al., 1990; Kuehn et al., 2000; Newell et al., 1995; Newell et al., 1989).

Observed breakdown rates for both leaf sheaths and stems are close to the rates of those mentioned by Gessner (2000) in a eutrophic hardwater lake. Furthermore, in accordance with the results of Gessner (2000) stems resisted detectable breakdown for up to six months after placement in the litter layer. However, litter mass loss probably started sooner but was underestimated because of the accumulation of fungal carbon (see Jones & Worrall, 1995).

Nutrient and plant polymer dynamics: fungal involvement

Increased total nutrient stocks in the litter bags (Fig. 3) and the absence of any substantial nutrient loss (by leaching and/or mineralization) suggest, notwithstanding the firm loss of dry mass during the study period (Fig. 2), that nutrients were either immobilized into microbial biomass, bound to the remaining plant matter, or that losses were (over)compensated by fresh inputs.

The large nitrogen sequestration by both plant parts may be largely accounted for by immobilization in fungal biomass (Fig. 8). Using a conversion factor of 6.5% N of fungal dry mass (Findlay et al. 2002) we estimated that nearly all of total litter nitrogen was present as fungal biomass in leaf sheaths at the start of the experiment and that it levelled off to an average proportion of 64 ± 13% (mean ± STDEV, N = 33) (Fig. 8a). Furthermore, litter N associated with fungal mycelia is probably higher because our reported fungal mass is measured as living biomass. A substantial portion of detrital N could by associated with residual dead fungal mycelium (i.e. N-acetyl-glucosamine). Nonliving fungal mass within decaying litter may be twice that of living fungal biomass (Newell et al., 1995; Newell, 1996). Within stems the contribution of fungal N to the total N pool in litter gradually increases and is estimated to retain most of the available nitrogen (102 ± 15%; mean fungal N for month 6-16, ± STDEV, N = 37) in the decomposing stem litter (Fig. 8b). The steady increase in the proportion of fungal N, while total nitrogen concentrations remained stable in the stem tissue, indicates a gradual translocation of plant litter nitrogen into fungal mycelia. Some of the high estimates for fungal N in stem litter could be due to conversion factors being slightly too high for our fungal community.

Similar to nitrogen, total litter phosphorus was also estimated for fungal P amount. We used a conversion factor at the lower end of the values mentioned in Table 1 of Beever & Burns (1980) for eumycotic mycelial growth in nutrient poor conditions (P = 0.4% fungal dry mass). Comparable to nitrogen, it was estimated that nearly all of the litter P was incorporated in fungal tissue before standing leaf sheaths entered the litter layer. After placement in the litter layer, the average proportion of fungal P was reduced and estimated to be on average 37 ± 9% (mean ± STDEV, N = 33) (Fig. 8c).

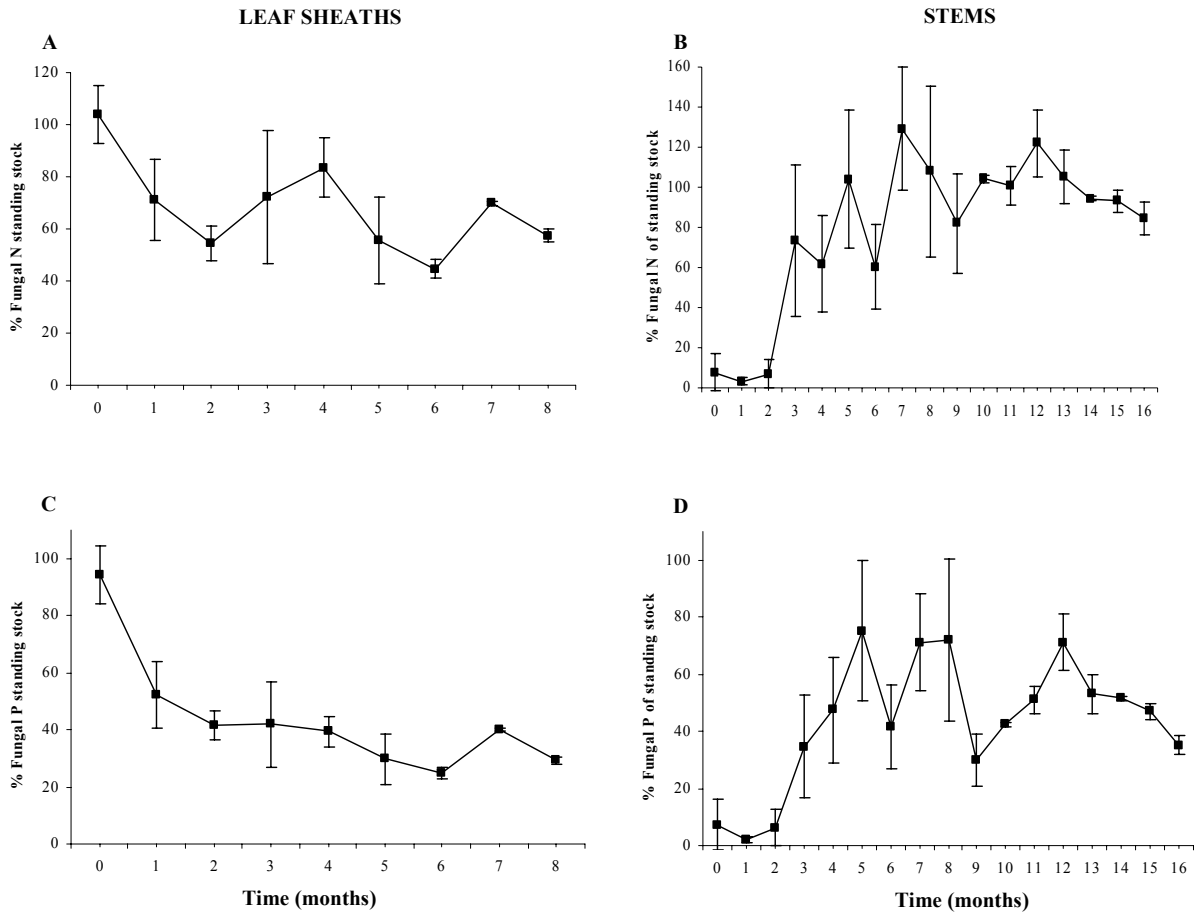


Figure 8. Estimated amount of the standing stock of total detrital nitrogen incorporated in fungal biomass in leaf sheaths (A) and stems (B) and estimated amount of the standing stock of total detrital phosphorus incorporated in fungal biomass in leaf sheaths (C) and stems (D) of *Phragmites australis* incubated on the sediment surface of a tidal brackish marsh. Error bars indicate means ± STDEV [*N* = 1 (no error bar) to 4].

In stem tissue the proportion of P incorporated in fungal biomass reflects the increasing fungal biomass (Fig. 5). This resulted in a maximal P capture of 75 ± 25% in May by the fungal hyphae in the litter (mean ± STDEV, *N* = 4) (Fig. 8d). As gradually total P concentrations increased, the proportion of P in fungal living mass during breakdown was slightly reduced and accounted for 52 ± 15% (mean ± STDEV, *N* = 37) of total P during decay.

Fungi can immobilize nutrients from the external environment (Suberkropp, 1995; Suberkropp & Chauvet, 1995) or they manage their growth by translocation of internal nutrient sources. If we accept as a first approximation an overall stable nutrient concentration within fungal tissues between species and during decay, there are no indications for nutrient immobilization from the environment within the leaf sheaths by fungi. Sheaths are nutrient rich compared to stems. Within this nutrient rich tissue it appears that the amount of original

nutrients stocks are always higher than the amounts of nutrients incorporated within fungal living mass during decay (Fig. 8a, c). Within leaf sheaths, fungal biomass was estimated to contain on average 57 ± 11% and 51 ± 10% of the original stock of N and P respectively. This suggests that leaf sheath mycota are not limited by nitrogen or phosphorus, and possibly other factors (such as C availability) limit the growth of fungi. Contrary to leaf sheaths, stems with lower initial N and P concentrations seem to show a net immobilization from the external environment of both N and P, which seems at least partly due to fungal colonization. This is suggested by the higher nutrient concentrations in fungal biomass during decay compared to the initial nutrient pool available in reed stems. Fungal biomass was estimated to contain on average 129 ± 21% and 123 ± 20% respectively of the original stock of N and P. However, within the stems N seems to be the limiting factor for fungal growth as all available N seems to be incorporated in the fungal tissue (see above). In contrast to N, the proportion of the standing stock of phosphorus incorporated in fungal biomass was far lower than the amounts present in the detrital mass. This suggests that other mechanisms are responsible for increased P concentrations in stems (Fig. 3b). P concentrations are characterized by a rise in August, and are maintained for a couple of months. This maximal P concentration in the stem tissue coincides with abundant ascocarp formation followed by vigorous sporulation of *Massarina arundinacea* (Van Ryckegem & Verbeken, 2005c; pers. obs.). Because ascocarps and spores generally have a higher phosphorus concentration compared to the vegetative mycelium (Beever & Burns, 1980) it is tempting to suggest that fungi have a higher phosphorus demand during reproduction. This observation may imply that the conversion factor was too low during periods of fungal sporulation, and, hence the estimated fungal P in the detrital mass itself may be too low. Another indication that our P concentration in fungal tissues might be higher during the summer comes from observations made by Grattan & Suberkropp (2001). The authors found an increased P concentration in fungal tissue with increased P availability in the surroundings. P availability is also higher in our study site during summer: i) P load of the tidal exchange water is increasing during summer; during this season a rise of about 0.2 mg/l PO_4^{-3} compared to the winter is noticed (Van Damme et al., 1995). ii) At the moment of a sharp increase in P concentrations in the stems (coinciding with a start of abundant sporulation) leaf sheaths start to mineralize phosphorus (Fig. 3c).

However, other factors such as complexation and adsorption of P to the detrital mass may explain the summer rise in P concentrations in stems (Fig. 3a). Whichever factor(s) is (are) responsible for the observed patterns, the above discussion illustrates the need to obtain, and test, available conversion factors during decomposition in relation to changing environmental circumstances.

Furthermore, fungi may impact carbon and nutrient dynamics within the studied site by their luxurious sporulation (Van Ryckegem & Verbeken, 2005b,c) and hence a substantial part of the assimilated carbon and nutrients may be expulsed in the form of spores or conidia within the environment (Dowding, 1981; Newell & Wasowski, 1995; Newell, 2001). Newell & Wasowski (1995) estimated the fraction of total fungal production allocated to ascomata at 9%,

and Newell (2001) estimated a minimal allocation of 4.5% fungal year production towards spores (7.5 g spore mass m^{-2} y^{-1}). Although the influence of carbon loss from an ecosystem by means of spore expulsion can thus be substantial, the impact on nutrient amounts within the detrital pool is proportionally higher, as they are incorporated in high concentrations within the diasporas (e.g. Beever & Burns, 1980; Dowding, 1981).

Factors related to fungal succession

Multivariate analysis of both species data sets indicated that cellulose concentration is the best variable explaining in a statistical way the sequential sporulation of the species on the decaying resources (stems and leaf sheaths) (Tables 7, 8). Nutrients (N, P) were indicated to be the driving forces behind fungal growth and productivity if carbon is not limiting (see e.g. Newell et al., 1996). Perhaps carbon availability is a variable that is more important in regulating fungal life history and eventually succession. Depletion of carbon resources and/or changes in the proportions of the different carbon substrates (mainly cellulose, lignin, tannins and chitin) may trigger fungal reproduction. Furthermore, carbon depletion will increase niche overlap and hence increases competition between species. This will possibly favour species with high antibiotic activity and the widest range of enzymatic capabilities (Swift, 1976). Illustrative for this changed carbon availability is the appearance of hypersaprotrophic species. Such a species is *Halosphaeria hamata* (Kohlmeyer & Kohlmeyer, 1979) which becomes dominant in the latest phase of decay (Table 1; Van Ryckegem & Verbeken, 2005b,c). These species use the luxurious amount of dead fungal tissues (mainly chitin) in the depleted litter as carbon source. This dead fungal mass is probably substantial and may be 2-fold that of living mass (e.g. Newell, 1992; Newell et al., 1995).

Acknowledgements

We thank R. Heynderickx and A. Van Heuverswijn for assistance with making litter bags and Dirk Van Gansbeke (Ghent University, Laboratory of Marine Biology) for nitrogen and phosphorus analyses. Prof. Dr. Mark Stevens (Ghent University, Laboratory of Wood Technology) kindly offered facilities to perform acid detergent fibre analysis. Bernhard De Meyer is warmly acknowledged for practical assistance during lignin determination. Thanks to Steven Newell for commenting on a previous version of this manuscript. This research was funded by the Institute for the Promotion of Innovation by Science and Technology in Flanders, Belgium, through a grant to G.V.R.

References

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1972). Colonisation of *Phragmites communis* leaves by fungi. Nova Hedwigia 23: 113-124.

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1975). Microfungi colonizing nodes and internodes of aerial standing culms of *Phragmites communis* Trin. Nova Hedwigia 26: 495-507.

BEEVER, R.E. & BURNS, D.J.W. (1980). Phosphorus uptake, storage and utilization by fungi. Adv. Bot. Res. 8: 127-219.

BILLS, G.F. (1996). Isolation and analysis of endophytic fungal communities from woody plants. *In* REDLIN, S.C. & CARRIS, L.M. (EDS.) Endophytic Fungi in Grasses and Woody Plants. American Phytopathological Press, St.-Paul, MN. 31-65.

BOULTON, A.J., BOON, P.I. (1991). A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over an old leaf? Aust. J. Mar. Freshwat. Res. 42: 1-43.

DOWDING, P. (1981). Nutrient uptake and allocation during substrate exploitation by fungi. *In* WICKLOW, D.T. & CAROLL, G.C. (EDS.) The fungal community, its organization and role in the ecosystem. Marcel Dekker, New York. 621-635.

ENRIQUEZ, S., DUARTE, C.M. & SAND-JENSEN, K. (1993). Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94: 457-471.

FINDLAY, S., HOWE, K. & AUSTIN, H.K. (1990). Comparison of detritus dynamics in two tidal freshwater wetlands. Ecology 71: 288-295.

FINDLAY, S.E.G., DYE, S. & KUEHN, K.A. (2002). Microbial growth and nitrogen retention in litter of *Phragmites australis* compared to *Typha angustifolia*. Wetlands 22: 616-625.

GESSNER, M.O. (2000). Breakdown and nutrient dynamics of submerged *Phragmites* shoots in the littoral zone of a temperate hardwater lake. Aquatic Bot. 66: 9-20.

GESSNER, M.O. (2001). Mass loss, fungal colonisation and nutrient dynamics of *Phragmites australis* leaves during senescence and early decay. Aquatic Bot. 69: 325-339.

GESSNER, M.O. & CHAUVET, E. (1994). Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75: 1807-1817.

GESSNER, M.O. & SCHMITT, A.L. (1996). Use of solid-phas extraction to determine ergosterol concentrations in plant tissue colonized by fungi. Appl. Environ. Microbiol. 62: 415-419.

GESSNER, M.O. & VAN RYCKEGEM, G. (2003). Water fungi as decomposers in freshwater ecosystems. *In* BITTON, G. (ED.) Encyclopedia of Environmental Microbiology. Wiley, New York. 3353-3364.

GESSNER, M.O., SCHIEFERSTEIN, B., MÜLLER, U., BARKMANN, S. & LENFERS, U.A. (1996). A partial budget of primary organic carbon flows in the littoral zone of a hardwater lake. Aquatic Bot. 55: 93-105.

GESSNER, M.O., SUBERKROPP, K. & CHAUVET, E. (1997). Decomposition of plant litter by fungi in marine and freshwater ecosystems. *In* WICKLOW, D.C. & SÖDERSTRÖM, B. (EDS.) The Mycota IV, Environmental and microbial relationships. Springer-Verlag, Berlin Heidelberg. 303-322.

GRAÇA, M.A., NEWELL S.Y., KNEIB, R.T. (2000). Grazing rates of organic matter and living fungal biomass of decaying *Spartina alterniflora* by three species of salt-marsh invertebrates. Mar. Biol. 136: 281-289.

GRANÉLI, W. (1990). Standing crop and mineral content of reed, *Phragmites australis* (Cav.) Trin. ex Steud., in Sweden - management of reed stands to maximize harvestable biomass. Folia Geobot. Phytotax. 25: 291-302.

GRATTAN II, R.M. & SUBERKROPP, K. (2001). Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. J. N. Am. Benthol. Soc. 20: 33-43.

HASLAM, S.M. (1972). Biological flora of the British Isles no. 128, *Phragmites communis* Trin. J. Ecol. 60: 585-610.

HATFIELD, R.D., JUNG, H.-J. G., RALPH, J., BUXTON, D.R. & WEIMER, P.J. (1994). A comparison of the insoluble residues produced by the Klason lignin and acid detergent lignin procedures. J. Sci. Food Agric. 65: 51-58.

HIETZ, P. (1992). Decomposition and nutrient dynamics of reed (*Phragmites australis* (Cav.) Trin. ex Steud.) litter in Lake Neusiedl, Austria. Aquatic Bot. 43: 211-230.

JONES, H.L. & WORRALL, J.J. (1995). Fungal biomass in decayed wood. Mycologia 87: 459-466.

JONGMAN, R.H.G., TER BRAAK, C.J.F. & VAN TONGEREN, O.F.R. (1987). Data analysis in community and landscape ecology. Pudoc, Wageningen.

JUNG, H.-J. G., VAREL, V.H., WEIMER, P.J. & RALPH, J. (1999). Accuracy of Klason lignin and acid detergent lignin methods as assessed by bomb calorimetry. J. Agric. Food Chem. 47: 2005-2008.

KIRK, T.K. & OBST, J.R. (1988). Lignin determination. *In* WOOD, W.A. & KELLOGG, S.T. (EDS.) Methods in enzymology - biomass, part b. Lignin, pectin, and chitin. Academic Press, San Diego. 161: 87-101.

KJØLLER, A. & STRUWE, S. (1982). Microfungi in ecosystems: fungal occurrence and activity in litter and soil. Oikos 39: 391-422.

KOHLMEYER, J. & KOHLMEYER, E. (1979). Marine Mycology, the Higher Fungi. Academic Press, New York. 690 pp.

KOMÍNKOVÁ, D., KUEHN, K.A., BÜSING, N., STEINER, D. & GESSNER, M.O. (2000). Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of a large lake. Aquatic Microb. Ecol. 22: 271-282.

KUEHN, K.A., GESSNER, M.O., WETZEL, R.G., SUBERKROPP, K. (1999). Decomposition and CO2 evolution from standing litter of the emergent macrophyte *Erianthus giganteus*. Microb. Ecol. 38: 50-57.

KUEHN, K.A., LEMKE, M.J., SUBERKROPP, K. & WETZEL, R.G. (2000). Microbial biomass and production associated with decaying leaf litter of the emergent macrophyte *Juncus effesus*. Limnol. Oceanogr. 45: 862-870.

KUEHN, K.A., STEINER, D. & GESSNER, M.O. (2004). Diel mineralization patterns of standing-dead plant litter: implications for CO_2 flux from wetlands. Ecology 85: 2504-2518.

MCCUNE, B. & GRACE, J.B. (2002). Analysis of ecological communities. MjM Software design, Gleneden Beach, Or. 304 pp.

MCCUNE, B. & MEFFORD, M.J. (1999). PC-ORD for Windows. Multivariate Analysis of Ecological Data. Version 4.0.. MjM Software, Gleneden Beach, Oregon, U.S.A.

MEGANCK, B. (1998). The morphology of *Phragmites australis* (Cav.) Steud. in relation to hydrodynamics, management and salinity in the Scheldt estuary. Unpublished master thesis (in Dutch). Ghent University, Gent, 97 pp.

MELILLO, J.M., ABER, J.D. & MURATORE, J.F. (1982). Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63: 621-626.

MELILLO, J.M., NAIMAN, R.J., ABER, J.D. & LINKINS, A.E. (1984). Factors controlling mass loss and nitrogen dynamics of plant litter decaying in northern streams. Bull. Mar. Sci 35: 341-356.

MITSCH, W.J. & GOSSELINK, J.G. (2000). Wetlands (3rd edition). Van Nostrand Reinhold, New York. 920 pp.

NEWELL, S.Y. (1992). Estimating fungal biomass and productivity in decomposing litter. *In* CAROLL G.C. & WICKLOW, D.T. The fungal community. Its organisation and role in the ecosystem. 2de ed. Marcel Dekker, New York. 521-561.

NEWELL, S.Y. (1996). Established and potential impacts of eukaryotic mycelial decomposers in marine/ terrestrial ecotones. J. Exp. Mar. Biol. Ecol. 200: 187-206.

NEWELL, S.Y. (2001). Spore-expulsion rates and extents of blade occupation by Ascomycetes of the smooth-cordgrass standing-decay system. Bot. Mar. 44: 277-285.

NEWELL, S.Y., MORAN, M.A., WICKS, R. & HODSON, R.E. (1995). Procutivities of microbial decomposers during early stages of decomposition of leaves of a freshwater sedge. Freshwater Biol. 34: 135-148.

NEWELL, S.Y. & WASOWSKI, J. (1995). Sexual productivity and spring intramarsh distribution of a key saltmarsh microbial sec. producer. Estuaries 18: 241-249.

NEWELL, S.Y., ARSUFFI, T.L. & PALM, L.A. (1996). Misting and nitrogen fertilization of shoots of a saltmarsh grass: effects upon fungal decay of leaf blades. Oecologia 108: 495-502.

NEWELL, S.Y., FALLON, R.D. & MILLER, J.D. (1989). Decomposition and microbial dynamics for standing, naturally positioned leaves of the salt-marsh grass *Spartina alterniflora*. Mar. Biol. 101: 471-481.

NEWELL, S.Y., MORAN, M.A., WICKS, R. & HODSON, R.E. (1995). Procutivities of microbial decomposers during early stages of decomposition of leaves of a freshwater sedge. Freshwater Biol. 34: 135-148.

PALM, C.A. & ROWLAND, A.P. (1997). A minimum dataset for characterization of plant quality for decomposition. *In* CADISCH, G. & GILLER, K.E. Driven by nature: plant litter quality and decomposition. CAB International, Oton. 379-392.

PALMER, M.W. (1993). Putting things in even better order: the advantages of canonical correspondence analysis. Ecology 74: 2215-2230.

PARBERY, D.G. (1996). Trophism and ecology of fungi associated with plants. Biol. Rev. Cambridge Philos. Soc. 71: 473-527.

PIECZYŃSKA, E. (1972). Ecology of the eulittoral zone of lakes. Ekol. Polska 20: 637-732.

POLUNIN, N.V.C. (1982). Processes contributing to the decay of reed (*Phragmites australis*) litter in freshwater. Arch. Hydrobiol. 94: 182-209.

POLUNIN, N.V.C. (1984). The decomposition of emergent macrophytes in fresh water. Adv. Ecol. Res. 14: 115-166.

RICE, W.R. (1990). A consensus combined P-value test and the family-wide significance of component tests. Biometrics 46: 303-308.

RODEWALD, L. & RUDESCU, L. (1974). Das Schilfrohr. Die Binnengewässer Band XXVII: 1-302.

SOETAERT, K., HOFFMANN, M., MEIRE, P., STARINK, M., VAN OEVELEN, D., VAN REGENMORTEL, S. & COX, T. (2004). Modeling growth and carbon allocation in two reed beds (*Phragmites australis*) in the Scheldt estuary. Aquatic Bot. 79: 211-234.

SUBERKROPP, K. (1995). The influence of nutrients on fungal growth, productivity, and sporulation during leaf breakdown in streams. Canad. J. Bot. Supll. 1: 1361-1369.

SUBERKROPP, K. & CHAUVET, E. (1995). Regulation of leaf breakdown by fungi in streams: influences of water chemistry. Ecology 76: 1433-1445.

SWIFT, M.J. (1976). Species diversity and the structure of microbial communities in terrestrial habitats. *In* ANDERSON, J.M. & MACFADYEN, A. (EDS.) The role of terrestrial and aquatic organisms in decomposition processes. Blackwell Scientific, Oxford. 185-222.

TANAKA, Y. (1991). Microbial decomposition of reed (*Phragmites communis*) leaves in a saline lake. Hydrobiologia 220: 119-129.

TANAKA, Y. (1993). Aerobic cellulolytic bacterial-flora associated with decomposing *Phragmites* leaf-litter in a seawater lake. Hydrobiologia 263: 145-154.

TER BRAAK, C.J.F. & SMILAUER, P. (2002). CANOCO Reference manual and CanoDrow for Windows User's guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power, Ithaca, NY, USA. 500 pp.

VAN DAMME, S., MEIRE, P., MAECKELBERGHE, H., VERDIEVEL, M., BOURGOING, L., TAVERNIERS, E., YSEBAERT, T. & WATTEL, G. (1995). De waterkwaliteit van de Zeeschelde: evolutie in de voorbije dertig jaar. Water 85: 244-256.

VAN DAMME, S., YSEBAERT, T., MEIRE, P. & VAN DEN BERGH, E. (1999). Habitatstructuren, waterkwaliteit en leefgemeenschappen in het Schelde-estuarium. Rapport IN 99/24, Instituut voor Natuurbehoud, Brussels, 119 pp.

VAN RYCKEGEM, G., GESSNER, M.O. & VERBEKEN, A. (2005). Decomposition and fungal diversity and succession on leaf blades of common reed (*Phragmites australis*) in a brackish tidal marsh. (target Mycologia).

VAN RYCKEGEM, G. & VERBEKEN, A. (2005a). Fungal diversity and community structure on *Phragmites australis* (Poaceae) along a salinity gradient in the Scheldt estuary (Belgium). Nova Hedwigia 80: 173-197.

VAN RYCKEGEM, G. & VERBEKEN, A. (2005b). Fungal ecology and succession on *Phragmites australis* in a brackish tidal marsh. I. Leaf sheaths. Fungal Div. 19 (in print).

VAN RYCKEGEM, G. & VERBEKEN, A. (2005c). Fungal ecology and succession on *Phragmites australis* in a brackish tidal marsh. II. Stems. Fungal Div. (accepted).

VAN RYCKEGEM, G. & VERBEKEN, A. (2005d). The complex fungal community structure associated with above ground plant parts of *Phragmites australis* in a brackish tidal marsh. (target Mycol. Res.).

VAN SOEST, P.J. & ROBERTSON, J.B. (1980). Systems of analysis for evaluation fibrous feeds. *In* PIGDEN, W.J., BALCH, C.C. & GRAHAM, M. Standardization of Analystical Methodology for Feeds. International Development Center, Ottowa, Canada. 49-60.

VAN SOEST, P.J. & WINE, R.H. (1967). Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. J. Assn. Offic. Anal. Chem. 50: 50-55.

WEBSTER, J. (1956). Succession of fungi on decaying cocksfoot culms. I. J. Ecol. 44: 517-544.

WEBSTER, J.R. & BENFIELD, E.F. (1986). Vascular plant breakdown in freshwater ecosystems. Ann. Rev. Ecol. Syst. 17: 567-594.

ZAR, J.H. (1996). Biostatistical analysis (3rd edition). Prentice-Hall, Upper Saddle River, New Jersey. 662 pp.

Metal accumulation in intertidal litter through decomposing leaf blades, sheaths and stems of P*hragmites australis*

Gunther Van Ryckegem[+#], Gijs Du Laing[*#], Filip M.G. Tack[*] and Marc G. Verloo[*]

#Authors contributing equally to this research paper

Submitted to Chemosphere

Abstract As many marshes are contaminated with metals, a better understanding of the influences of vegetation on metal retention is needed. This study aims to assess the role of decomposing reed plant material in wetland metal cycles. Metal contents of decomposing leaf blades, stems and sheaths of reed plants (*Phragmites australis*) were monitored by a standard litter bag method in an intertidal zone of the Scheldt estuary (Belgium). Most metal contents in reed litter increased considerably during decomposition. As reed biomass turnover is also very high, metal accumulation by litter could be a very important variable to monitor and take into account when discussing metal transfer at the base of the food chain in intertidal reed beds. There are indications that fungal activity is an important factor determining metal accumulation in decomposing stem tissue. This could also be the case for leaf blades, but for this tissue type the effect of fungal activity on metal concentrations is found to be overridden by passive metal sorption and trapping of sediment particles and associated metals. Both factors seem to be of intermediate importance for leaf sheath tissue.

Introduction

Heavy metals are introduced into wetlands by discharge of industrial and urban sewage or through atmospheric deposition. Wetland sediments are generally considered to be a sink for metals anthropogenically introduced into the environment (Hart, 1982). A major fraction of the elements entering the sediment system will rapidly be fixed onto the solid phase in the sediments, where a number of physical and chemical properties will determine the strength of metal retention. Physical properties include texture (proportion of sand, silt and clay), and to some extent the type of clay minerals. Chemical properties include organic matter content, pH, redox conditions and cation exchange capacity (Gambrell, 1994). A small proportion of the metals dissolves and becomes available for plant uptake. Plant uptake directly reduces the input of metals into adjacent waters. Plants can especially provide a sink if during decomposition metals are bound to the litter by passive sorption on organic surfaces or by physiological mechanisms of microbial colonizers of the litter. However, the litter can also act as a metal source when microbial activity mobilizes metals or when it becomes available to deposit feeders. Several studies suggest that metals in litter are available to deposit feeders and, thus, can enter estuarine food webs (Weis and Weis, 2004). Consumption of metal-laden detritus can

cause metal accumulation and deleterious effects in higher trophic levels (Dorgelo et al., 1995; Du Laing et al., 2002; Weis et al., 2002). The role of plant sequestration of metals into long-term sinks depends on the rate of uptake into the plant, rates of translocation and retention within individual tissue types, and the rate and mode of tissue decomposition (Catallo, 1993; Kadlec and Knight, 1996).

Metal uptake and distribution has been examined in *Phragmites australis* (Cav.) Trin. ex Steud. (common reed) (Larsen and Schierup, 1981; Schierup and Larsen, 1981; Gries and Garbe, 1989; Peverly et al., 1995; Keller et al., 1998; Windham et al., 2003). All studies report the highest amounts of metals in the roots, while leaf tissue has the second highest concentrations followed by stems and rhizomes. A small fraction of metals is released in the environment through leaf tissue during the growing season (Burke et al., 2000). Once the reed tissue enters the litter layer and decomposition proceeds, increasing metal concentrations can be observed (Larsen and Schierup, 1981). Besides the uptake of metals by organisms involved in decay, the fate of metals after organic matter decomposition is unsure. Several authors claim that upon mineralization, the metals previously bound to the organic matter will be remobilised into the environment (Alloway, 1995). Others claim that metals will be transferred from the more available fractions to e.g. highly insoluble organic complexes with strongly humified litter and eventually become buried as long-term sinks (Paré et al., 1999).

This study aims to assess the role of decomposing organic matter in wetland metal cycles. Heavy metal contents of decomposing leaf blades, stems and sheaths were monitored by a standard litter bag method in an intertidal zone of the Scheldt estuary. Common reed was selected as the test plant species as this is a widespread, dominant species in many aquatic ecosystems. It forms dense stands that are among the most productive ecosystems in temperate areas. Moreover, reed plants are only lightly grazed in the living state and the greatest part of the primary production ultimately enters detrital systems (Polunin, 1982).

Materials and methods

Study site

The study was carried out in a brackish tidal marsh of the Scheldt estuary called 'Schor van Doel'. It faces the 'Hertoginpolder' close to 'Saeftinghe Marsh', just across the Belgium border in The Netherlands (51° 21' N, 4° 14' E). It is vegetated by a monospecific stand of common reed, *Phragmites australis*. Dynamics of the reed vegetation in our study site is modelled by Soetaert et al. (2004). Leaf blade, leaf sheath and stem biomass production was found to be 320 ± 131, 125 ± 16 and 445 ± 16 g m^{-2}, respectively (mean ± standard deviation; Van Ryckegem et al., in preparation). The estimated sedimentation rate is about 3 cm $year^{-1}$ (Meganck, 1998; Van Damme et al., 1999). Metal contents in the upper 1 m sediment layer were found to be in the range 0.11 - 4.47 mg kg^{-1} DW for Cd, 19 - 213 mg kg^{-1} DW for Cr, 0.2

- 90.9 mg kg^{-1} DW for Cu, 3.9 - 98.9 mg kg^{-1} DW for Ni, 7 - 152 mg kg^{-1} DW for Pb and 24 - 377 mg kg^{-1} DW for Zn. Organic matter and carbonate contents varied from 1.9 to 22.1% and from 3.3 to 12.9%, respectively (w/w). Ground water had conductivities between 0.42 and 7.96 mS cm^{-1}, pH between 7.1 and 8.2 and chloride contents between 0.4 and 11.4 g kg^{-1} DW. All metal, carbonate, organic matter and chloride contents were highest in the upper 5 cm sediment layer. The lowest concentrations were observed at higher depths (between 60 and 100 cm below the surface).

Experimental setup

The presented results in this study are part of a larger project describing biotic and abiotic dynamics associated with *P. australis* during decay. The organic matter breakdown dynamics, with changing nitrogen, phosphorus and plant polymer concentrations and specific microbial colonization patterns are discussed by Van Ryckegem et al. (in preparation) and Van Ryckegem and Verbeken (in preparation). Summarized the experimental setup was as follows: brown lower leaves were collected from the marsh prior to shedding in October 2001; culms were collected in December 2001 by cutting out sections comprising two nodes and one internode 1 m above the sediment, with the leaf sheath surrounding the stem. Plastic litter bags (35x20 cm; mesh 4 mm) were filled with 5.0 g fresh weight of leaves and 50.0 g fresh weight of cut culm sections and anchored by hooked bars on the sediment the day after collecting. On monthly intervals 2 litter bags of each type (leaves and culms) were retrieved from the marsh. Samples were immediately transported in a cool box to the laboratory, where they were gently but thoroughly rinsed with distilled water to remove adhering clay and macro-invertebrates. Stems and leaf sheaths were separated and processed separately during the entire study. All samples were dried at 40°C during 72 h, weighed and analysed for ash and heavy metal contents. Whereas during the experiment two samples were retrieved from the marsh and analysed at each sampling time, initial ash and heavy metal contents were determined on five samples of leaves, stems and sheaths. Monitoring was continued over a period of 11 months for the leaves and 16 months for the culms.

Analyses

Mass loss data were fitted to the exponential model, $m_t = m_0 e^{-kt}$, where m_t is the litter dry mass remaining after time t, m_0 is the original mass and k the breakdown coefficient* (Van Ryckegem et al., in preparation; Van Ryckegem and Verbeken, in preparation). Analysis of covariance (ANCOVA; general linear model procedure, SAS Statistical Package version 8.2, 1999) on log-transformed data was used to compare the breakdown rates between litter types (Boulton and Boon, 1991). Ash contents were determined by measuring the weight loss after incineration of the oven dried samples (4 h at 450°C). For the analyses of metal contents, samples were

* Same litterbags used as those reported on in Chapter 5 and 6

ground in a hammer-cross beater mill and homogenised. Five gram of each sample was weighed to the

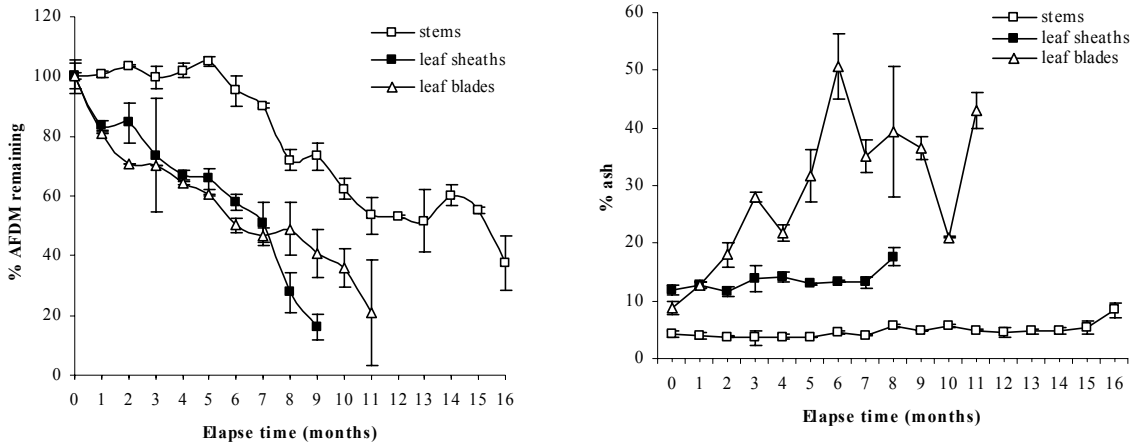


Figure 1. Ash-free dry mass (AFDM) remaining and ash content (%, mean ± standard deviation) in the litter bags during decomposition of stems, leaf sheaths and leaf blades of *Phragmites australis* in a brackish tidal marsh. Experiment started in December 2001 for leaf sheaths and stems and in October 2001 for leaf blades.

nearest 0.1 mg on an analytical balance (Sartorius BP221S, Sartorius, Göttingen, Germany) into a 100 mL pyrex beaker and treated with 10 mL ultra-pure 65 % HNO_3. The beaker was covered with a watch-glass and the suspension was heated up to 130°C for 1 h. A total amount of 4 ml 20 % H_2O_2 was added in aliquots of 1 mL. After cooling, the suspension was filtered (S&S, blue ribbon) in a 50-mL volumetric flask and diluted to the mark. In all of the extracts, Cd, Cu, Cr, Ni, Pb and Zn contents were measured using F-AAS (flame atomic absorption spectrometry, Varian SpectrAA-1475, Palo Alto, CA, USA) or GF-AAS (graphite furnace atomic absorption spectrometry, Varian SpectrAA-800 / GTA-100, Palo Alto, CA, USA). The analyses were performed according to Du Laing et al. (2003). Du Laing et al. (2003) also extensively reported analysis results of standard reference material and the method detection levels. The concentrations of Fe, Mn, Ca, Mg, K and Na were also measured as they can interact with adsorbed fractions of heavy metals. F-AAS was used to measure Fe, Mn, Ca and Mg contents and flame emission photometry (Eppendorf Elex 6361, Hamburg, Germany) to measure K and Na contents. Statistical analyses consisted of the calculation of correlations according to the Pearson correlation coefficients method, using SPSS 12.0 (2003). Modified boxplots were constructed using SPSS 12.0 (2003) to represent distribution of data graphically.

Results and discussion

Weight loss and organic matter decomposition

The residual ash-free dry mass (AFDM) is depicted in Fig. 1. The time for 50% weight loss was approximately 7 months for sheaths, 7 months for leaf blades and 15 months for stems. This coincides with exponential breakdown rates k of 0.0039, 0.0035 and 0.0026 for respectively leaf sheaths, leaf blades and stems (Van Ryckegem et al., in preparation; Van Ryckegem and Verbeken, in preparation). The breakdown coefficient given for stems presents the decay rate after the initial lag period of six months during which there was no decomposition. ANCOVA suggested no difference in breakdown rate between leaf sheaths and leaf blades ($P = 0.10$, $F_{1,46} = 2.75$), while stems showed a significantly slower breakdown compared to both leaf blades and sheaths ($P = 0.0002$, for stem-leaf sheath comparison, $F_{1, 41} = 16.38$, plantpart*time; $P = 0.02$, for stem-leaf blade comparison, $F_{1, 45} = 5.57$, plantpart*time; with the lag period kept out of the analysis), a feature noticed before (e.g. Hietz, 1992; Gessner, 2000).

The ash content of the leaf blades increased considerably during the first six months of decomposition, from 7 to around 50%, whereas the ash contents of the stems and the sheaths did not vary much during the experimental period, 4.7 ± 1.2% and 12.6 ± 1.1% for stems and sheaths, respectively (mean ± SD) (Fig 1). The high ash content of leaf blade samples compared to other plant parts is probably caused by the large surface area and specific decay characteristics. Leaf blades show a decay pattern which is visually characterized by a collapse and removal by shredder invertebrates of leaf mesophyll between the longitudinal vascular bundles (see Fig. 8 in Polunin, 1982). Between those fine ridges of vascular bundles, fine clayey sediments infiltrate and accumulate, making leaves efficient traps for inorganic substances or mud particles difficult to remove. Ash content of stems and, more pronounced, of leaf sheaths gradually increases during the decomposition process. This is probably due to the softening of the tissue becoming more susceptible to mud infiltration and invertebrate shredder activity causing micro relief at the tissue surface.

Metal contents

Initial metal contents in leaf blades, leaf sheaths and stems are presented in Table 1. Except for Cr and Zn, initial metal concentrations are lowest in stem tissue.

The evolutions of the metal contents in the leaf blades, sheaths and stems are depicted in Fig. 2. Most concentrations expressed on dry weight basis increased considerably during the decomposition. As reed biomass turnover is also very high, metal accumulation by litter could be a very important variable to monitor and take into account when discussing metal transfer at the base of the food chain in intertidal reed beds. In the studied marsh one of the main primary consumers of reed litter, and hence an important channel of metal transfer in the ecosystem, is the gastropod *Assiminea grayana* (Van Ryckegem et al., in preparation).

Table 1. Initial metal contents of leaf blades, stems and sheaths (mg kg^{-1} DW, mean ± standard deviation, n = 5)

	Leaf blades	Stems	Sheaths
Cd	0.159 ± 0.016	0.033 ± 0.008	0.146 ± 0.023
Cr	1.48 ± 0.53	1.42 ± 0.29	1.39 ± 0.37
Cu	6.26 ± 0.25	2.95 ± 0.55	8.89 ± 1.54
Ni	1.32 ± 0.35	0.45 ± 0.14	0.82 ± 0.23
Pb	4.62 ± 0.87	0.34 ± 0.08	3.12 ± 0.51
Zn	40.9 ± 6.1	38.4 ± 14.1	35.3 ± 1.7

Increases of metal concentrations in plant litter were also observed by other authors. Larsen and Schierup (1981) found increasing Zn, Pb and Cd concentrations in *Phragmites* leaf blades during decomposition in the littoral zone of a sewage-polluted and a non-polluted lake. However, Cu contents were relatively constant.

Increasing metal concentrations do not prove that the litter bags act as a sink for metals. Therefore, the overall metal pools in the decomposing organic matter were estimated by multiplying the measured litter weights by their metal concentrations. The calculated amounts after 7 months, associated with leaf blades, stems and sheaths, are presented in Table 2 as a proportion to the initial amounts. Generally, a very important net metal inflow could be observed during these first 7 months. The inflow was highest for leaf blades. After 7 months, metal amounts associated with leaf blades were between 5.3 and 12.9 times higher than the initial amounts. Leaf sheath tissue accumulated up to between 1.5 and 13.3 times the initial amounts, whereas this proportion was found to be between 0.9 and 7.4 for stems. These data indicate that the litter bags were indeed important sinks for heavy metals.

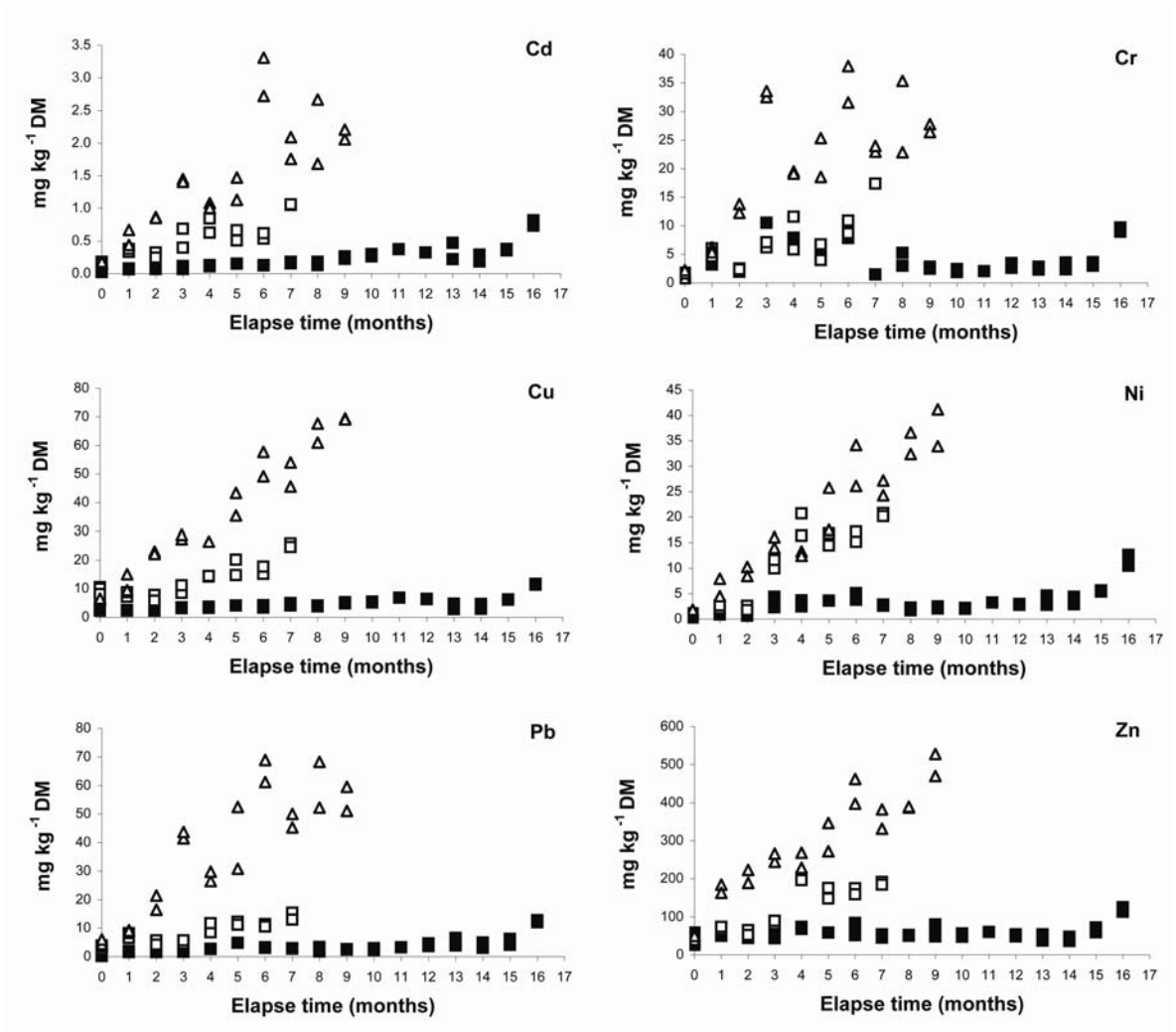


Figure 2. Evolution of metal contents (mg kg^{-1} DW) during decomposition of stems, leaf sheaths and leaf blades of *Phragmites australis* in a brackish tidal marsh. Experiment started in December 2001 for leaf sheaths and stems and in October 2001 for leaf blades.

Table 2. Calculated mean metal amounts after 7 months, associated with leaf blades, stems and sheaths, expressed as a proportion to their original stock

	Leaf blades	Stems	Sheaths
Cd	8.0	4.5	3.8
Cr	10.5	0.9	6.6
Cu	5.3	1.4	1.5
Ni	12.9	5.4	13.3
Pb	6.8	7.4	2.4
Zn	5.8	1.2	2.8

Increases of the metal contents could be attributed to different factors, such as contamination by sediment particles, passive sorption onto recalcitrant organic fractions and active accumulation by e.g. microbial colonizers (Breteler et al., 1981; Gadd, 1993; Zawislanski et al., 2001; Kovacova and Sturdik, 2002; Weis and Weis, 2004).

Kufel (1991) however observed decreasing Pb and Mo amounts per litter bag during decomposition. The litter bags used by Kufel however were submerged in littoral water with low metal pollution without contact with the sediment surface, which may account for a less significant inflow of metals. It should be noted that metal accumulation observed in litter bag experiments significantly depends upon the environmental conditions, the location (e.g. submerged or littoral) and mesh size of the litter bags, which makes comparison between different studies very difficult.

The top layer of the marsh sediments can be periodically resuspended by tidal wave action, which increases the risk of infiltration of mud particles into the litter bags. The concentrations of metals in the top layer of the marsh sediment are much higher than those recorded in the initial plant litter. Contamination with sediment particles can therefore easily constitute a major source of error in determining low litter metal concentrations. To test whether metal contents of the litter could be influenced by adhering mud particles, correlations between ash contents and metal concentrations were calculated (Table 3). If increasing ash contents (as presented in Fig. 1) cannot be related to increasing metal contents, trapping of sediment particles is expected to be of little importance. For leaf blades with highly increasing ash contents (Fig. 1), the correlations were significant at the 0.01 level for all elements, whereas for the sheaths and stems the correlations were not significant for most of the elements. The increase of leaf blade metal contents may thus be due to an important infiltration of mud particles, which were not removed by rinsing the leaf blades with distilled water preceding the analyses.

Table 3. Correlations between ash and heavy metal contents in litter bags for leaf blades, stems and sheaths

	Leaf blades	Stems	Sheaths
Cd	0.986 **	0.638 *	0.525 ns
Cr	0.899 **	-0.449 ns	0.540 ns
Cu	0.886 **	0.587 *	0.430 ns
Ni	0.906 **	-0.058 ns	0.707 *
Pb	0.979 **	0.032 ns	0.512 ns
Zn	0.906 **	0.106 ns	0.674 ns

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed); ns Correlation is not significant.

The proportion of metals in ash inflow was also estimated. To this aim, ash inflow at each sampling time was calculated as the difference between ash amounts and initial ash amounts in the litter bags, associated with leaf blades, stems and sheaths, respectively. Similarly, metal inflow at each sampling time was calculated as the difference between metal amounts and initial metal amounts in the litter bags, associated with leaf blades, stems and sheaths, respectively. The percentage of metals in the ash flowed into the litter bags was then calculated at each sampling time for leaf blades, stems and sheaths, respectively: 100*(metal inflow/ash inflow). The values were also calculated for Fe, Mn, Ca, Mg, K and Na as these elements can interact with adsorbed fractions of heavy metals. The distribution of the results is presented in Fig. 3 for leaf blades by means of modified boxplots. For the leaf blades, a substantial ash inflow was observed. The metal concentration in the inflowing ash fraction was found to be extremely high for most of the elements during the first month. Percentages of metals in the ash inflow were much higher for this first month than for the following months. After the first month, the percentages decreased substantially and remained relatively stable (Fig. 3). Assuming that the inflow of ash with relatively low and stable metal concentrations from the second up to the ninth month is primarily due to trapping of certain sediment fractions which do not only contain heavy metals, the inflow of ash with higher metal concentrations during the first month could be attributed to combined trapping of these sediment fractions and a faster physicochemical sorption of dissolved metals onto the remaining organic matter. This hypothesis is strengthened by the fact that simultaneous leaching of K was observed during the first month, resulting in a negative K concentration in the ash inflow and suggesting metal exchange at the litter surface (Fig. 3).

The combination of leaching of K and high inflow of other elements during the first month was also observed for the stems, also suggesting metal exchange at the litter surface in the beginning of the experiment. For leaf sheaths and stems, however, smaller amounts of inflowing ash, and for stems even continuously outflowing ash amounts, were found during the remainder of the experiment. Metal contents in the inflowing and outflowing ash are fluctuating, which again suggests that inflow of inorganic particles is not the major factor

determining metal accumulation by leaf sheaths and stems on medium term. Other mechanisms such as active accumulation by microbial organisms could be more important.

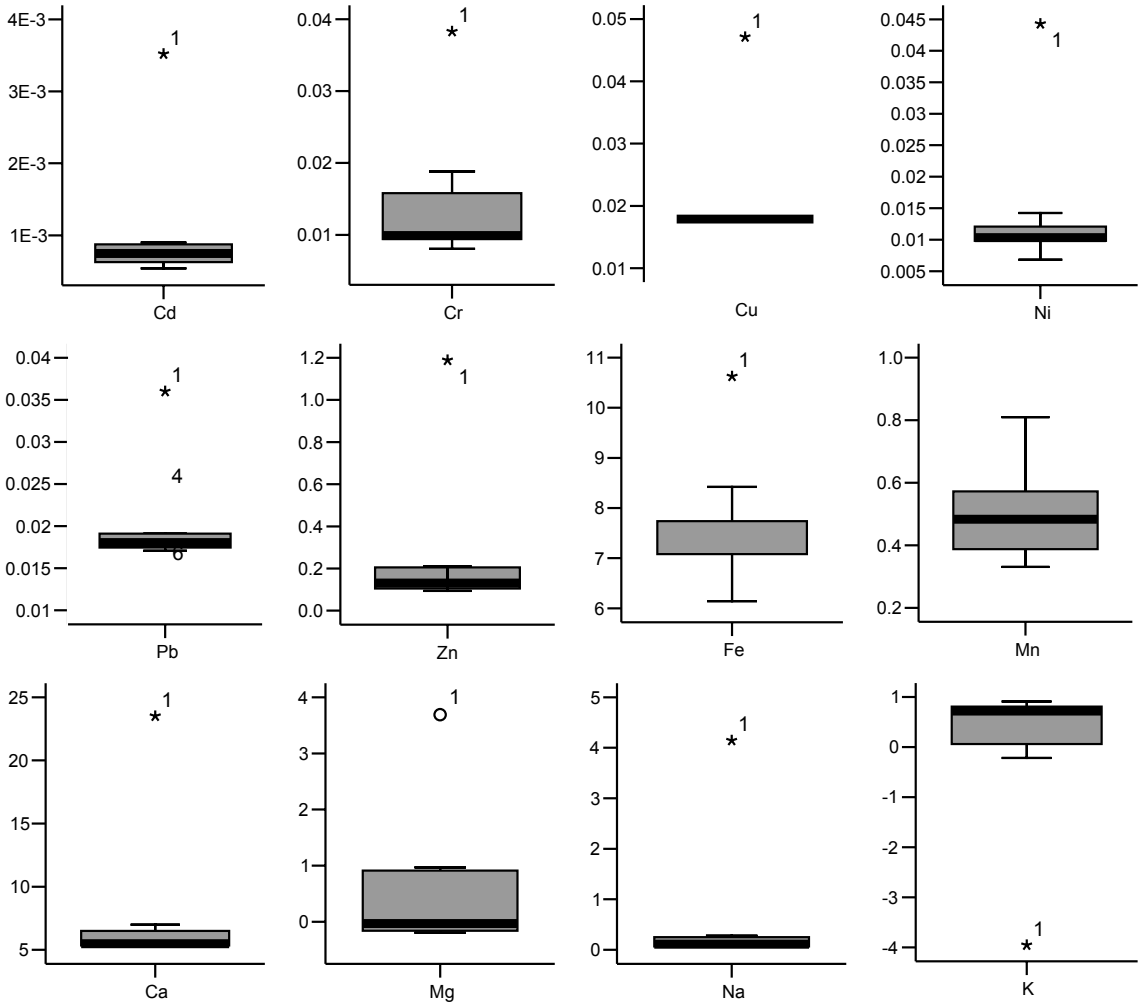


Figure 3. Modified box-plot diagrams showing for leaf blades the distribution of metal concentrations in the ash inflow (%) with the initial values as a basis. The centre line shows the median of the values. Outliers are indicated with a circle and extremes with an asterix. They are labeled by sampling time (number of months from the beginning of the experiment).

To test whether metal accumulation could be due to incorporation by microbial litter colonizers, correlations between metals and fungal biomass (ergosterol concentrations) within the same plant litter were determined. Fungal dynamics in the decomposing litter are discussed by Van Ryckegem et al. (in preparation) and Van Ryckegem and Verbeken (in preparation), demonstrating that fungi are dominant decomposers during aerobic decay of *Phragmites australis*. They contribute up to 10% of ash free litter mass. However, patterns are different between plant parts. Leaf blades had highest fungal colonization in the canopy in a hanging

position prior to shedding. After entering the litter layer fungal biomass decreased spectacular but recovered partially. Leaf sheaths showed a similar pattern but the drop in fungal biomass

Table 4. Correlations between fungal biomass (ergosterol) and heavy metal contents in litter bags for leaf blades, stems and sheaths

	Leaf blades	Stems	Sheaths
Cd	-0.393^{ns}	0.741**	0.660*
Cr	-0.106^{ns}	0.003^{ns}	0.640*
Cu	-0.341^{ns}	0.624**	0.274^{ns}
Ni	-0.280^{ns}	0.491**	0.336^{ns}
Pb	-0.246^{ns}	0.577**	0.201^{ns}
Zn	-0.314^{ns}	0.295^{ns}	0.211^{ns}

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed); ^{ns} Correlation is not significant

after falling was less pronounced. Final concentrations were the highest recorded during decay. Stems showed no detectable fungal colonization in a standing position and fungal biomass gradually increased during the decomposition process in the litter layer. Correlations between fungal biomass (ergosterol concentrations) and metals are depicted in Table 4. No significant correlations were found for leaf blades and low correlations were demonstrated for the leaf sheaths. However, fungal biomass proved to be highly correlated with metal contents in stem tissue except for Cr and Zn, both elements of which the contents stayed relatively stable during the experimental period (Table 2). This correlation suggests an involvement of fungal activity in metal accumulation in stem tissue by a) direct incorporation in fungal mass; b) enhanced binding of metals to the decomposing litter due to complexation between extracellular fungal products and metals (Gadd, 1993); c) by induced changes in litter quality by mineralization, e.g. increasing availability of phenolic units as lignin depolymerises, offering many potential metal-binding sites (e.g. Senesi et al., 1987). Furthermore, correlations could have been higher if an index for total fungal mass was used to asses fungal contribution to metal detainment in litter. This is suggested by the fact that chitin, the main constituent of the fungal cell wall, received attention as a significant metal-biosorbing element (Gadd, 1993). To clarify which mechanisms could drive metal accumulation under influence of microbial decomposers, more specific research is needed.

Conclusion

Most metal contents in reed litter increased considerably during decomposition. As reed biomass turnover is also very high, metal accumulation by litter could be a very important variable to monitor and take into account when discussing metal transfer at the base of the food chain in intertidal reed beds. Fungal activity may be an important factor in immobilizing metals in decomposing stem tissue. This could also be the case for leaf blades, but for this tissue type the effect of fungal activity on metal concentrations is found to be overridden by passive metal sorption and trapping of sediment particles and associated metals. Both factors seem to be of intermediate importance for leaf sheaths.

References

ALLOWAY, B.J. (1995). Soil processes and the behaviour of heavy metals. In: Alloway, B.J. (Ed.). Heavy metals in soils. Blackie Academic & Professional, London, UK, pp. 11-37.

BOULTON, A.J. & BOON, P.I. (1991). A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over an old leaf? Aust. J. Mar. Freshwat. Res. 42: 1-43.

BRETELER, R.J., TEAL, J.M., GIBLIN, A.E. & VALIELA, I. (1981). Trace element enrichments in decomposing litter of *Spartina alterniflora*. Aquatic Bot. 11: 111-120.

BURKE, D.J., WEIS, J.S. & WEIS, P. (2000). Release of metals by the leaves of salt marsh grasses *Spartina alterniflora* and *Phragmites australis*. Estuar. Coast. Shelf S. 51: 153-159.

CATALLO, W.J. (1993). Exotoxicology and wetland ecosystems: current understanding and future needs. Environ. Toxicol. Chem. 12: 2209-2224.

DORGELO, J., MEESTER, H. & VANVELZEN, C. (1995). Effects of diet and heavy metals on growth rate and fertility in the deposit-feeding snail *Potamopyrgus jenkinsi* (Smith) (Gastropoda: Hydrobiidae). Hydrobiologia 316: 199-210.

DU LAING, G., BOGAERT, N., TACK, F.M.G., VERLOO, M.G. & HENDRICKX, F. (2002). Heavy metal contents (Cd, Cu, Zn) in spiders (*Pirata piraticus*) living in intertidal sediments of the river Scheldt estuary (Belgium) as affected by substrate characteristics. Sci. Total Environ. 289: 71-81.

DU LAING, G., TACK, F.M.G. & VERLOO, M.G. (2003). Performance of selected destruction methods for the determination of heavy metals in reed plants (*Phragmites australis*). Anal. Chim. Acta 497: 191-198.

GADD, G.M. (1993). Interactions of fungi with toxic metals. New Phytol. 124: 25-60.

GAMBRELL, R.P. (1994). Trace and toxic metals in wetlands – a review. J. Environ. Qual. 23: 883-891.

GESSNER, M.O. (2000). Breakdown and nutrient dynamics of submerged *Phragmites* shoots in the littoral zone of a temperate hardwater lake. Aquatic Bot. 66: 9-20.

GRIES, C. & GARBE, D. (1989). Biomass, and nitrogen, phosphorus and heavy metal content of *Phragmites australis* during the third growing season in a root zone waste water treatment. Arch. Hydrobiol. 117: 97-105.

HART, B.T. (1982). Uptake of trace metals by sediments and suspended particulates: a review. Hydrobiologia 91: 299-313.

HIETZ, P. (1992). Decomposition and nutrient dynamics of reed (*Phragmites australis* (Cav.) Trin. ex Steud.) litter in Lake Neusiedl, Austria. Aquatic Bot. 43: 211-230.

KADLEC, R.H. & KNIGHT, R.L. (1996). Treatment wetlands. CRC Press, Boca Raton.

KELLER, B.E.M., LAJTHA, K. & CRISTOFOR, S. (1998). Trace metal concentration in the sediments and plants of the Danube delta, Romania. Wetlands 40: 42-50.

KOVACOVA, S. & STURDIK, E. (2002). Interactions between microorganisms and heavy metals including radionuclides. Biologia 57: 651-663.

KUFEL, I. (1991). Lead and molybdenum in reed and cattail – open versus closed type of metal cycling. Aquatic Bot. 40: 275-288.

LARSEN, V.J. & SCHIERUP, H.H. (1981). Macrophyte cycling of zinc, copper, lead and cadmium in the littoral zone of a polluted and a non-polluted lake. II. Seasonal changes in heavy metal content of above-ground biomass and decomposing leaves of *Phragmites australis* (Cav.) Trin. Aquatic Bot. 11: 211-230.

MEGANCK, B. (1998). De morfologie van *Phragmites australis* (Cav.) Steud. in relatie tot hydrodynamiek, beheer en saliniteit in het Schelde-estuarium. Ghent University, Ghent, Belgium.

PARÉ, T., DINEL, H. & SCHNITZER, M. (1999). Extractability of trace metals during co-composting of biosolids and municipal solid wastes. Biol. Fertil. Soils 29: 31-37.

PEVERLY, J.H., SURFACE, J.M. & WANG, T. (1995). Growth and trace metal absorption by *Phragmites australis* in wetlands constructed for landfill leachate treatment. Ecol. Eng. 5: 21-35.

POLUNIN, N.V.C. (1982). Processes contributing to the decay of reed (*Phragmites australis*) litter in fresh water. Arch. Hydrobiol. 94: 182-209.

SCHIERUP, H.H. & LARSEN, V.J. (1981). Macrophyte cycling of zinc, copper, lead and cadmium in the littoral zone of a polluted and a non-polluted lake. I. Availability, uptake and translocation of heavy metals in *Phragmites australis* (Cav.) Trin. Aquatic Bot. 11: 197-210.

SENESI, N., SPOSITO, G. & MARTIN, J.P. (1987). Copper (II) and iron (III) complexation by humic acid-like polymers (melanins) from soil fungi. Sci. Total Environ. 62: 241-252.

SOETAERT, K., HOFFMANN, M., MEIRE, P., STARINK, M., VAN OEVELEN, D., VAN REGENMORTEL, S. & COX, T. (2004). Modeling growth and carbon allocation in two reed beds (*Phragmites australis*) in the Scheldt estuary. Aquatic Bot. 79: 211-234.

VAN DAMME, S., YSEBAERT, T., MEIRE, P. & VAN DEN BERGH, E. (1999). Habitatstructuren, waterkwaliteit en leefgemeenschappen in het Schelde-estuarium. - Rapport IN 99/24, Instituut voor Natuurbehoud, Brussels, Belgium.

VAN RYCKEGEM, G., GESSNER, M.O. & VERBEKEN, A. (2005). Fungi on leaf blades of *Phragmites australis* in a brackish tidal marsh: diversity, succession and leaf decomposition. Mycologia (to be submitted).

VAN RYCKEGEM, G., VAN DRIESSCHE, G., VAN BEEUMEN, J.J. & VERBEKEN, A. (2005). Fungal dynamics during decomposition of *Phragmites australis* leaf sheaths and stems in a brackish tidal marsh. Microb. Ecol. (to be submitted).

WEIS, J.S. & WEIS, P. (2004). Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. Environ. Int. 30: 685-700.

WEIS, J.S., WINDHAM, L., SANTIAGO-BASS, C. & WEIS, P. (2002). Growth, survival, and metal content of marsh invertebrates fed diets of detritus from *Spartina alterniflora* Loisel. and *Phragmites australis* Cav. Trin. ex Steud. from metal-contaminated and clean sites. Wetlands Ecol. Manag. 10: 71-84.

WINDHAM, L., WEIS, J.S. & WEIS, P. (2003). Uptake and distribution of metals in two dominant salt marsh macrophytes, *Spartina alterniflora* (cordgrass) and *Phragmites australis* (common reed). Estuar. Coast. Shelf S. 56: 63-72.

ZAWISLANSKI, P.T., CHAU, S., MOUNTFORD, H., WONG, H.C. & SEARS, T.C. (2001). Accumulation of selenium and trace metals on plant litter in a tidal marsh. Estuar. Coast. Shelf S. 52: 589-603.

PART 2

FUNGI IN AN ESTUARY

Fungal diversity and community structure on *Phragmites australis* (Poaceae) along a salinity gradient in the Scheldt estuary (Belgium)

Adapted from: Van Ryckegem, G. & Verbeken, A. (2005). Fungal diversity and community structure on *Phragmites australis* (Poaceae) along a salinity gradient in the Scheldt estuary (Belgium). Nova Hedwigia 80: 173-197.

Abstract We examined fungal communities on stems and leaf sheaths of common reed (*Phragmites australis*) in four tidal marshes along a salinity gradient (mesohaline to freshwater) in the Scheldt estuary (Belgium) by direct observation. One hundred and fourteen taxa, the highest number of taxa ever recorded during a survey of phragmiticolous fungi, were found, with 56 taxa (49%) in the ascomycetes, 6 taxa (5%) in the basidiomycetes and 52 taxa (46%) of anamorphic fungi, the latter comprising 30 (26%) coelomycetes and 22 (19%) hyphomycetes. Fungal diversity in tidal marshes compared is lower than that in non-tidal reed marshes. Species distribution on reed litter, investigated by multivariate analysis, was strongly correlated with flooding frequency and salinity. Influences of salinity on the fungal community are discussed and tested by a culture experiment. Taxa were divided into three basic distributional types: taxa present only in the saline-brackish water zone, species present only in the freshwater zone, and a group of eurytopic species found over the entire salinity gradient sampled. Indicator species for each group were determined. In two sites, mesohaline brackish and freshwater, the vertical distribution of mycota was analysed. Higher similarities between the same vertical zones, especially in middle and apical parts of the culms, indicated that terrestrial fungi (no direct contact with tidal exchange water) were less influenced by site-specific characteristics. However, in both sites, a few common indicator species for standing *Phragmites* culms could be identified. Flooding height (and frequency) is demonstrated to influence vertical species distribution. Generally rather complicated interactions and a narrow niche differentiation among species is revealed for the phragmiticolous fungal community.

Introduction

The ecological importance of microfungi decomposing litter in (semi)-aquatic habitats is well recognized. However, relatively few studies report on the diversity and ecology of these interesting organisms (Wong et al., 1998; Gessner & Van Ryckegem, 2003). Knowledge about fungal diversity, ecology and secondary production during decomposition is essential, as fungal activity and species composition could affect the dynamics of other microorganisms and invertebrate detritivores and influence the loss of litter mass, nutrient immobilization and mineralization. (Webster & Benfield, 1986; Mann, 1988; Newell, 1996; Findlay et al., 2002).
In brackish and freshwater tidal marshes of the Scheldt estuary, common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) is the dominant macrophyte, reaching an annual biomass production of 10 ± 3.8 ton/ha (Meganck, 1998). Only a small proportion of this plant mass is

consumed by herbivores (e.g. van der Toorn & Mook, 1982; Mann, 1988; Odum, 1988), leaving a large pool of detritus for decay by micro-organisms and detritus-feeding invertebrates. There are major indications that fungi are important in this decomposition process: (1) the high *species richness* of fungi recorded on reed (Taligoola, 1969; Rodewald & Rudescu, 1974; Bán et al., 1996, 1998; Beyer, 1997; Poon & Hyde, 1998a; Peláez et al., 1998; Wirsel et al., 2001; Wong & Hyde, 2001); (2) a considerable fungal *biomass* develops during decay (Tanaka, 1991; Gessner, 2001; Findlay et al., 2002), and (3) high fungal *productivity* measured during decay (Findlay et al., 2002). In addition to their role as decomposers in reed stands, fungi are associated with common reed as endophytes (Peláez et al., 1998; Wirsel et al., 2001), pathogens (Bán et al., 1996) and arbuscular mycorrhiza (Oliveira et al., 2001).

The purpose of this study was to determine fungal species richness, diversity and community structure on common reed in tidal marshes of the river Scheldt to provide the necessary background information for decomposition studies and process-related research. The specific research objectives were to: (1) determine fungal species occurrence on above-ground *litter layer culms*- (4 sites) and *standing dead culms* (2 sites) of *P. australis* at sites representing different salinity and flooding regimes; (2) these data were used for spatial analysis along a salinity gradient and along the vertical axes of the standing reed culms; (3) a small *culture experiment* was undertaken to correlate the NaCl salinity effects on fungal vegetative growth in Petri dishes with field observations of reproductive structures.

Materials and methods

Study area, sites and datasets

The Scheldt estuary (Fig. 1) comprises a continuum of tidal marshes along a salinity gradient. The wetlands range from salt, brackish (transitional) to freshwater tidal marshes, the latter being endangered natural wetland habitats in Europe (Meire et al., 1997). The factors causing this gradient are the entrance of seawater twice a day and the outflow of freshwater from upstream areas. The tidal effect is apparent up to the city of Ghent (160 km inland). An oligohaline zone (~0.5-5 g $Cl^- l^{-1}$)* (seasonally shifting along the longitudinal axis of the estuary) extends roughly from the Dutch-Belgian border [57 km inland, near Doel (S1)] towards Temse (100 km inland). Downstream from Antwerp city, this oligohaline zone shows a rapid increase in salinity from about zero to approximately 5 g $Cl^- l^{-1}$ at the Dutch border. From the Dutch-Belgian border to Hansweert (38 km inland) a mesohaline zone (~5-18 g $Cl^- l^{-1}$) extends (Van Damme et al., 1999).

* We express salinity in this chapter as NaCl salinity (g NaCl l^{-1})

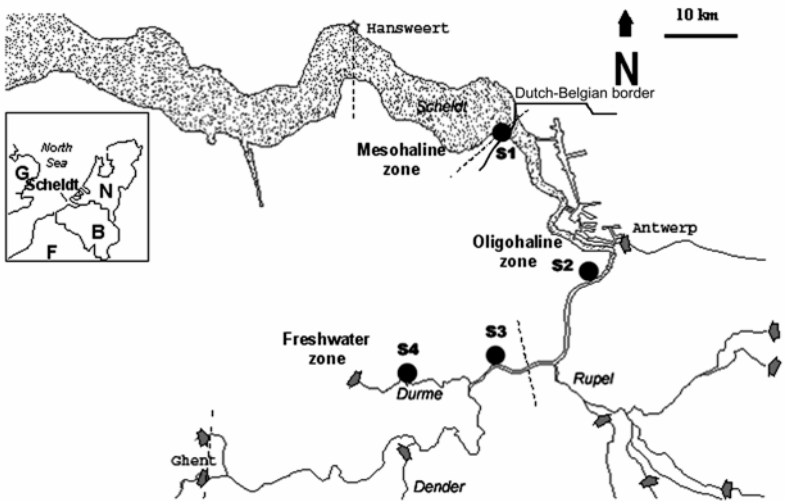


Figure 1. Map of the Scheldt estuary showing the locations of the sampling stations (black dots S1-S4) and salinity zones delimited by dashed lines. River names are in italics. Upper limit of tidal influence is indicated with grey arrows. S1 = Saeftinghe; S2 = Burcht; S3 = Kijkverdriet, Tielrode; S4 = Rietsnijderij, Waasmunster.

A first dataset (**DATA1**) contains information on 28 *litter layer samples* (lying dead culms without leaves) taken from four sites on seven collecting dates along the salinity gradient of the Scheldt (Fig. 1). Sample sites were located in permanent quadrats of 100 m^2 marked for vegetation research (Muylaert, 1996; Meganck 1998). The four sites ordered from downstream to upstream were: 'Doel', S1, 51° 21´ N, 4° 14´ E; 'Burcht', S2, 51° 13´ N, 4° 23' E; 'Temse-Kijkverdriet', S3, 51° 07´N, 4° 16´ E; 'Waasmunster-Rietsnijderij', S4, 51° 06´ N, 004° 08´ E. Sites are characterized in Table 1. Due to their geographical proximity, environmental conditions such as insolation, rainfall, and seasonal temperature regimes do not differ significantly among the sites. All tidal marshes along the Scheldt are highly polluted with heavy metals (Pb, Cu, Zn, Cd, Hg), and have tidal exchange water that is characterized by (seasonally shifting) high nitrogen (for example $N0_3^-$ in S1 varying between 3.7 and 6.3 mg l^{-1}) and PO_4^{-3} loads, the latter scoring 1-2 orders of magnitudes higher than in unpolluted situations (Van Damme et al., 1999). All sites show a pH 7.5-8 of the tidal exchange water (Van Damme et al., 1999). Three sites (S1, S2, S4) were sampled on the same dates (July-98, Aug-98, Oct-98, Nov-98, Dec-98, Feb-99, Mar-99), collections from S3 were spread over time (Jan-00, Feb-00, May-00, Sep-00, Apr-01, Nov-02 and Feb-03). Fifteen reed culms were random collected each time at each location and cut into approximately 30 cm lengths. A culm is considered to consist of two plant parts: stem and leaf sheaths. Samples were stored at 4 °C in plastic bags and screened for fungal presence within 3 weeks after collecting under a dissecting microscope (magnification 180x); stems and leaf sheaths were processed separately. All observations are based on the presence of mature fruiting structures formed *in situ*. It was not feasible to identify all taxa to species level, as monographs or up-to-date identification keys are not

available for several genera. Furthermore, some genera contain numerous species and/or consist of species-complexes difficult to unravel by a non-specialist or without molecular work. Several specimens were described as new species (Van Ryckegem & Verbeken 2000; Van Ryckegem & Aptroot 2001; Van Ryckegem et al., 2002) and some are waiting for further research. However, all distinguishable taxa were considered and indicated as unique. The number of 15 30-cm-long culm parts, needed to provide representative data on fungal diversity of a site at one moment, was determined by a cumulative species recovery experiment at the start of the inventory. DATA1 serves to explore dominant mycota in the litter layer and is considered to cover the seasonal differences of the fungi present. The different sampling dates for S3 are of little influence on the ecological interpretation of the results as many species were retrieved in more than one month each year. No temporal aspects are further taken into consideration and no monthly abundances are used to compare the sites. DATA1 is based on presence/absence data for the species found in each site at each collection date, giving a pseudo-abundance value on a scale of 1-7 according to the number of times a species was detected during the seven samplings (7 = max. recovery of a species from a site).

The second dataset (**DATA2**) combines data on 23 *standing-dead* reed samples from two sites (S1 and S3). Both sites, a brackish and a freshwater site, were sampled at the same dates as given above for S3 and all samples were processed as described above. Ten standing dead culms (stems + leaf sheaths) from the previous growing season were cut at the base on six sampling dates (Jan-00, Feb-00, May-00, Apr-01, Nov-02 and Feb-03) and/or 10 old, basal parts of stems still standing from at least two growing seasons ago were cut on five sampling dates (Jan-00, Feb-00, May-00, Sep-00, Apr-01). As above, the number of ten culm parts, necessary to provide representative data on fungal diversity of a site at one moment, was determined by a cumulative species recovery experiment. Standing dead culms from the previous growing season were sampled as follows: culms were cut at bottom level and divided into three standardized parts: basal, middle and upper part (excluding inflorescence). Each part had a length of 30 cm and consisted of both stem and leaf sheath (except sometimes for basal parts which quickly lose leaf sheaths by tidal wave action). Reed culms can remain in standing position for several years but generally the upper part of standing culms fall onto the sediment after approximately one year, leaving only the lower part for further standing decay. Consequently the collection of old standing culms consisted of only the basal stem part without leaf sheaths, the latter were already decomposed. A total of 230 culm pieces was investigated from each site (180 standing dead parts from the previous growing season and 50 old standing dead basal parts). DATA2 serves to explore the fungal community on standing dead culms of different ages and at different heights. It provides a representative survey of the dominant fungal taxa on standing dead reeds at two sites with different salinity exposure. Fungal frequency of occurrence is expressed as the number of collections of a species at each site, divided by the total number of standardized culm pieces examined. For the ecological gradient analyses, no distinction was made between stem and leaf sheath data.

Culture experiment

Four multiple-spore isolates from newly collected species were obtained and deposited in MUCL: *Massarina arundinacea* (MUCL 45115) from S1 (brackish), *M. arundinacea* (MUCL 45116) from S3 (fresh), *Halosphaeria hamata* (MUCL 45117) from S1, and *Massariosphaeria* sp. (MUCL 45118) from S3. All were grown free of contamination on cornmeal agar (CMA, Corn Meal Agar, Difco, pH 6.0) (17 g l^{-1}) with 50 ppm chloramphenicol to suppress bacterial growth. Colonies were plated in three replicas after 14 days on CMA medium (50 ppm chloramphenicol) with 8 different NaCl salinities (0 (control), 2, 4, 6, 8, 10, 20, 50 g NaCl l^{-1}). Plastic Petri dishes (9 cm diam.) containing an equal amount of medium (20 ml) were centrally inoculated with a 3 mm diam. plug cut from the leading edge of an actively growing colony. Plates were sealed with parafilm to minimize evaporation and stored at ambient room temperature [20.9 °C (STDEV= 0.8)] and light conditions. Diameters of the colonies were measured in the same directions using a vernier calliper (accuracy = 0.1 mm) after one and two weeks (data not shown). Growth recorded in the second growth week was used for colony radial growth rate calculation to avoid the lag phase after plating.

Statistics and ordination

Species occurring with relative abundances higher than 1 (55 taxa/ DATA1 and 53 taxa/ DATA2) were used for the (in)direct ordination technique without down-weighting rare taxa (since these were already omitted) or transformation. Detrended Correspondance Analysis (DCA performed with PC-ORD version 4.26, McCune & Mefford, 1999) was chosen as indirect gradient analysis to explore the fungal community structure, as the data show a modest to clear unimodal model with maximum length of gradient (SD) axis 1: 2.535 SD DATA1 and 4.535 SD DATA2 (ter Braak & Smilauer, 2002). The gradient length indicates the amount of β-diversity (species turnover) along the axes, whereas the eigenvalue is a measure of the amount of variation extracted by the axes.

Detrended Canonical Correspondence Analysis (DCCA; CANOCO, ter Braak & Smilauer, 2002), was used to check if two environmental variables (salinity and flooding frequency) as constraining factors for the ordination axes would give a similar ordination as did the indirect ordination based only on species data. To calculate species similarity (performed with PC-ORD) between culm parts of different heights, the Sørensen similarity index [2W/(A+B)] was used, where W is the sum of shared abundances and A+ B are the sum of abundances in the individual sample units.

Table 1. Characterization of the four sampling sites.

Site	Distance to sea (km)	Cl^- (mg l^{-1}) conc. of surface water*			Type of tidal marsh classified toward salinity+	Inundation frequency (%)$	Average height of inundation (cm)$	Sedimentation (mm. yr^{-1})!	Reed characteristics			
		Mean	Standard deviation	Max.					Stem diam. at first internode (mm) (n = 10)†	Culm height (cm) (n = 10)↑	Density of living culms† #/m^2 (n = 6)	Biomass of culms† g/m^2 (n = 6)
S1	53.9	3270	2362 (n = 24)	6864	Mesohaline	15.2	17	34	4.0	197	192	568
S2	79.5	325	282 (n = 12)	1100	Oligohaline	22.5	14	45	3.6	228	80	306
S3	94.4	99	41 (n = 5)	166	Fresh	52.7	27	93	6.3	309	100	620
S4	109.5	89	42 (n = 5)	137	Fresh	45.7	27	82	7.6	405	40	772

* Values derived form physico-chemical measuring network of the Flemish Environment Agency (S1 = VMMNR154100; S2 = VMMNR160500; S3 = VMMNR162500; S4 = VMMNR494700), http://www2.vmm.be/, data from S1 & S2 are from 1999 (not available for S3 & S4), S3 & S4 are data from 2001. Mean % salinity is indicative to compare values with culture experiment.

+ Terminology marsh type according to Mitch & Gosselink (2000).

$ Percentage of flooding at high tides, values from Meganck (1998).

! Based on: sedimentation = 157.44x(flooding frequency) + 10.029, $r^2 = 0.518207$, p-level: 0.000001 (Van Damme et al., 1999).

† Values from Meganck (1998).

↑ Own measurement (2001).

Results

Litter layer subset (DATA1)

Table 2 shows the results of the survey in four tidal marshes (S1-S4) along the river Scheldt on seven different occasions. Species are ranked in the table according to relative frequencies, ordination results (see below) and additional findings from the results of DATA2. Seventy-seven taxa (374 records) of fungi were recorded from decaying culms (stems and leaf sheaths) in the litter layer; 41 (53%) taxa of ascomycetes, 6 (8%) taxa of basidiomycetes, 30 taxa (39%) of anamorphic fungi of which 14 taxa (18%) were coelomycetes and 16 taxa (21%) were hyphomycetes. Twenty-two taxa were found only once during the 28 field trips (Table 3). Distributional patterns of fungal species collected are listed in Table 3 and illustrated in Figure 2. A high number of site-specific species was found, but approximately 72% of those species were collected in only one sample, indicating that these species probably were rare or may not be a regular part of the phragmiticolous mycota. Three groups of taxa can be derived from Table 3: a group of ten taxa occurring in all sites; a group of eight taxa common to sites S3 and S4; and a third group of eight taxa common to sites S2 and S3. The third group contains three terrestrial taxa found in the litter layer and one species, *Schizothecium hispidulum*, which was found on *Phragmites* but is considered to be a coprophilous fungus.

The DCA ordination (raw data; axes 1 and 2 respectively: eigenvalues 0.299 and 0.168; length of gradient 2.535 and 2.587) resulted in two interpretable axes (Fig. 2). Both axes are well correlated with two environmental variables, the first axis reflecting a coenocline of inundation frequency (Pearson correlation axis 1: 0.986, axis 2: -0.318[*]; PCORD) opposed to salinity influence (Pearson correlation axis 1: -0.704, axis 2: 0.806) which also has a high correlation with the second axis. A DCCA ordination (CANOCO; ter Braak & Smilauer, 2002) based on raw data (axes 1 and 2 respectively: eigenvalues 0.299 and 0.128; length of gradient 1.325 and 1.303), showed similar results (not illustrated) indicating that the two variables included (inundation frequency and maximum salinity, see Table 1) possibly explain a significant part of the observed variation in the data.

All four sites, based on average DCA-ordination scores of the seven samples from each site, differ clearly from each other (Fig. 2). However, five clusters are discerned in Figure 2. Group A, central at the lower part of the plot, contains the species occurring on the reed over the whole salinity gradient (see analyses of DATA2 for comments on this group of taxa). To the left of group A, group B1 contains species that are more typical for the litter flooded by brackish water. Radiating from site two (S2) are groups (B1, B2, B3) with species in common to S2 and the other sites. This shows the intermediate position of S2 in the salinity gradient.

[*] In the original publication erroneously a Pearson correlation of -3.18 was given.

Species in B2 and B3 are found frequently in the oligohaline tidal marshes but most of the taxa are also found in the limnetic (< 0.1 g Cl^{-} l^{-1}) parts of the Scheldt estuary. Group C, which is spread out over the right side of Figure 2, contains taxa that occur exclusively in the freshwater tidal marshes (S3 and S4), and are indicative of these habitats.
In order to better understand the ecology and distribution of the different taxa found in the litter layer and especially to investigate the group A taxa, which show no preferential distribution in the estuary, DATA2 with information on the standing dead reed culms, was analyzed.

Standing dead subset (DATA2)

A total of 71 taxa (947 records) was found on standing dead culms. Thirty-three taxa (46%) were in ascomycetes, 3 taxa (4%) were in basidiomycetes, 35 taxa (49%) were anamorphic fungi of which 27 taxa (38%) were coelomycetes and 8 taxa (11%) were classified as hyphomycetes. In Table 4, species are ranked according to their occurrence in the two sites studied (calculated as the number of records of a taxon on 230 culm pieces examined) and results obtained from DATA1. Further analyses of DATA2 shows that a vertical stratification of taxa occurs on the stems. This is demonstrated in Figure 3 and Table 5. In Figure 3, the different heights of the two sites are plotted within a DCA-ordination diagram (raw data), axis 1 shows a coenocline of vertical height with eigenvalues 0.74 and axis 2 with eigenvalues 0.12.
There is a clear differentiation in species occurrence between the top, middle and basal part of the culms (Table 5) and a higher similarity in species occurrence between the sites within each vertical zone (Table 6).

Table 2. (DATA1) Fungal frequencies in 28 litter layer samples (= max. total frequency) on *Phragmites australis* at four sites (S1-S4) in the Scheldt estuary. Seven sampling dates (= max. recovery of a species at a given site), systematic position (A= ascomycetes, B = basidiomycetes, C = coelomycetes, H = hyphomycetes) followed by the plant part with highest species recurrence (st = stem and ls = leaf sheath). Code stands for the species abbreviation used in Fig. 2. Species groups were recognized according to the occurrence of the taxa in the four sites and based on personal observations in 4 additional sites along the salinity gradient.

Taxa	Systematic position and colonized plant part	Code	S1 (meso-haline)	S2 (oligo-haline)	S3 (fresh)	S4 (fresh)	Total frequency
Eurytopic taxa							
Massarina arundinacea (Sowerby: Fr.) Leuchtm.	A, st	AMASARU	7	7	7	5	26
Lophiostoma arundinis (Pers.) Ces. & De Not.	A, st	ALOPARU	7	4	5	5	21
Phomatospora berkeleyi Sacc.	A, st & ls	APHOBER	6	7	5	3	21
Lophiostoma semiliberum (Desm.) Ces. & De Not.	A, st	ALOPSEM	4	4	6	5	19
Septoriella phragmitis Oudem.	C, st & ls	CSEPPHR	6	4	4	0	14
Arthrinium phaeospermum (Corda) M.B. Ellis	H, st	HARTPHA	6	4	1	3	14
Cytoplacosphaeria rimosa (Oudem.) Petrak s.l.	C, st & ls	CCYTRIM	6	3	3	2	14
Stagonospora incertae sedis I	C, st	CSTAINA	1	2	6	0	9
Phaeosphaeria pontiformis (Fuckel) Leuchtm.	A, ls & st	APHAPON	4	2	2	0	8
Myrothecium cinctum (Corda) Sacc.*	H, st & ls	HMYRCIN	3	2	1	1	7
Dictyosporium oblongum (Fuckel) S. Hughes	H, st	HDICOBL	2	2	1	0	5
Lachnum controversum (Cooke) Rehm*	A, ls & st	ALACCON	1	2	0	1	4
Mollisia retincola (Rabenh.) P. Karst.	A, st	AMOLRET	0	1	1	3	5
* species restricted to litter which is not flooded (hanging litter or litter from higher part of permanent quadrats)							
Stenotopic taxa: mesohaline							
Halosphaeria hamata (Höhnk) Kohlm.	A, st & ls	AHALHAM	6	4	3	1	14
Botryosphaeria festucae (Lib.) Arx & E. Müll.	A, st	ABOTFES	5	4	0	2	11
Maireina monacha (Speg.) W.B. Cooke	B, st	BMARMON	3	0	0	0	3
Haligena spartinae E.B.G. Jones	A, st	AHALSPA	0	2	0	0	2
Stenotopic esturarine taxa: oligohaline- freshwater							
Phomatospora dinemasporium J. Webster	A, st	APHODIN	0	4	3	0	7
Lophiostoma caudatum Fabre	A, st	ALOPCAU	0	4	0	2	6
Passeriniella discors (Sacc. & Ellis) Apinis & Chesters	A, st	APASDIS	0	3	1	2	6
Periconia minutissima Corda*	H, ls	HPERMIN	0	3	0	0	3
Dendrodochium microsorum var. *phragmitis* Fautrey*	H, st & ls	HDENMIC	0	4	0	1	5
* species restricted to litter which is not flooded (hanging litter or litter from higher part of permanent quadrats)							
Stenotopic taxa: freshwater							
Massarina aquatica J. Webster	A, st	AMASAQU	0	0	6	3	9
Massariosphaeria typhicola (P. Karst.) Leuchtm.	A, st	AMASTYP	0	0	5	2	7
Massariosphaeria sp.	A, st	AMASSPE	0	0	4	2	6
Phoma sp. I	C, st	CPHOMAA	0	0	4	1	5
Buergenerula typhae (Fabre) Arx	A, st	ABUETYP	0	0	3	1	4
Mollisia hydrophila (P. Karst.) Sacc.	A, st & ls	AMOLHYD	0	0	2	2	4
Stagonospora elegans (Berk.) Sacc. & Traverso	C, st	CSTAELE	0	1	1	1	3
Gibberella zeae (Schwein.) Petch	A, st	AGIBZEA	0	0	0	2	2
Massariosphaeria grandispora (Sacc.) Leuchtm.	A, st	AMASGRA	0	0	0	2	2
Massariosphaeria palustris (E. Müll.) Leuchtm.	A, st		0	0	0	1	1
Massariosphaeria mosana (Mouton) Leuchtm.	A, st		0	0	1	0	1
Acrocordiopsis sp.	A, st		0	0	1	0	1
Terrestrial taxa (atypic in litter layer)*							
Phoma sp. III	C, ls	CPHOMAC	5	3	4	2	14
Septoriella sp(p).	C, ls	CSEPTOR	4	2	2	1	9
Didymella glacialis Rehm	A, ls	ADIDGLA	2	2	1	0	5
Neottiosporina australiensis B. Sutton & Alcorn	C, ls	CNEOAUS	1	0	4	0	5
Hendersonia culmiseda Sacc.	C, ls	CHENCUL	1	0	1	2	4
Phaeosphaeria eustoma (Fuckel) L. Holm s.l.	A, ls	APHAEUS	2	1	1	0	4
Puccinia magnusiana Körn.[1]	B, ls	URPUCMA	0	1	0	3	4
Keissleriella linearis E. Müll.	A, st	AKEILIN	2	1	0	0	3

Taxa	Systematic position and colonized plant part	Code	S1 (meso-haline)	S2 (oligo-haline)	S3 (fresh)	S4 (fresh)	Total frequency
Stagonospora vexata Sacc. sensu Diedicke	C, st & ls	CSTAVEX	0	1	1	0	2
Didymella sp.	A, ls	ADIDYME	0	1	0	1	2
Puccinia phragmitis (Schumach.) Körn.[1]	B, ls	URPUCPH	0	1	0	1	2
Torula herbarum (Pers.) Link	H, ls	HTORHER	0	2	0	0	2
[1]biotrophic taxa, but telia found in litter layer							
[*]based on analysis of DATA2							
taxa with unknown ecological characteristics							
Arthrinium state of *Apiospora montagnei* Sacc.	H, st	HARTAPI	0	4	0	0	4
Phaeosphaeria luctuosa (Niessl) Otani & Mikawa	A, ls	APHALUC	0	1	0	2	3
Schizothecium hispidulum (Speg.) N.Lundq.	A, st & ls	ASCHHIS	0	1	0	2	3
Apiospora montagnei Sacc.	A, st	AAPIMON	0	1	0	1	2
Fusarium sp. I	H, st	HFUSSP1	0	1	0	1	2
Bactrodesmium atrum M.B. Ellis	H, st	HBACATR	2	0	0	0	2
Keissleriella culmifida (P. Karst.) Bose	A, st	AKEICUL	0	0	1	1	2
Anthostomella punctulata (Roberge) Sacc.	A, ls	AANTPUN	0	0	1	1	2
Aposphaeria sp.	C, st	CAPOSPH	0	0	2	0	2
Mollisia cf. *palustris* (Roberge ex Desm.) P. Karst.	A, ls & st	AMOLPAL	1	1	0	0	2
Tremella spicifera Van Ryck., Van de Put & P.Roberts	B, st	BTREMEL	1	1	0	0	2
Ascochyta sp. I	C, ls	CASCOCI	1	1	0	0	2
Didymella proximella (P. Karst.) Sacc.	A, st		0	0	1	0	1
Phaeosphaeria sp. III	A, ls		0	0	1	0	1
Phomatospora sp. I	A, st		0	0	1	0	1
Hyphomycete sp. II	H, st		0	0	1	0	1
Monodictys cf. *putredinis* (Wallr.) S. Hughes	H, st		0	0	1	0	1
Cistella fugiens (Pholl. ex Bucknall) Matheis	A, st		1	0	0	0	1
Lophodermium arundinaceum (Schrad.) Chevall.	A, ls		1	0	0	0	1
Stagonospora sp. II	C, st		1	0	0	0	1
Arthrobotrys sp.	H, st		1	0	0	0	1
Bisporella scolochloae (De Not.) Spooner	A, st		1	0	0	0	1
Rhinocladiella sp.	H, st		1	0	0	0	1
Psilachnum eburneum (Roberge ex Desm.) Baral	A, st		0	0	0	1	1
Dinemasporium graminum (Lib.) Lév.	C, ls		0	1	0	0	1
Myrothecium masonii M.C. Tulloch	H, ls		0	1	0	0	1
Rosellinia musispora Van Ryck. & Verbeken	A, st		0	1	0	0	1
Trimorphomyces papilionaceus Bandoni & Oberw.	B, ls		0	1	0	0	1
Drechslera biseptata (Sacc. & Roum.) M.J. Richardson & E.M. Fraser	H, ls		0	1	0	0	1
Corynespora sp.	H, st		0	1	0	0	1
Pellidiscus pallidus (Berk. & Broome) Donk	B, ls		0	1	0	0	1
			S1	**S2**	**S3**	**S4**	**TOTAL**
Taxa richness in each site			32	47	39	37	77
Ascomycetes (A)			15	22	23	25	41
Basidiomycetes (B)			2	4	0	2	6
Coelomycetes (C)			9	9	11	6	14
Hyphomycetes (H)			6	11	5	4	16
Ratio Ascomycetes/ anamorphic fungi			1	1.1	1.44	2.5	

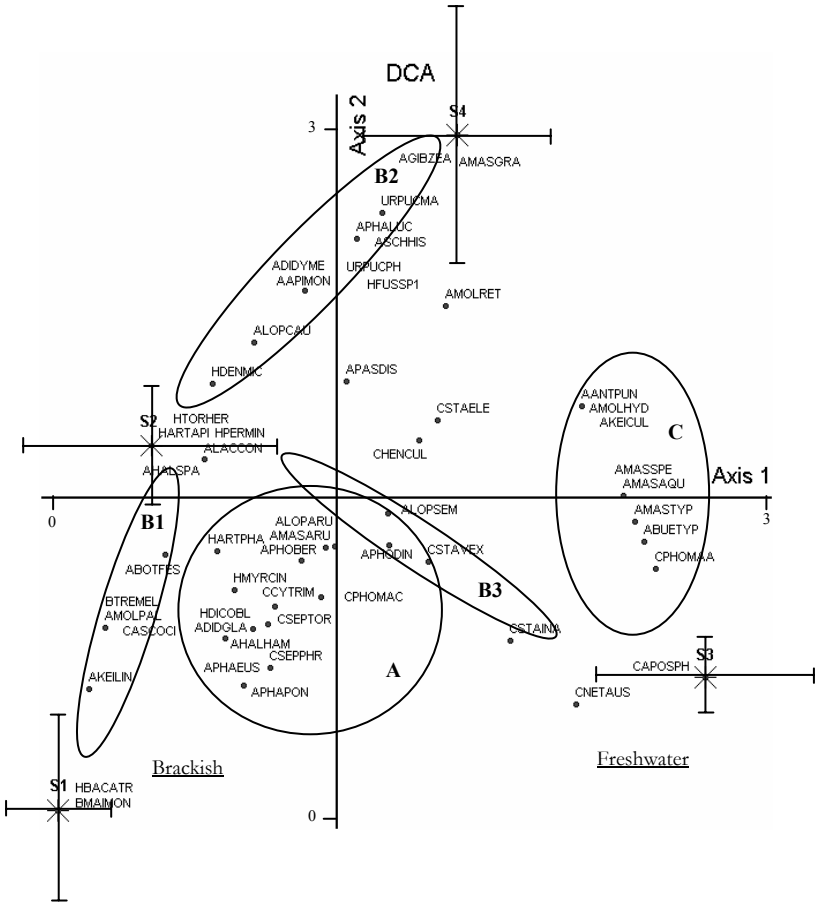


Figure 2. DCA-ordination plot of species occurrences on *Phragmites australis* litter along a salinity gradient in the Scheldt estuary. DATA1, 4 sites (S1-S4), 55 taxa, 28 samples. Abbreviations of sites and groups as in text, for abbreviations of species names see Table 2. Standard deviations calculated from DCA-ordination scores of 7 samples from each site. Axes are scaled in SD units.*

* Species clusters were drawn based on groups recognized in Table 2 (this note was not included in the original publication)

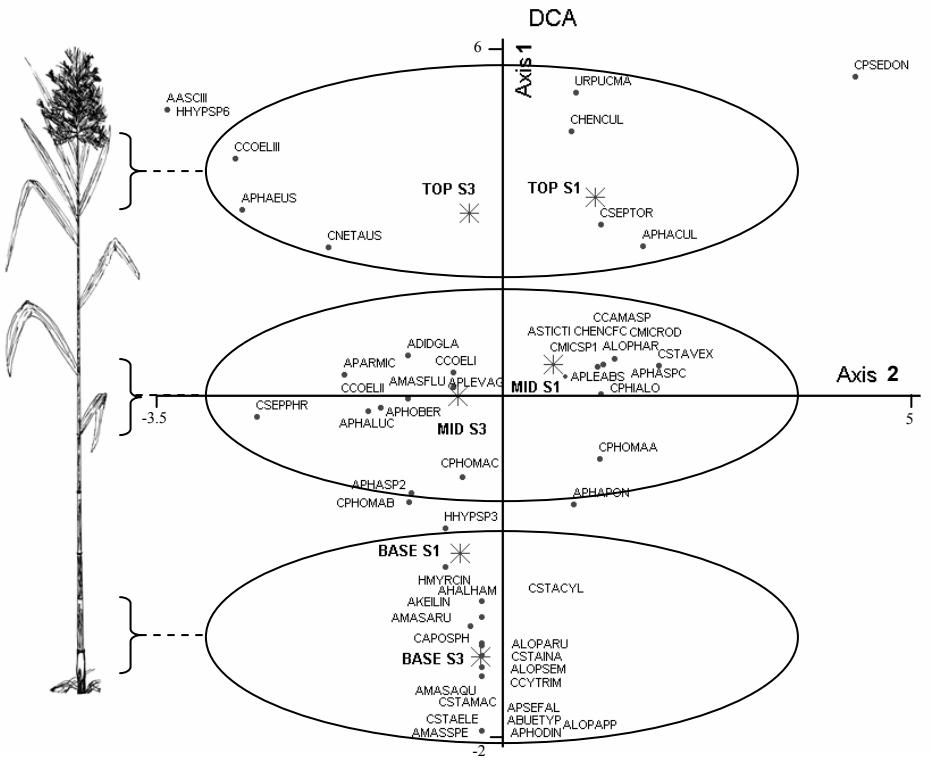


Figure 3. DCA-ordination plot of species occurrences at three different vertical regions on standing stems of *Phragmites australis*. DATA2, 2 sites (S1 and S3), 53 taxa, 23 samples. Vertical regions are apical part (TOP), middle part (MID) and basal part (BASE) of standing shoots. Clusters indicate species groups with high occurrence in a vertical region. For abbreviations of species names see Table 5. Axes are scaled in SD-units.

Table 3. (DATA1) Distributional patterns of fungi in the litter layer of four sites along a salinity gradient in the Scheldt estuary. Sites as in text. Total number of litter taxa exclusively collected in site(s). Unique taxa were only found once. * Including 3 species atypical for the litter layer and one species atypical on *Phragmites*.

Distribution pattern	Total number of taxa exclusive	Unique taxa
S1 (mesohaline)	8	6
S2 (oligohaline)	9	7
S3 (fresh)	8	7
S4 (fresh)	4	2
S1+S2	4	
S1+S2+S3	5	
S1+S2+S3+S4	10	
S2+S3+S4	2	
S3+S4	8	
S1+S3	1	
S1+S4	0	
S2+S3	2	
S2+S4	8*	

Table 4. (DATA2) Fungal species associated with standing dead culms (23 samples, 180 standing culm parts from the previous growing season and 50 older standing stem bases) of *Phragmites australis* in a brackish and a fresh water tidal marsh along the river Scheldt. Systematic position (A= ascomycetes, B = basidiomycetes, C = coelomycetes, H = hyphomycetes). Percentage occurrence calculated as numbers of records divided by total number of samples investigated (= 230 for each site).

Taxa	Systematic position	Brackish (S1)		Fresh (S3)	
		# records	% occurrence	# records	% occurrence
Eurytopic taxa					
Phoma sp. III	C	76	33.0	52	22.6
Septoriella sp(p).	C	63	27.4	55	23.9
Hendersonia culmiseda Sacc.	C	53	23.0	51	22.2
Massarina arundinacea (Sowerby: Fr.) Leuchtm.*	A	46	20.0	19	8.3
Myrothecium cinctum (Corda) Sacc.*	H	32	13.9	7	3.0
Lophiostoma arundinis (Pers.) Ces. & De Not.*	A	13	5.7	6	2.6
Stagonospora incertae sedis I	C	7	3.0	5	2.2
Lophiostoma semiliberum (Desm.) Ces. & De Not.	A	4	1.7	4	1.7
Phoma sp. II	C	3	1.3	3	1.3
Didymella glacialis Rehm	A	4	1.7	3	1.3
Aposphaeria sp.	C	4	1.7	2	0.9
Phaeosphaeria eustoma (Fuckel) L. Holm s.l.	A	3	1.3	5	2.2
Cytoplacosphaeria rimosa (Oudem.) Petrak s.l.	C	3	1.3	4	1.7
* eurytopic taxa unsampled in freshwater part due to an upwards shift caused by tidal amplitude					
Taxa with highest occurrence in brackish tidal marsh					
Stagonospora vexata Sacc. sensu Diedicke	C	30	13.0	7	3.0
Phaeosphaeria culmorum (Auersw.) Leuchtm.	A	29	12.6	6	2.6
Stictis sp.	A	24	10.4	2	0.9
Phialophorophoma sp.	C	18	7.8	1	0.4
Coelomycete sp. I	C	15	6.5	6	2.6
Hyphomycete sp. III	H	15	6.5	5	2.2
Phaeosphaeria sp. II	A	10	4.3	5	2.2
Camarosporium sp.	C	10	4.3	0	0.0
Lophodermium arundinaceum (Schrad.) Chevall.	A	9	3.9	0	0.0
Halosphaeria hamata (Höhnk) Kohlm.	A	8	3.5	1	0.4
Microsphaeropsis sp. I	C	7	3.0	1	0.4
Stagonospora cylindrica Cunnell	C	6	2.6	0	0.0
Pleospora abscondita Sacc. & Roum.	A	5	2.2	2	0.9
Phaeosphaeria pontiformis (Fuckel) Leuchtm.	A	5	2.2	0	0.0
Pseudoseptoria donacis (Pass.) B. Sutton	C	5	2.2	0	0.0
Microdiplodia sp.	C	5	2.2	0	0.0
Taxa with highest occurrence in freshwater tidal marsh					
Coelomycete sp. II	C	7	3.0	27	11.7
Septoriella phragmitis Oudem.	C	9	3.9	17	7.4
Neottiosporina australiensis B. Sutton & Alcorn	C	8	3.5	21	9.1
Buergenerula typhae (Fabre) Arx	A	0	0.0	16	7.0
Phomatospora berkeleyi Sacc.	A	1	0.4	10	4.3
Stagonospora elegans (Berk.) Sacc. & Traverso	C	0	0.0	11	4.8
Massarina aquatica J. Webster	A	0	0.0	10	4.3
Coelomycete sp. III	C	1	0.4	6	2.6
Paraphaeosphaeria michotii (Westend.) O.E. Erikss.	A	1	0.4	5	2.2
Hyphomycete sp. VI	H	0	0.0	5	2.2
Massarina fluviatilis Aptroot & Van Ryck.	A	2	0.9	5	2.2
Massariosphaeria sp.	A	0	0.0	4	1.7
Asco sp. Dothideales incertae sedis III	A	0	0.0	4	1.7
Phomatospora dinemasporium J. Webster	A	0	0.0	2	0.9
Lophiostoma appendiculatum Fuckel	A	0	0.0	2	0.9
Stagonospora macropycnidia Cunnell	C	0	0.0	2	0.9
Pseudohalonectria aff. *falcata* Shearer	A	0	0.0	2	0.9
Taxa with unknown ecological needs (not enough information in dataset)					
Phoma sp. I	C	3	1.3	1	0.4
Phaeosphaeria sp. III	A	3	1.3	0	0.0

Taxa	Systematic position	Brackish (S1)		Fresh (S3)	
		# records	% occurrence	# records	% occurrence
Hendersonia aff. *culmiseda* Sacc.	C	2	0.9	0	0.0
Keissleriella linearis E. Müll.	A	2	0.9	0	0.0
Puccinia magnusiana Körn.	B	1	0.4	1	0.4
Pleospora vagans Niessl	A	1	0.4	1	0.4
Phaeosphaeria luctuosa (Niessl) Otani & Mikawa	A	0	0.0	2	0.9
Hendersonia sp. II	C	0	0.0	1	0.4
Microsphaeropsis arundinis (J. Ahmad) B. Sutton	C	0	0.0	1	0.4
Phaeosphaeria phragmitis (Hollós) Leuchtm.	A	1	0.4	0	0.0
Acrocordiopsis sp.	A	0	0.0	1	0.4
Maireina monacha (Speg.) W.B. Cooke	B	1	0.4	0	0.0
Lewia infectoria (Fuckel) M.E. Barr & E.G. Simmons	A	1	0.4	0	0.0
Phoma sp. IIa	C	0	0.0	1	0.4
Hyphomycete sp. V	H	0	0.0	1	0.4
Ophioceras cf. *dolichostomum* (Berk. & M.A.Curtis) Sacc.	A	0	0.0	1	0.4
Hendersonia sp. I	C	1	0.4	0	0.0
Massariosphaeria typhicola (P. Karst.) Leuchtm.	A	0	0.0	1	0.4
Pseudohalonectria adversaria Shearer	A	0	0.0	1	0.4
Cladosporium sp(p).	H	1	0.4	0	0.0
Arthrinium phaeospermum (Corda) M.B. Ellis	H	1	0.4	0	0.0
Puccinia phragmitis (Schumach.) Körn.	B	0	0.0	1	0.4
Fusarium sp. III	H	1	0.4	0	0.0
Deightoniella roumegueri (Cavara) Constant.	H	0	0.0	1	0.4
Asco sp. Dothideales incertae sedis II	A	0	0.0	1	0.4
Summary		**Brackish**		**Fresh**	**Total**
Species richness		49		55	71
Number of records		530		417	947
Ascomycetes		20		27	33
Basidiomycetes		2		2	3
Coelomycetes		22		21	27
Hyphomycetes		5		5	8

Culture experiment

The results obtained from the culture experiment using selected species correlate with the observed occurrences of species in the field (Fig. 4). Growth is expressed as relative growth compared to the maximal growth at a certain NaCl concentration. *Massarina arundinacea* is a eurytopic species in the Scheldt estuary and shows almost a maximal growth from 0 to 10 g NaCl l^{-1} CMA. No significant differences (Two-Sample t-Test, % data transformed asin [sqrt(X/100)], p-value 0.9153) were found between strains isolated from the freshwater and brackish sites. *Halosphaeria hamata*, a species typically isolated from brackish environments, shows an optimum growth rate at about 10 g NaCl l^{-1} which is close to the highest salinity values of the exchange water at site 1 (Table 1). On the other hand, *Massariosphaeria* sp., a freshwater ascomycete, already showed reduced growth from the lowest NaCl concentration tested (2 g NaCl l^{-1}) (Fig. 4).

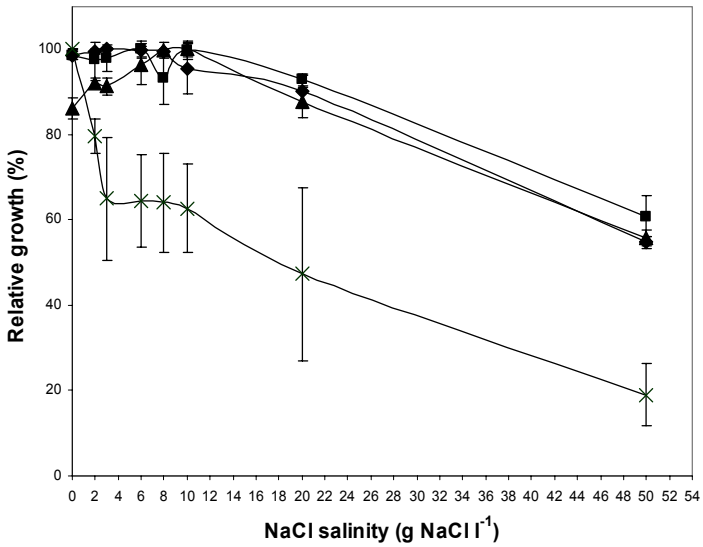


Figure 4. Relative growth of four selected taxa, measured as colony diameter growth in the second incubation week. ▲*Halosphaeria hamata*; ■ *Massarina arundinacea*, freshwater isolate; • *Massarina arundinacea*, brackish isolate; x *Massariosphaeria* sp. Dots indicate average values and bars the standard deviation (n = 3).

Table 5. (DATA2) Vertical distribution of fungi occurring on standing dead culms (from previous growing season and older stem bases) of *Phragmites australis* in two tidal marsh sites [S1 (brackish) and S3 (fresh)] along the river Scheldt. Substrate: plant part with highest species recurrence (st = stem and ls = leaf sheath). 53 taxa listed, exclusive of 18 unique records. Species groups were recognized according to the occurrence of the taxa in the two sites and based on personal observations in 6 additional sites along the salinity gradient.

Taxa	substrate	Code	Base (records)	Middle (records)	Top (records)
Taxa occurring more frequently in the basal parts of culms: litter layer species*					
Massarina arundinacea (Sowerby: Fr.) Leuchtm.	st	AMASARU	62	3	0
Myrothecium cinctum (Corda) Sacc.	st & ls	HMYRCIN	32	7	0
Lophiostoma arundinis (Pers.) Ces. & De Not.	st	ALOPARU	19	0	0
Buergenerula typhae (Fabre) Arx	st	ABUETYP	16	0	0
Hyphomycete sp. III	ls	HHYPSP3	13	7	0
Stagonospora incertae sedis I	st	CSTAINA	12	0	0
Stagonospora elegans (Berk.) Sacc. & Traverso	st	CSTAELE	11	0	0
Massarina aquatica J. Webster	st	AMASAQU	10	0	0
Halosphaeria hamata (Höhnk) Kohlm.	st & ls	AHALHAM	9	0	0
Lophiostoma semiliberum (Desm.) Ces. & De Not.	st	ALOPSEM	8	0	0
Cytoplacosphaeria rimosa (Oudem.) Petrak s.l.	st	CCYTRIM	7	0	0
Stagonospora cylindrica Cunnell	st	CSTACYL	6	0	0
Aposphaeria sp.	st	CAPOSPH	6	0	0
Massariosphaeria sp.	st	AMASSPE	4	0	0
Phomatospora dinemasporium J. Webster	st	APHODIN	2	0	0

* called 'estuarine saprotrophic species' in original publication

Taxa	substrate	Code	Base (records)	Middle (records)	Top (records)
Keissleriella linearis E. Müll.	st	AKEISLIN	2	0	0
Lophiostoma appendiculatum Fuckel	st	ALOPAPP	2	0	0
Pseudohalonectria aff. *falcata* Shearer	st	APSEFAL	2	0	0
Stagonospora macropycnidia Cunnell	st	CSTAMAC	2	0	0
Phoma sp. II	st	CPHOMAB	3	3	0
Phaeosphaeria pontiformis (Fuckel) Leuchtm.	st & ls	APHAPON	3	2	0
Taxa occurring more frequently in basal and middle parts of culms: canopy species*					
Phoma sp. III	ls	CPHOMAC	49	77	2
Phaeosphaeria sp. II	ls	APHASP2	8	6	1
Taxa occurring more frequently in middle parts of culms: canopy species					
Coelomycete sp. II	ls	CCOELII	1	30	3
Stagonospora vexata Sacc. sensu Diedicke	st & ls	CSTAVEX	2	28	7
Stictis sp.	ls	ASTICTI	0	26	0
Phialophorophoma sp.	ls	CPHIALO	2	17	0
Coelomycete sp. I	ls	CCOELI	1	18	2
Phomatospora berkeleyi Sacc.	ls & st	APHOBER	0	11	0
Camarosporium sp.	ls	CCAMASP	0	10	0
Lophodermium arundinaceum (Schrad.) Chevall.	ls	ALOPHAR	0	9	0
Microsphaeropsis sp. I	ls	CMICSP1	0	8	0
Pleospora abscondita Sacc. & Roum.	ls	APLEABS	0	7	0
Massarina fluviatilis Aptroot & Van Ryck.	ls & st	AMASFLU	0	7	0
Didymella glacialis Rehm	ls	ADIDGLA	0	6	1
Paraphaeosphaeria michotii (Westend.) O.E. Erikss.	ls	APARMIC	0	5	1
Microdiplodia sp.	ls	CMICROD	0	5	0
Phoma sp. I	ls	CPHOMAA	1	3	0
Phaeosphaeria sp. III	ls	APHASPC	0	3	0
Phaeosphaeria luctuosa (Niessl) Otani & Mikawa	ls	APHALUC	0	2	0
Pleospora vagans Niessl	ls	APLEVAG	0	2	0
Hendersonia aff. *culmiseda* Sacc.	ls	CHENCFC	0	2	0
Taxa occurring more frequently in middle and top part of culm: canopy species					
Neottiosporina australiensis B. Sutton & Alcorn	ls & st	CNEOAUS	0	12	17
Phaeosphaeria culmorum (Auersw.) Leuchtm.	ls	APHACUL	0	19	16
Phaeosphaeria eustoma (Fuckel) L. Holm s.l.	ls	APHAEUS	0	3	5
Taxa occurring more frequently in top part of culm: canopy species					
Septoriella sp(p).	ls	CSEPTOR	6	29	83
Hendersonia culmiseda Sacc.	ls	CHENCUL	0	12	92
Coelomycete sp. III	ls	CCOELIII	0	1	6
Hyphomycete sp. VI	ls	HHYPSP6	0	0	5
Pseudoseptoria donacis (Pass.) B. Sutton	ls	CPSEDON	0	0	5
Asco sp. Dothideales incertae sedis III	ls	AASCIII	0	0	4
Puccinia magnusiana Körn.	ls	BPUCMAG	0	0	2
No pattern observed					
Septoriella phragmitis Oudem.*	st, ls	CSEPPHR	10	3	13
* probably two species are involved here					

* called ' terrestrial saprotrophic species' in original publication

Discussion

Influence of salinity on the fungal community

Salinity is an important factor in explaining multiple biodiversity patterns in several ecosystems including that of the Scheldt estuary (Van Damme et al., 1999). Comparable studies describing the fungal saprotrophic community in tidal marshes along a salinity gradient are not available but salinity influences on fungal growth are well documented (e.g. Luard & Griffin, 1981; Adler, 1996) and differences in species composition were noted on wood panels in the water along salinity gradients (Gold, 1959; Schaumann, 1968; Shearer, 1972). Salinity can have a direct effect on fungal mycelia causing osmotic stress or it may act as a toxic component interfering with enzymatic and membrane activities (Adler, 1996). Indirectly, different salinities and flooding regimes cause changes in *Phragmites* morphology, as shoots in brackish conditions and in less flooded stands are smaller (Rodewald & Rudescu, 1974; Hellings & Gallagher, 1992). These morphological changes are noticeable in substrate quality and micro-climatological conditions (Rodewald & Rudescu, 1974). In the Scheldt estuary these morphological differences were also observed (Table 1), where shoot morphology showed the highest correlation with salinity and secondarily with flooding regime (Muylaert, 1996). Shoots in brackish water are much shorter (about 2 m versus up to 4 m high in the freshwater areas), form denser vegetation, have narrower stems (lowest node about 6 mm diam. versus about 1 cm in freshwater part) and smaller leaves compared to freshwater clones (Muylaert, 1996). Furthermore, as the reed stands in the Scheldt estuary are lower and dense, in the brackish zone, the microclimate is altered in these reed belts (Rodewald & Rudescu, 1974), changing fungal colonization and growth conditions.

Our results show that distinct fungal communities occur in tidal marshes along a salinity gradient (Fig. 2, Tables II, V). Whether these differences were due to direct effects of salinity exposure or indirect effects causing changes in substrate morphology and quality is hard to tell from our data*. Considering fungal community composition as purely a result of salinity exposure, however, is over-simplifying things, as other important abiotic [e.g. inundation frequency, tidal height, tidal exchange water quality (nutrients, heavy metals, etc.), microclimate] and biotic factors (e.g., reed quality, competition and invertebrate grazing) might also influence local fungal community structure.

In any case, salinity is an important environmental factor because it is well correlated with the first and second axes of the ordination as a passive variable (Fig. 2, see above). Additionally, simple culture experiments showed growth response curves corresponding to field distribution patterns for some selected taxa (Fig. 4). Furthermore, direct ordination (DCCA) with salinity and flooding frequency as selected constraining variables has comparable output as the indirect ordination. The results of the, although statistically more powerful, direct ordination are less reliable because only four sites are involved and less objective as environmental variables are

* because of autocorrelation problems and additional variables not considered (note not included in orginal publication)

highly correlated. The above points suggest that salinity has a direct influence on fungal community composition.

Distinct communities of *Phragmites* leaf fungi were noticed by Apinis et al. (1972a) comparing sites with different salinities. A higher salinity corresponds to a species-impoverished community on substrate influenced by flooding (lower parts of standing culms and litter layer) (Table 2). The brackish communities are characterized by few commonly occurring species, while freshwater communities contain more commonly occurring species. This trend is most obvious for ascomycetes, where several genera are frequently encountered in the freshwater habitat, such as *Massariosphaeria* and *Pseudohalonectria* (confirmed by additional records from a personal database).

A decreasing ratio of ascomycetes to anamorphic fungi with increasing salinity (Table 2) agrees with the culture observations of Byrne & Jones (1975), showing that asexual reproduction (except for true freshwater hyphomycetes) was less severely affected by high salinities compared to sexual reproduction. However, these ratio observations are in contrast to the observations on wood blocks exposed in estuarine water (e.g. Shearer, 1972). Reasons for these differences could be (1) intertidal euhaline *Phragmites* stands were not sampled during this study, so possibly a characteristic mycota for this salinity concentration was not found, (2) fungal communities on riparian tidal vegetation are structured by different environmental variables and show different species composition compared to wood blocks exposed in the water. The latter point is illustrated by higher numbers of terrestrial fungi on reed and lower numbers of anamorphic taxa in the freshwater part which is mainly due to the low species richness of hyphomycetes probably caused by tidal dynamics and high sedimentation, making the habitat unfavourable for both terrestrial and aquatic hyphomycetes (see below).

Whether differences in species composition caused by salinity differences have an effect on fungal productivity and biomass and, subsequently, on decomposition, is unknown. An effect of salinity on the decomposition of *Phragmites* leaves was noticed by Reice & Herbst (1982) and in a recent study a markedly lower fungal biomass was recorded in the most brackish of several reed stands (Findlay et al., 2002).

An indicator species for the brackish habitat is *Halosphaeria hamata*, a species regarded as a marine ascomycete (Kohlmeyer & Kohlmeyer, 1979). This species occurs over the entire gradient sampled but shows a clear decrease in abundance from brackish towards freshwater (Table 2). A comparable distribution was noticed by Schaumann (1968) in the Weser Estuary (Germany) and by Shearer (1972) in the Patuxent Estuary (U.S.A.) (no freshwater tidal system present) with maximal occurrence in the brackish zone and no records in the limnetic part. Byrne & Jones (1975) found that *H. hamata* fruited on balsa wood in yeast extract over a range of salinities from 0-100% seawater. In contrast with the above observations, Gold (1959; Newport Estuary, U.S.A.) recorded this species only in the freshwater tidal system. The relative growth curve of *H. hamata* in culture (Fig. 4) shows a reduced growth in pure distilled water culture and maximal growth at a salinity of about 10 g NaCl l^{-1}, roughly corresponding with maximal salinity in the most brackish site (S1) from which the isolate originated. This growth

curve correlates with patterns known for marine fungi, showing reduced growth at freshwater conditions (Byrne & Jones, 1975).

Most species found in the mesohaline brackish site are common species with a wide ecological amplitude occurring over the entire salinity gradient sampled. Culture experiments with such a eurytopic species, *Massarina arundinacea*, confirms the ability of this species to grow over a wide salinity amplitude and no differentiation occurred in growth response to salinity concentrations among taxa isolated from brackish or freshwater sites. Isolates of this species show a constant growth rate with NaCl concentrations up to 10 g NaCl l^{-1} (Fig. 4). The fact that no significant differences were found between isolates obtained from different sites indicate that the isolates are not locally adapted ecotypes with respect to salinity.

Typical tidal freshwater saprotrophic fungal species are *Massarina aquatica*, *Phoma* sp. I, several species of the genus *Massariosphaeria*, *Buergenerula typhae* and *Stagonospora* incertae sedis I. All these taxa are known to have representatives in freshwater aquatic habitats (Shearer, 1993, http://fm5web.life.uiuc.edu:23523/ascomycete/). Ascomycete taxa richness is higher in the freshwater tidal marshes compared to the most brackish site (S1) (Table 2). Although the two freshwater sites share a similar, typical freshwater mycota, some differences in species composition were observed between the two sites, probably due to the collection of rare taxa and the fact that some coelomycetes were not found in S4. An important observation here is that there is a common species pool in the freshwater tidal marshes with no species overlap with the mesohaline brackish water site.

A culture experiment with the freshwater species *Massariosphaeria* sp. (Fig. 4) shows strong negative influences of increasing NaCl concentrations, with an average growth reduction of more than 40% at a salinity of 10 g NaCl l^{-1}. This effect agrees with a reduced vegetative growth of freshwater fungi under saline conditions (Byrne & Jones, 1975). These authors also showed a reduced spore germination and reproduction with increasing salinity, a process that could explain some of the observed fungal distribution patterns

None of the common species was found characteristic of the oligohaline site (S2). In this part of the estuary, with shifting salinity levels, species are possibly found in suboptimal conditions. For example, *Botryosphaeria festucae* is probably a species found in the estuary in an optimal niche in the mesohaline zone, but found occasionally in the oligohaline habitat.

Fungal tolerance towards salinity seems to increase with rising temperature in culture (Ritchie, 1957; Schaumann, 1974; Castillo & Demoulin, 1997). If this experimental evidence holds in nature, this temperature-dependent tolerance could result in seasonal/temporal adaptations, a point that makes sense in an estuary where seasonal differences in salinity prevail due to a different flow of freshwater from upstream areas and to evaporation of tidal exchange water on the marsh. Consequently, fungi in the same sites of the brackish part of the estuary encounter higher salinities during summer when there is high evaporation, but due to the higher temperatures they may shift their growth optimum.

In agreement with Gold's (1959) classification of estuarine fungi, phragmiticolous tidal marsh fungi are split up into three basic distributional types: taxa adapted to saline-brackish

circumstances, species typical for the freshwater zone, and a group of eurytopic species found over the entire salinity gradient (Tables II, IV).

Table 6. (DATA2) Sørensen similarity index of fungal spectra on standing reed culms of *Phragmites australis* in a mesohaline brackish reed vegetation (S1) and a freshwater reed vegetation (S3) sampled at three different heights.

	Base S3	Middle S1	Middle S3	Top S1	Top S3
Base S1	0.322	0.247	0.384	0.048	0.109
Base S3		0.093	0.136	0.026	0.016
Middle S1			0.454	0.238	0.204
Middle S3				0.259	0.284
Top S1					0.725

Fungal diversity on tidal marshes

The combination of the two datasets, one comprising litter samples and another standing dead samples, yields 114 taxa found on *Phragmites* in the Scheldt estuary. Fifty-six taxa (49%) in the ascomycetes, 6 taxa (5%) in the basidiomycetes and 52 taxa (46%) of anamorphic fungi, the latter comprising 30 (26%) coelomycetes and 22 (19%) hyphomycetes. Most of these taxa are saprotrophic while some are biotrophic. For example telia of parasitic rusts (Uredinales) are frequently found in the litter layer and some endophytic species seem to be secondary saprotrophs and appear during initial decomposition (for example *Phaeosphaeria* spp.) (Guo et al., 2000; Wong & Hyde, 2001; Van Ryckegem, pers. obs.).

The number of taxa found during the study is the highest record of any survey of phragmiticolous fungi (Taligoola, 1969; Rodewald & Rudescu, 1974; Bán et al., 1996, 1998; Beyer, 1997; Poon & Hyde, 1998a; Peláez et al., 1998; Wirsel et al., 2001; Wong & Hyde, 2001). However a full discussion of the biodiversity will be treated elsewhere based on a larger database with non-systematically gathered data and sampling a broader ecological range of *Phragmites*. The species list presented is therefore incomplete, and conclusions on diversity and a biogeographic comparison of reed mycota are somewhat premature. Nevertheless, we can state that the estuarine reed belts are characterized by an impoverished mycota compared to non-tidal reed vegetations with comparable salinity concentrations (from freshwater to mesohaline) in the same climatic zone (Van Ryckegem, unpublished data). This is obvious from the low numbers of Agaricales and a lower abundance of terrestrial hyphomycetes retrieved from the tidal marsh litter layer. It is thought that these species are not able to resist the tidal dynamics, which affect reproductive structures mechanically and introduce severe osmotic stress by periodical wetting with brackish or fresh water. The water regime in non-tidal marshes fluctuates less drastically as it is affected mainly by seasonal hydropulses from precipitation and runoff, creating a more stable humid atmosphere in the litter layer in summer and often inundated circumstances in winter and early spring. Additionally, the tidal dynamic causes high sedimentation rates (Table 1) on the lower parts of standing culms and litter,

Table 7. (DATA2) Selected fungal species on standing *Phragmites australis* with detailed information of occurrence at different heights of the culm in the two tidal marshes in the Scheldt estuary [S1 (brackish) and S3 (fresh)]. Numbers of records are given.

Species	Height	S1	S3
Hendersonia culmiseda Sacc.	Top	46	46
	Middle	7	5
	Base	0	0
Septoriella sp(p).	Top	47	36
	Middle	10	19
	Base	6	0
Massarina arundinacea (Sowerby: Fr.) Leuchtm.	Top	0	0
	Middle	0	3
	Base	46	16
Myrothecium cinctum (Corda) Sacc.	Top	0	0
	Middle	0	7
	Base	32	0
Phoma sp. III	Top	1	1
	Middle	38	39
	Base	37	12

especially in the freshwater tidal marshes (Van Damme et al., 1999). The negative influences of hydropulses and/or mud sedimentation on fructification are illustrated in Table 7 and further discussed below. The absence of discomycetes is a further indication that tidal systems show a poorer mycota. This fungal group is well represented in humid marsh habitats and found on substrates in freshwater (Shearer, 1993); still it is less represented on the tidal marsh probably because the 'swamp' or 'marsh' species do not withstand the tidal dynamics and pollution stress. Most aquatic discomycetes are thought to need constant inundation in clean aerated water (Lamore & Goos, 1978; Shearer & Von Bodman, 1983; Révay & Gönczöl, 1990), conditions not fulfilled in the Scheldt marshes. The same combination of factors, periodic flooding with polluted, oxygen-depleted water, probably create unfavourable conditions for true aquatic hyphomycetes, which were never observed on the tidal marsh *Phragmites* litter.
However, other methods such as incubation of litter samples in aerated water could perhaps reveal some (semi)-aquatic hyphomycetes on the Scheldt tidal marshes.
The observed diversity pattern on tidal marsh reed, with higher species richness in freshwater sites than in brackish sites, resembles the vascular plant species diversity curve on tidal marshes which gradually decreases towards salt marshes (Odum, 1988). Some indications point to a slightly enriched species composition at the transition zone (oligohaline), but more elaborate sampling is needed to confirm this as there are many rare species causing a high stochastic effect on species richness.

Vertical distribution along the reed culm: estuarine and terrestrial fungi

Comparing the species list from S1 and S3 for litter and standing dead samples of *Phragmites*, we find 31 out of 93 taxa in common. This is quite a low proportion of taxa considering the possibility to retrieve, in theory, all taxa on standing dead reed in the litter layer after the standing culms fall onto the sediment. *Hendersonia* spp., *Septoriella* sp(p)., *Phoma* sp. III and

Didymella spp. are such typical terrestrial taxa found in the litter layer, but they are replaced by other species fruiting on the reed litter after it falls over. Distinctly different fungal communities on standing dead culms and on litter were also observed by Apinis et al. (1972a, b) and failure of terrestrial taxa to grow on leaves once they have fallen in the water was suggested by a decline in fungal biomass (Tanaka, 1991). These observations suggest that most fungi associated with standing-dead shoots cannot survive in submerged or periodically flooded conditions and are gradually replaced by taxa better adapted to the (semi)-aquatic environment.

The study of the standing dead mycota (DATA2) enables us to discriminate between accidental records of terrestrial fungi found in the litter layer and the true litter colonizers (Table 2). These immigrant fungi are often listed, because of insufficient knowledge on their ecology, as (semi)-aquatic fungi (Shearer, 1993; Thomas, 1996; http://fm5web.life.uiuc.edu:23523/ascomycete/).

Furthermore, the results show a vertical distribution of the taxa on the culms (Tables 5, 7, Fig. 3). Ordination results (Fig. 3) and a high similarity between the same vertical zones of the two sites (Table 6) show that the vertical distribution is the primary factor in modelling fungal community composition, overruling site specificity. This pattern is enforced because most of the abundant species are eurytopic with consequent vertical distribution between sites. Details on three taxa showing a similar vertical pattern in both sites are given in Table 7. *Hendersonia culmiseda* and *Septoriella* sp(p). occur exclusively on the top section of standing culms, while *Phoma* sp. III only forms conidiomata on the lower and middle parts of standing shoots. Although more site-specific species are retrieved from reed parts influenced by tidal exchange water, some indicator species for brackish- and freshwater sites can be found on the middle and upper parts of standing dead reed. For example *Stictis* sp. is a very common brackish saprotroph on standing reed almost absent from freshwater tidal marshes, while Coelomycete sp. II is a typical colonizer of the upper parts of freshwater reeds. These remarkable differences between the constantly air-exposed reed parts in brackish and freshwater stands could be caused by multiple chemical and anatomical differences of the reed plant and micro-environmental properties of the *Phragmites* stands (see Table 1). Influences on aerial leaf mycota of different, site-specific water availabilities and salinities were noticed by Apinis et al. (1972a) who found different fungal communities in more terrestrial reed stands, aquatic reed stands, both brackish and freshwater, and freshwater tidal reed belts. Unfortunately, their sampling effort was unequal among sites. Although there are true differences in species composition between basal standing parts (brackish or fresh) (Table 5), some observed patterns need cautious interpretation. This is the case with *Massarina arundinacea*, *Lophiostoma arundinis* and *Myrothecium cinctum*, which are also very common in non-tidal freshwater reed stands (Apinis et al., 1972b; Van Ryckegem unpublished data), but which seem to show highest occurrence in the estuary for the basal parts of stems in the mesohaline zone, while they were almost absent in the freshwater site. This is illustrated for two species in Table 7. These site differences are caused by an upward shift of species due to the tidal amplitude (see also Poon

& Hyde 1998b), which is much higher in the freshwater tidal system (Table 1) and causes slightly higher similarities between basal parts of S1 and middle parts of S3 (Table 6). These three species form fruitbodies just above (*Myrothecium cinctum*) high water level or just in the upper zone of flooding (*Massarina arundinacea, Lophiostoma arundinis*). This zone was not sampled in the freshwater site, hence giving a bias in data and misleading us to conclude that *M. arundinacea*, *L. arundinis* and *M. cinctum* occur more commonly in brackish than in freshwater. For the same reasons, we find no hyphomycetes at all on the basal parts of the freshwater reeds. The fragile conidiophores probably cannot withstand high sedimentation and high water dynamics in this zone of the estuary.

Evaluation of the method

Direct observation of microfungi on freshly collected material, without incubation or indirect culturing of surface-sterilized substrate, is an unusual method and related to techniques used for the study of macrofungi (Arnolds, 1992). This technique gives more information on the fungal ecology and community, as prolonged incubation in moist chambers or plating on selected media could mask the presence of species and alter the natural species composition, caused by changed growth conditions in unnatural laboratory circumstances. Moreover, the periodical direct screening offers possibilities of quantification assessing the spatial frequency, the abundance and phenology of representative microfungi. In moist chambers, mainly the development of small and simple fruitbodies is promoted, for example asexual hyphomycetes are favoured (also the most common group recovered in culture experiments) as these fungi have fairly simple architecture and develop faster than the sexual stages (Kendrick, 1979; Van Ryckegem, pers. obs.). Ubiquitous genera such as *Penicillium*, *Cladosporium*, *Epiccocum* and *Alternaria* sporulate often after moistening (also in culture) while they are less frequently found in the field using direct microscopy (Hudson & Webster, 1958; Van Ryckegem, pers. obs.). On the other hand, species recorded by indirect methods could easily be overlooked when screening for reproductive structures on freshly collected samples. In this group we expect species with mycelial presence that rarely fruit (in field conditions) or species forming ephemeral reproductive structures (ascomata, basidiomata, conidiomata or conidiophores). Both cases occur: e.g. *Lachnum controversum* (Cooke) Rehm, one of the most common litter saprotrophs of reed litter (Apinis et al., 1972a; Van Ryckegem pers. obs.), was rarely found in the estuary, but when reed samples were incubated in moist chambers it sporulated on almost all of them. This proves that mycelia are present but (sexual) fructification fails due to ecological constraints of the habitat. Still it is not clear whether this species plays an important role in reed decay in the Scheldt estuary. The rare field collections of this species were all on 'hanging' litter in the reed stands or on parts of marshes rarely flooded. *Coprinus kubickae* Pilát & Svrček is another case. This common species has basidiocarps lasting only a few hours (it manages to sporulate between two tides!). One must come at the right moment to collect this species and then often many fruitbodies are found. Bills & Polishook (1990) regarded traditional surveys by fruitbody observation as being of limited use for inventories of fungi on

plants; but we stress that rigorous but painstaking mycological determination of fruitbodies formed can yield much useful information on taxonomy, ecology and phenology of fungal communities on plants and plant litter (although less useful for soil mycota). These observations should serve as a basis for further indirect observations using culturing, chemical or genetical techniques. A combination of direct (where possible) and indirect observation with genetical techniques is considered to be the best approach to detailed fungal ecological work (Buchan et al., 2002).

Conclusions

Tidal marshes are very dynamic, transitional habitats at the interface of land and water. In addition to the longitudinal factor of salinity, we distinguish a vertical factor on the substrate and a transverse gradient on the marsh, creating a patchwork of niches influenced by height and frequency of flooding. This creates suitable habitats for the whole range from truly aquatic through xerophilic, terrestrial fungi. Qualitative differences of the reed substrate grown under different ecological conditions, probably induces additional differentiation of the mycota. Generally, colonizers of unflooded reeds (terrestrial fungi) show a higher uniformity in species composition along the entire salinity gradient, while estuarine fungi (species which can be placed on a scale from truly aquatic to terrestrial) show a greater diversity in species composition.

Acknowledgements

We thank C.A. Shearer (Dept. of Plant Biology, University of Illinois, U.S.A.) for critical commentary on the manuscript and for improving the English. G. Dewaele and M. Vlerick are thanked for assisting in the culture experiment. The research was funded by the Institute for the Promotion of Innovation by Science and Technology in Flanders, Belgium (to G.V.R.).

References

ADLER, L. (1996). Fungi and salt stress. *In* FRANKLAND, J.C., MAGAN, N. & GADD, G.M. (Eds.). Fungi and environmental change. Cambridge University Press, Cambridge. 217-234.

APINIS, A.E., C.G.C. CHESTERS & H.K. TALIGOOLA (1972a). Colonization of *Phragmites communis* leaves by fungi. Nova Hedwigia 23: 113-124.

APINIS, A.E., C.G.C. CHESTERS & H.K. TALIGOOLA (1972b). Microfungi colonizing submerged standing culms of *Phragmites communis* Trin. Nova Hedwigia 23: 473-480.

ARNOLDS, E. (1992). The analysis and classification of fungal communities with special reference to macrofungi. *In* WINTERHOFF, W. (ed.): Fungi in Vegetation Science: 7-47. Kluwer Academic Publishers, Dordrecht.

BÁN, R., G. FISCHL & F. VIRANYI (1996). A spatio-temporal analysis of fungal pathogens on reed in natural habitats. Acta Phytopathol. Entomycol. Hung. 31: 219-227.

BÁN, R., F. VIRANYI & C. OBADOVICS (1998). Survey of pathogenic fungi in different reed stands. Növénytermelés 47: 407-416.

BEYER, W. (1997). Ascomyceten und einige Basidiomyceten an *Typha latifolia* L. and *Phragmites australis* (Cav.) Trin. Beitr. Kenntn. Pilze Mitteleuropas 11: 49-113.

BILLS, G.F. & J.D. POLISHOOK (1990). Microfungi from *Carpinus caroliniana*. Canad. J. Bot. 69: 1477-1482.

BUCHAN, A., S.Y. NEWELL, J.I.L. MORETA & M.A. MORAN (2002). Analysis of internal transcribed spacer (ITS) regions of rRNA genes in fungal communities in a southeastern U.S. Salt Marsh. Microbial Ecol. 43: 329-340.

BYRNE, P.J. & E.B.G. JONES (1975). Effect of salinity on the reproduction of terrestrial and marine fungi. Trans. Brit. Mycol. Soc. 65: 185-200.

CASTILLO, G. & V. DEMOULIN (1997). NaCl salinity and temperature effects on growth of three wood-rotting basidiomycetes from a Papua New Guinea coastal forest. Mycol. Res. 101: 341-344.

FINDLAY, S.E.G., S. DYE & K.A. KUEHN (2002). Microbial growth and nitrogen retention in litter of *Phragmites australis* compared to *Typha angustifolia*. Wetlands 22: 616-625.

GESSNER, M.O. (2001). Mass loss, fungal colonisation and nutrient dynamics of *Phragmites australis* leaves during senescence and early decay. Aquatic Bot. 69: 325-339.

GESSNER, M.O. & G. VAN RYCKEGEM (2003). Water fungi as decomposers in freshwater ecosystems. *In* BITTON, G. (ed.). Encyclopedia of Environmental Microbiology: (online edition: DOI: 10.1002/0471263397.env314). Wiley, New York.

GOLD, H.S. (1959). Distribution of some lignicolous Ascomycetes and Fungi Imperfecti in an estuary. J. Elisha Mitchell Sci. Soc. 75: 25-28.

GUO, L.D., K.D. HYDE & E.C.Y. LIEW (2000). Identification of endophytic fungi from *Livistona chinensis* based on morphology and rDNA sequences. New Phytol. 147: 617-630.

HELLINGS, S.E. & J.L. GALLAGHER (1992). The effects of salinity and flooding on *Phragmites australis*. J. Appl. Ecol. 29: 41-49.

HUDSON, H.J. & J. WEBSTER, J. (1958). Succession of fungi on decaying stems of *Argopyron repens*. Trans. Brit. Mycol. Soc. 41: 165-177.

KENDRICK, B. (1979). Dialogue on the ecology of anamorphs and teleomorphs. The Conference. *In* KENDRICK, B. (ed.). The Whole Fungus. The Sexual-Asexual Synthesis. Volume 2: 623-634. National Museum of Natural Sciences and National Museums of Canada, Ottawa.

KOHLMEYER, J. & E. KOHLMEYER (1979). Marine Mycology, the Higher Fungi. Academic Press, New York. 690 pp.

LAMORE, B. J. & R. D. GOOS (1978). Wood-inhabiting fungi of a freshwater stream in Rhode Island. Mycologia 70: 1025-1034.

LUARD, E. & D.M. GRIFFIN (1981). Effect of water potential on fungal growth and turgor. Trans. Brit. Mycol. Soc. 76: 33-40.

MANN, K.H. (1988). Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. Limnol. Oceanogr. 33: 910-930.

MCCUNE, B. & M.J. MEFFORD (1999). PC-ORD for Windows. Multivariate Analysis of Ecological Data. Version 4.0. MjM Software, Gleneden Beach, Oregon, U.S.A.

MEGANCK, B. (1998). De morfologie van *Phragmites australis* (Cav.) Steud. in relatie tot hydrodynamiek, beheer en saliniteit in het Schelde-estuarium. Ghent University, master thesis, unpublished, Ghent. 97 pp.

MEIRE, P., M. STARINK & M. HOFFMANN (1997). Integratie van ecologie en waterbouwkunde in de zeeschelde: aanleiding tot en situering van het onderzoek milieueffecten sigmaplan (OMES). Water 95: 147-165.

MITSCH, W.J. & J.G. GOSSELINK (2000). Wetlands, 3th edition. Van Nostrand Reinhold, New York. 920 pp.

MUYLAERT, W. (1996). Morfologisch-ecologische studie van *Phragmites australis* (Cav.) Steud. in het Schelde-estuarium. Ghent University, master thesis, unpublished, Ghent. 120 pp.

NEWELL, S.Y. (1996). Established and potential impacts of eukaryotic mycelial decomposers in marine/ terrestrial ecotones. J. Exp. Mar. Biol. Ecol. 200: 187-206.

ODUM, W.E. (1988). Comparative ecology of tidal freshwater and salt marshes. Ann. Rev. Ecol. Syst. 19: 147-176.

OLIVEIRA, R.S., J.C. DODD & P.M.L. CASTRO (2001). The mycorrhizal status of *Phragmites australis* in several polluted soils and sediments of an industrialised region of Northern Portugal. Mycorrhiza 10: 241-247.

PELÁEZ, P., J. COLLADO, F. ARENAL, A. BASILIO, A. CABELLO, M.T. DIEZ MATAS, J.B. GARCÍA, A. GONZÁLEZ DEL VAL, V. GONZÁLEZ, J. GORROCHATEGUI, P. HERNÁNDEZ, I. MARTIN, G. PLATAS & F. VICENTE (1998). Endophytic fungi from plants living on gypsum soils as a source of secondary metabolites with antimicrobial activity. Mycol. Res. 102: 755-761.

POON, M.O.K. & K.D. HYDE (1998a). Biodiversity of intertidal estuarine fungi on *Phragmites* at Mai Po marshes, Hong Kong. Bot. Mar. 41: 141-155.

POON, M.O.K. & K.D. HYDE (1998b). Evidence for the vertical distribution of saprophytic fungi on senescent *Phragmites australis* culms at Mai Po marshes. Bot. Mar. 41: 285-292.

REICE, S.R. & G. HERBST (1982). The role of salinity in decompostion of leaves of *Phragmites australis* in desert streams. J. Arid Environm. 5: 361-368.

RÉVAY, A. & J. GÖNCZÖL (1990). Longitudinal distribution and colonization patterns of wood-inhabiting fungi in a mountain stream in Hungary. Nova Hedwigia 51: 505-520.

RITCHIE, D. (1957). Salinity optima for marine fungi affected by temperature. Amer. J. Bot. 44: 870-874.

RODEWALD, L. & L. RUDESCU (1974). Das Schilfrohr. Binnengewässer 27: 1-302.

SCHAUMANN, K. (1968). Marine höhere Pilze (Ascomycetes und Fungi imperfecti) aus dem Weser-Ästuar. Veröff. Inst. Meeresf. Bremerhaven 11: 93-117.

SCHAUMANN, K. (1974). Experimentelle Untersuchungen zum Einfluss des Salzgehaltes und der Temperatur auf das Mycelwachstum höherer Pilze aus dem Meer-und Brackwasser. Veröff. Inst. Meeresf. Bremerhaven Suppl. 5: 443-474.

SHEARER, C.A. (1972). Fungi of the Chesapeake Bay and its tributaries. III. The distribution of wood-inhabiting Ascomycetes and Fungi imperfecti of the Patuxent river. Amer. J. Bot. 59: 961-969.

SHEARER, C.A. (1993). The freshwater Ascomycetes. Nova Hedwigia 56: 1-33.

SHEARER, C.A. & S.B. VON BODMAN (1983). Patterns of occurrence of Ascomycetes associated with decomposing twigs in a midwestern stream. Mycologia 75: 518-530.

TALIGOOLA, H.K. (1969). Stages in the colonization of *Phragmites communis* Trin. by microfungi. University of Nottingham, unpublished doctoral thesis, Nottingham. 153 pp.

TANAKA, Y. (1991). Microbial decomposition of reed (*Phragmites communis*) leaves in a saline lake. Hydrobiologia 220: 119-129.

TER BRAAK, C.J.F. & P. SMILAUER (2002). CANOCO Reference manual and CanoDraw for Windows User's guide. Software for Canonical Community Ordination (version 4.5). Microcomputer Power, Ithaca, NY, USA. 500 pp.

THOMAS, K. (1996). Freshwater fungi. *In* GRGURINOVIC (Ed.). Introductory volume to the fungi. Part 2C. Fungi of Australia Vol. 1B: 1-37. Australian Biological Research Studies, Canberra.

VAN DAMME, S., T. YSEBAERT, P. MEIRE & E. VAN DEN BERGH (1999). Habitatstructuren, waterkwaliteit en leefgemeenschappen in het Schelde-estuarium. Rapport IN 99/24, Instituut voor Natuurbehoud, Brussel. 119 pp.

VAN DER TOORN, J. & J.H. MOOK (1982). The influence of environmental factors and managment on stands of *Phragmites australis*. J. Appl. Ecol. 19: 477-499.

VAN RYCKEGEM, G. & A. APTROOT (2001). A new *Massarina* and a new *Wettsteinina* (Ascomycota) from freshwater and tidal reeds. Nova Hedwigia 73: 161-166.

VAN RYCKEGEM, G., K. VAN DE PUT & P. ROBERTS (2002). *Tremella spicifera* sp. nov., a parasite of *Massarina arundinacea*. Mycotaxon 81: 185-189.

VAN RYCKEGEM G. & A. VERBEKEN (2000). *Rosellinia musispora* sp. nov., een nieuw tepelkogeltje op riet (*Phragmites australis*). Sterbeeckia 19: 3-5.

WEBSTER, J.R. & E.F. BENFIELD (1986). Vascular plant breakdown in freshwater ecosystems. Ann. Rev. Ecol. Syst. 17: 567-594.

WIRSEL, S.G.R., W. LEIBINGER, M. ERNST & K. MENDGEN (2001). Genetic diversity of fungi closely associated with common reed. New Phytol. 149: 589-598.

WONG, M.K.M., T.K. GOH, I.J. HODGKISS, K.D. HYDE, V.M. RANGHOO, C.K.M. TSUI, W.H. HO, W.S.W. WONG & T.K. TUEN (1998). Role of fungi in freshwater ecosystem. Biodivers. Conserv. 7: 1187-1206.

WONG, M.K.M. & K.D. HYDE (2001). Diversity of fungi on six species of Gramineae and one species of Cyperaceae in Hong Kong. Mycol. Res. 105: 1485-1491.

The role of fungi in tidal reed marshes: their established and potential impact in the Scheldt estuarine ecosystem

Abstract Research providing evidence for the important role accomplished by phragmiticolous fungi is summarized and extrapolated to evaluate the potential impact of fungi in the Scheldt estuarine ecosystem. A huge diversity of 166 fungal taxa is recorded on *Phragmites australis* in the Scheldt estuary. Besides substantial diversity, fungi sustain a great biomass and within a sample fungi could contain up to 10% of reed carbon. Moreover within decaying stems and leaf sheaths fungi contain the bulk of nutrients (N and P) present in the canopy and generally more than half of the nutrient stock in the litter layer. Fungi incorporate a considerable proportion of nutrients (N) (~37%) in leaf blades in the canopy and on average 14% of leaf blade N in the litter layer. The mycelial decomposers are thought to immobilize external nutrients and heavy metals potentially raising the sink function of a tidal marsh. Furthermore extrapolation predicts that fungi sustain a high biomass on a unit marsh base in the canopy in for example 1) leaves (blades and sheaths) before shedding of ≈ 45 g fungal biomass m^{-2} and 2) stems ≈ 10.8 and 11.2 g fungal biomass m^{-2} after one year decay respectively in the litter layer and in the canopy. The potential importance of fungi as a food source for detritus feeding invertebrates and the potential importance of fungi for plants vigour is briefly discussed. This paper stresses the need to include fungi in ecosystem studies within the Scheldt and other wetland ecosystems.

Introduction

Members of the Kingdom Fungi are mycelial decomposers highly adapted for capture of solid resource by pervasion and assimilation from within. With their hyphae comparable to self-extending tubular reactors they manage to penetrate deep into the plant tissue (e.g. Carlile, 1994). Within this solid resource away from disturbance, predators and competing organoosmotrophs such as bacteria, they manage to build up a considerable biomass (Van Ryckegem et al. 2005a, b). A portion of it is converted and exported as propagules (Newell & Wasowski, 1995; Newell, 2001) and some portion is consumed by shredder invertebrates (Newell & Bärlocher, 1993; Graça et al. 2000; pers. obs.).

There are several methods for measuring fungal mass and rates of fungal production (Newell, 1992; Gessner & Newell, 2002). Both of the most widespread and elegant methods for estimating biomass on the one hand and fungal productivity on the other hand are based on the presence of ergosterol, a nearly specific eumycotic fungal membrane molecule (Gessner & Newell, 2002). Both techniques include a lipid – sterol – extraction, followed by high performance liquid chromatographic (HPLC) quantification of the ergosterol. This molecule is unique in its UV-absorption spectrum (Vanden Bossche, 1990). The final, most controversial step is a conversion from ergosterol concentrations towards fungal biomass in the resource (Gessner & Newell, 2002). The productivity method extends the biomass assay by initial

incubation of fungi in a radiolabeled [1-^{14}C]acetate environment. This label is incorporated during ergosterol synthesis for a timed period and results in estimates for instantaneous growth rates for fungi in the field. Although ergosterol based techniques received criticism (e.g. Bermingham et al., 1995) they are considered to be fairly robust (Fell & Newell, 1998; Charcosset & Chauvet, 2001; Newell, 2001a) and the application has resulted in considerable advances in our understanding of litter decomposition and microbial interactions in several environments.

Although fungal importance in ecosystem processes is evident, they are considered rarely in ecosystem and habitat studies (Watling, 1997). Recently, fungi have been found to be the predominant decomposers in several wetlands and of several macrophytes including *Phragmites australis* (Cav.) Trin. ex Steud., *Spartina* spp., *Carex* spp., *Juncus* spp. and *Typha* spp.. In these macrophyte dominated systems, fungi (Eumycota) accounted for up to 99% of the total microbial biomass and production (e.g. Newell et al., 1995; Komínková et al., 2000; Kuehn et al., 2000; Newell & Porter, 2000; Findlay et al., 2002).

Fungi perform critical functions as symbionts (both endophytes and (vesicular) arbuscular mycorrhiza) and parasites in reed stands (Tewksbury et al., 2002; Ernst et al., 2003; Wirsel, 2004). Although these are ecologically important, the main focus in this paper will be on fungal saprotrophs. It might be clear that such dominant organisms can influence decay patterns in multiple ways, however specific information on their involvement is scarce (Gessner et al., 1997; Gessner & Van Ryckegem, 2003). A significant portion of carbon and nutrients (N, P) of the decaying plant material could be converted into microbial mass (e.g. Newell, 1993) and they could even immobilize external nutrients (e.g. Sinsabaugh et al., 1993; Suberkropp & Chauvet, 1995). Therefore, Fungi potentially regulate wetland decomposition process (Newell, 1996; Gessner et al., 1997).

With this paper I would like to demonstrate the important function of fungi within an estuarine, reed dominated ecosystem. I will focus on a single decomposition system associated with the above ground shoots of *Phragmites australis*, within the Scheldt estuary (The Netherlands – Belgium), with most of the results derived from a single brackish marsh ('Doel-Saefinghe' 51° 21' N, 4° 14' E).

Phragmites australis within the Scheldt estuary

In the Scheldt estuary, *P. australis* is one of the dominant macrophytes and detailed information on the annual above ground biomass and seasonal dynamics of common reed is available (Hoffmann, 1993; Muylaert, 1996; Meganck, 1998; Soetaert et al., 2004). On a whole of 3011 ha of tidal marsh along the Scheldt, 219 ha (7%) is covered by common reed. About 160 ha monotypic reed vegetation and 59 ha mixed with herbaceous vegetation, especially in the freshwater part (± 42 ha), with *Urtica dioica*, *Calystegia sepium* and *Impatiens glandulifera* being the other dominant plant species (Hoffmann, 1993). Along the Sea Scheldt (Belgian part of the estuary) 179 ha (35%) of the tidal marches are covered with *P. australis* (Van Damme et al.,

1999). Most of these reed marshes (70%, 125 ha) are situated in the brackish part of the Sea Scheldt. This results in a brackish zone of which 67% of the vegetation cover is common reed. In the freshwater zone, more than half of the tidal marshes are covered by willow (*Salix* spp.) while *Phragmites* covers 54 ha (13.4%) of the freshwater tidal marsh (Van Damme et al., 1999). The presented figures indicate that *Phragmites* is mainly of importance as dominant vegetation along the Sea Scheldt, and in particular in the oligohaline and in the lower end of the mesohaline brackish part.

Within the Scheldt estuary common reed was estimated to have an average biomass production of 10 ton/ha (Meganck, 1998), resulting in a total reed biomass of about 2190 ton yr^{-1} in the entire Scheldt estuary (exclusive tributaries) of which 1787 ton yr^{-1} is produced along the Sea Scheldt.

Plant parts take different proportions in this bulk, with the stem material accounting for the greatest part (~60%). The remaining mass is divided over (~25%) leaf blades and (~14%) leaf sheaths (Meganck, 1998; Van Ryckegem et al., 2005b). Although leaf sheaths take a substantial fraction of plant production, they are often neglected in ecosystem organic budgets or culm material is not separated from each other and threatened as a single entity. However, as was shown by Gessner (2000) and further illustrated during our studies those plant parts have completely different decay patterns and bear different mycota (Van Ryckegem & Verbeken, 2005b,c, d). Hence, all plant parts should be considered separately. Inflorescences were not studied but these take generally less than 5% of total shoot mass (Meganck, 1998) and have comparable species composition compared to upper canopy parts (Taligoola, et al., 1975). Rhizomes and roots account for more than 60% of total reed production (Soetaert et al., 2004) but their decay can be controlled by totally different assemblages of organisms (Wirsel et al., 2001), which have not been considered during our research.

Fungal Biodiversity within the Scheldt estuary

Fungal community structure on *P. australis* proved to be species rich and complex (Van Ryckegem & Verbeken, 2005d). In a single brackish marsh, intensively studied ('Doel-Saeftinghe'), we discovered 95 taxa on the reed host (Van Ryckegem & Verbeken, 2005d). Within the Scheldt estuary we discovered 166 fungal taxa occurring on *P. australis* during plants growth and decay (Van Ryckegem & Verbeken, 2005a; Van Ryckegem, 2005). Notwith-standing this number is already higher than total plant diversity within the estuary (see Hoffmann, 1993), the real number of fungal taxa on reed will be much higher (Van Ryckegem, 2005). This astonishing diversity is mainly caused by small, specific ecological niches captured by different taxa, fungal succession during breakdown and by a changing species composition along the salinity gradient of the Scheldt estuary (Van Ryckegem & Verbeken, 2005a, d). A full taxonomic treatment of the fungi associated with common reed found during four years of research is presented in Van Ryckegem (2005).

Fungal decomposers in the Scheldt estuary and of *Phragmites australis* in general are mainly ascomycetes. Together with their asexual stages they account for roughly 95% of the taxa involved. However, some basidiomycetes could be important in litter layer decay, such as *Coprinus* spp. in the Scheldt-estuary (Van Ryckegem & Verbeken, 2005a) and additionally *Mycena* and *Psathyrella* species in other wetlands.

Established and potential impact of fungi in the Scheldt estuary

Fungal biomass and carbon-nutrient incorporation

Fungi can sustain a high biomass both on the standing dead reed and in the litter layer of several ecosystems (Komínková et al., 2000; Gessner, 2001; Findlay et al., 2002; Kuehn et al., 2004) and in the Scheldt estuary (Van Ryckegem et al. 2005a, b). However, most of the results gathered in the Scheldt estuary deal with data originating from the litter layer. The conversion factors that have been used rely on the determinations by Findlay et al. (2002): 5.8 mg ergosterol/g fungal dry mass, 65 mg N/g fungal biomass and 431 mg C/g fungal biomass. The conversion factor used for phosphorus calculations, 4 mg P/g fungal biomass, was depicted from Beever & Burns (1980) (see Van Ryckegem et al., 2005b for comments on those conversion factors).

All below extrapolations should be considered low because only living fungal mass was included for calculations. In a further stage of decay, non-living fungal mass in a resource was estimated to equal or surpass the amount of living fungal mass resulting in a 2 to 3 fold greater total mass compared to living mass (Newell, 1992). Dead fungal mass, mainly glucosamine, also contains carbon and nitrogen, and a lesser amount of phosphorus. Unfortunately no fungal productivity was measured during this study but as pointed out by Komínková et al., (2000) and Findlay et al. (2002) total fungal production is much higher because of the turnover of fungal mass and species transitions. For example, Newell (2001) estimated the annual fungal production per-area to be about ten times higher than the average fungal biomass present on standing dead *Spartina* in a salt marsh.

In the canopy of a brackish tidal marsh ('Saeftinghe') we found high fungal biomass (104 ± 16 mg fungal biomass g^{-1} dry mass) in leaf blades prior to shedding. These fungal colonizers were estimated to incorporate at least 10% of leaf blade carbon, 37% of nitrogen standing stock (Van Ryckegem et al., 2005a) and 54 ± 8% (mean, ± STDEV, N = 4) of phosphorus standing stock (unpubl. data). For stems there are no data available in the reed canopy in combination with nutrient determination but after senescence of the culms (December), no fungal colonization was detected on the standing stem. However, after one year standing decay, a considerable biomass (50 ± 19 mg fungal biomass g^{-1} dry mass) was found. In contrast to stems, leaf sheaths had high fungal biomass (93 ± 10 mg fungal biomass g^{-1} dry mass) after senescence of the stems in December. Leaf sheath fungi contained at this stage the entire stock of nitrogen and phosphorus measured in the leaf sheaths (Van Ryckegem et al., 2005b).

Table 1. Rough average calculations of g organic living fungal mass m^{-2} marsh in the canopy and variables.

	Leaf blades Autumn	Leaf sheaths Autumn	Stems next year Autumn
Decaying material§	320 g m^{-2}	125 g m^{-2}	223 g m^{-2}
Percentage living fungal mass	10.4%	9.3%	5%
Fungal biomass	33.3 g m^{-2}	11.6 g m^{-2}	11.2 g m^{-2}
Percentage fungal N$	37	~100%	N/A
Percentage fungal P$	54	~100%	N/A

§ From Meganck (1998) and Van Ryckegem et al., 2005b specific for our studied site ('Saeftinghe'); 50% of the stems considered to be standing after one year standing decay; with little mass lost after one year standing decay.
$ The percentage of total N and P of the decay system estimated to be present in living fungal mass, using 6.5% N of fungal mass (Findlay et al., 2002) and 0.4% P of fungal mass (Beever & Burns, 1980).

In the litter layer fungal dynamics were followed during decay of leaf blades, sheaths and stems (Van Ryckegem et al. 2005a, b). For leaf blades and sheaths with considerable fungal presence in the canopy, a distinct drop in fungal biomass and a clear shift in species composition has been noticed after placement on the marsh surface. The mean fungal biomass on leaf blades in our study site was 45.3 mg fungal biomass g^{-1} dry mass in the litter layer. About 14% of the leaf blade nitrogen was associated with fungal biomass. Thus, fungi seem to play a role in N retention during leaf senescence and early decay in the canopy, but appear not to play a major role in litter nitrogen dynamics during decomposition on the marsh surface. Leaf sheaths had on average 85 ± 25 mg fungal biomass g^{-1} dry mass. This fungal mass accounted for about 64% of the nitrogen and 37% of the phosphorus stock during leaf sheath decay (Van Ryckegem et al., 2005b). After initial establishment of fungal colonizers on the stems lying on the marsh surface, an average of 80 ± 27 mg fungal biomass g^{-1} dry mass (excluding the initial samples without fungal colonization) was recorded on the decomposing stems. Those fungal communities comprised nearly total nitrogen stock and 52% (on average) of the phosphorus stock. Furthermore, besides an important impact on nutrient retention in decaying culms (sheaths and stems), culm fungi were suggested to immobilize external nitrogen and phosphorus from the tidal exchange water to sustain their growth (Van Ryckegem et al., 2005b).

Calculations, unit-marsh basis – Based on the above results and literature, we can roughly demonstrate the fungal impact as organic mass present and on carbon and nutrient budgets within our study site on a unit marsh base. For example we could calculate the amount of fungal mass present in the canopy of last year's reed cohort in late autumn. At this stage only leaf blades and sheath are colonized by fungi (Van Ryckegem et al., 2005b) (Table 1) and resorption of nutrients is largely completed (Granéli et al., 1992). We find about 45 g fungal biomass m^{-2} (Table 1) or extrapolating towards the entire estuary (using above areas of reed

marsh): 99 ton of fungal biomass in the reed canopy. These fungi would account for 38% and 50% of total standing crop of N and P respectively in all above ground plant parts of last year's reed cohort, using % N and % P for plant parts from Van Ryckegem et al. (2005a, b) (leaf blades: 1.86% N, 0.077% P; leaf sheaths: 0.58% N, 0.039% P; stems: 0.24% N, 0.015% P).
Another example illustrates the importance of fungi associated with stems. After one year of decay in the litter layer, nearly all leaf blades and leaf sheaths are decomposed. Assuming that 50% of the stems showed decay in the litter layer and that about half of this material decomposed one year after death (see Van Ryckegem et al., 2005b), we could retrieve about 111 g stem mass m^{-2} left on the marsh surface. This stem mass supports about 10.8 g fungal biomass m^{-2} estimated to incorporate about all of the litter associated nitrogen and 71% of litter phosphorus. Additionally equal fungal biomass is expected within stem tissue in the canopy (11.2 g m^{-2}).

Now that is established that fungal diversity and secondary production is substantial in reed dominated vegetations along the Scheldt River, we might ask what this could imply for the whole estuarine ecosystem. Because of the small proportion tidal (reed) marshes have in the Scheldt estuary (see above), their influence on the whole ecosystem is limited (Van Damme et al., 1999). However, because of the high plant productivity large amounts of litter are produced on relatively small areas making these zones real bioreactors for nutrients. In these processes fungi may have an important role as decomposers. Furthermore, fungi could immobilize nutrients and heavy metals from the river water flowing into the marsh (Van Ryckegem et al., 2005b; Du Laing et al., 2005). Although aquatic fungi were observed on plant material within the pelagial (Pers. obs.), their importance is unknown. It seems not unlikely to think that floating plant material will have extensive fungal colonization (perhaps also with oomycetes). However, a more quantitative approach is needed to establish fungal role in these processes.

Besides their role in carbon and nutrient cycles and a substantial contribution to the overall diversity on tidal marshes fungi could potentially be important as symbionts. Furthermore fungi could prove to be important in interacting with other host plant in the estuary.
Fungal-reed mutualism – recent research of Ernst et al. (2003) provides substantial evidence of a symbiotic relationship between phragmiticolous endophytes of the genus *Phaeosphaeria* and the reed plant. Fungi were demonstrated to increase plant's growth by mechanisms that need to be resolved. Furthermore, endophytes could enhance defences against herbivores and against microbial pathogens (Saikkonen et al., 1998). Thus, if this evidence holds, *Phragmites* vegetations might be influenced to a much greater extend by fungal colonizers than previously thought.
Fungal-depending organisms – Fungi could be an important food source for several invertebrates (see Bärlocher, 1992) and they are known to form mutualistic relationships with

several reed specific insects (Tscharntke, 1999). Hence there could be an important mass flow towards the animal food webs (Newell & Bärlocher, 1993; Graça et al., 2000; see Van Ryckegem et al., 2005a) and fungi could contribute indirectly to marsh biodiversity. Furthermore fungal-invertebrate relations are complex and species could dependent on fungal standing crop (Newell & Porter, 2000; Graça et al. 2000).

Fungi on other host plants in the estuary – Although unstudied so far, the compelling evidence of fungal dominated decay of above ground shoots of *Spartina alterniflora* in Georgia (U.S.A.) (Newell & Porter, 2000; Newell, 2001b) could be true for *Spartina anglica* dominated salt marshes within the Scheldt estuary. The only support for this hypothesis seems to be the stereomicroscopic observation of abundant fungal sporulation on *S. anglica* (Van Ryckegem, pers. obs.).

Conclusion

Fungi do not deserve to be ignored. More than 400 scientifically peer review papers appeared on the Scheldt ecosystem, however not a single paper seems to mention fungi. Although bacteria are considered to be the most important organoosmotrophs within the sediment (e.g. Boschker et al., 1999), fungi are the main actors in carbon mineralization and play important roles in nutrient cycles in the plant's canopy and during aerobic decay on the sediment surface. Fungi have a high diversity and have the ability to form a substantial part of the litter fraction. There are indications that fungi are involved in the retention and immobilization of nutrients and heavy metals and hence they could substantially contribute to the sink function of a (tidal) marsh. Although unknown within the Scheldt estuary fungi could be an important food source for invertebrates.

The established impacts of fungi in the Scheldt estuary presented in this paper are exclusively derived from the results from a Ph.D. study and are limited to the work of one person in 5 years. It is clear that the potential impact is far beyond the present knowledge and opens an exciting pathway for future research.

References

BÄRLOCHER, F. (ED.) (1992). The ecology of aquatic hyphomycetes. Ecol. Stud. Series, vol. 94. Springer-Verlag, New York.

BEEVER, R.E. & BURNS, D.J.W. (1980). Phosphorus uptake, storage and utilization by fungi. Advances in Bot. Res. 8: 127-219.

BERMINGHAM, S., MALTBY, L. & COOKE, R.C. (1995). A critical assessment of the validity of ergosterol as an indicator of fungal biomass. Mycol. Res. 99: 479-484.

BOSCHKER, H.T.S., DE BROUWER, J.F.C. & CAPPENBERG, T.E. (1999). The contribution of macrophyte-derived organic matter to microbial biomass in salt-marsh sediments: Stable carbon isotope analysis of microbial biomarkers. Limnol. Oceanogr. 44: 309-319.

CARLILE, M.J. (1994). The success of the hypha and mycelium. *In* GOW, N.A.R. & GADD, G.M. The growing fungus. Chapman & Hall, London. 3-19.

CHARCOSSET, J.-Y. & CHAUVET, E. (2001). Effect of culture conditions on ergosterol as an indicator of biomass in the aquatic hyphomycetes. Appl. Environ. Microb. 67: 2051-2055.

DU LAING, G., VAN RYCKEGEM, G., TACK, F.M.G. & VERLOO, M.G. (2005). Metal accumulation in intertidal litter through decomposing leaf blades, sheaths and stems of *Phragmites australis*. Chemosphere (submitted).

ERNST, M., MENDGEN, K.W. & WIRSEL, S.G.R. (2003). Endophytic fungal mutualists: seed-borne *Stagonospora* spp. enhance reed biomass production in axenic microcosms. Molec. Pl.-Microbe Interact. 16: 580-587.

FELL, J.W. & NEWELL, S.Y. (1998). Biochemical and molecular methods for the study of marine fungi. *In* COOKSEY, K.E. (ED.) Molecular approches to the study of the oceans. Chapman & Hall, London. .

FINDLAY, S.E.G., DYE, S. & KUEHN, K.A. (2002). Microbial growth and nitrogen retention in litter of *Phragmites australis* compared to *Typha angustifolia*. Wetlands 22: 616-625.

GESSNER, M.O. (2000). Breakdown and nutrient dynamics of submerged *Phragmites* shoots in the littoral zone of a temperate hardwater lake. Aquatic Bot. 66: 9-20.

GESSNER, M.O. & NEWELL, S.Y. (2002). Biomass, growth rate, and production of filamentous fungi in plant litter. *In* HURST, C.J., CRAWFORD, R.L., KNUDSON, G., MCINERNEY, M., STETZENBACH, L.D., (EDS.) Manual of Environmental Microbiology, 2nd ed. ASM Press, Washington, DC. 390-408.

GESSNER, M.O. & VAN RYCKEGEM, G. (2003). Water fungi as decomposers in freshwater ecosystems. *In* BITTON, G. (ED.) Encyclopedia of Environmental Microbiology. Wiley, New York. 3353-3364.

GESSNER, M.O., SUBERKROPP, K. & CHAUVET, E. (1997). Decomposition of plant litter by fungi in marine and freshwater ecosystems. *In* WICKLOW, D.C. & SÖDERSTRÖM, B. (EDS.) The mycota IV, Environmental and microbial relationships. Springer-Verlag, Berlin Heidelberg. 303-322.

GRAÇA, M.A., NEWELL S.Y., KNEIB, R.T. (2000). Grazing rates of organic matter and living fungal biomass of decaying *Spartina alterniflora* by three species of salt-marsh invertebrates. Mar. Biol. 136: 281-289.

GRANÉLI, W., WEISNER, S.E.B. & SYTSMA, M.D. (1992). Rhizome dynamics and resource storage in *Phragmites australis*. Wetlands Ecol. Manage. 1: 239-247.

HOFFMANN, M. (1993). Vegetatiekundig-ecologisch onderzoek van de buitendijkse gebieden langs de Zeeschelde met vegetatiekartering. Universiteit Gent, 223 pp.

KOMÍNKOVÁ, D., KUEHN, K.A., BÜSING, N., STEINER, D. & GESSNER, M.O. (2000). Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a litteral reed stand of a large lake. Aquatic Microb. Ecol. 22: 271-282.

KUEHN, K.A., LEMKE, M.J., SUBERKROPP, K. & WETZEL, R.G. (2000). Microbial biomass and production associated with decaying leaf litter of the emergent macrophyte *Juncus effesus*. Limnol. Oceanogr. 45: 862-870.

KUEHN, K.A., STEINER, D. & GESSNER, M.O. (2004). Diel mineralization patterns of standing-dead plant litter: implications for CO2 flux from wetlands. Ecology 85: 2504-2518.

MEGANCK, B. (1998). De morfologie van *Phragmites australis* (Cav.) Steud. in relatie tot hydrodynamiek, beheer en saliniteit in het Schelde-estuarium. Universiteit Gent, licentiaatsscriptie, unpublished, Gent. 97 pp.

MUYLAERT, W. (1996). Morfologisch-ecologische studie van *Phragmites australis* (Cav.) Steud. in het Scheldeestuarium. Universiteit Gent, Licentiaatsscriptie, unpublished, Gent. 120 pp.

NEWELL, S.Y. (1992). Estimating fungal biomass and productivity in decomposing litter. *In* CAROLL G.C. & WICKLOW, D.T. The fungal community. Its organisation and role in the ecosystem. 2de ed.. Marcel Dekker, New York. 521-561.

NEWELL, S.Y. (1993). Decomposition of shoots of a salt-marsh grass, methodology and dynamics of microbial assemblages. Adv. Microb. Ecol. 13: 301-326.

NEWELL, S.Y. (1996). Established and potential impacts of eukaryotic mycelial decomposers in marine/ terrestrial ecotones. J. Exp. Mar. Biol. Ecol. 200: 187-206.

NEWELL, S.Y. (2001a). Fungal biomass and productivity. Meth. Microbiol. 30: 357-372.

NEWELL, S.Y. (2001b). Spore-expulsion rates and extents of blade occupation by Ascomycetes of the smooth-cordgrass standing-decay system. Bot. Mar. 44: 277-285.

NEWELL, S.Y. & BÄRLOCHER, F. (1993). Removal of fungal and total organic matter form decaying cordgrass leaves by shredder snails. J. Exp. Mar. Biol. Ecol. 171: 39-49.

NEWELL, S.Y. & PORTER, D. (2000). Microbial secondary production from saltmarsh-grass shoots and its known and potential fates. *In* WEINSTEIN, M.P. & KREEGER, D.A. (EDS.) Concepts and controversion in tidal marsh ecology. Kluwer Academic Publishers, Dordrecht. 159-186.

NEWELL, S.Y. & WASOWSKI, J. (1995). Sexual productivity and spring intramarsh distribution of a key saltmarsh microbial sec. producer. Estuaries 18: 241-249.

NEWELL, S.Y., MORAN, M.A., WICKS, R. & HODSON, R.E. (1995). Procutivities of microbial decomposers during early stages of decomposition of leaves of a freshwater sedge. Freshwater Biol. 34: 135-148.

NEWELL, S.Y., PORTER, D. & LINGLE, W.L. (1996). Lignocellulolysis by ascomycetes (fungi) of a saltmarsh grass (smooth cordgrass). Microscopy Res. Techn. 33: 32-46.

SAIKKONEN, K., FAETH, S.H., HELANDER, M. & SULLIVAN, T.J. (1998). Fungal endophytes: a continuum of interactions with host plants. Rev. Ecol. Syst. 29: 319-343.

SINSABAUGH, R.L., ANTIBUS, R.K., LINKINS, A.E., MCCLAUGHERTY, C.A., RAYBURN, L., REPERT, D. & WIELAND, T. (1993). Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecology 74: 1586-1593.

SOETAERT, K., HOFFMANN, M., MEIRE, P., STARINK, M., VAN OEVELEN, D., VAN REGENMORTEL, S. & COX, T. (2004). Modeling growth and carbon allocation in two reed beds (*Phragmites australis*) in the Scheldt estuary. Aquatic Bot. 79: 211-234.

SUBERKROPP, K. & CHAUVET, E. (1995). Regulation of leaf breakdown by fungi in streams: influences of water chemistry. Ecology 76: 1433-1445.

TALIGOOLA, H.K., APINIS, A.E. & CHESTERS, C.G.C. (1975). Microfungi colonizing the inflorescences of *Phragmites communis* Trin. Nova Hedwigia 26: 483-494.

TEWKSBURY, L., CASAGRANDE, R., BLOSSEY, R., HAFLIGER, P. & SCHWARZLANDER, M. (2002). Potential for biological control of *Phragmites australis* in North America. Biol. Control 23: 191-212.

VAN DAMME, S., YSEBAERT, T., MEIRE, P. & VAN DEN BERGH, E. (1999). Habitatstructuren, waterkwaliteit en leefgemeenschappen in het Schelde-estuarium. Rapport IN 99/24, Instituut voor Natuurbehoud, Brussel. 119 pp.

VAN RYCKEGEM, G. (2005). Fungi on common reed (*Phragmites australis*): fungal diversity, community structure and decomposition processes. Ph.D. thesis. Ghent University, Ghent.

VAN RYCKEGEM, G. & VERBEKEN, A. (2005a). Fungal diversity and community structure on *Phragmites australis* (Poaceae) along a salinity gradient in the Scheldt estuary (Belgium). Nova Hedwigia 80: 173-197.

VAN RYCKEGEM, G. & VERBEKEN, A. (2005b). Fungal ecology and succession on *Phragmites australis* in a brackish tidal marsh I. Leaf sheaths. Fungal Diversity 19 (in print).

VAN RYCKEGEM, G. & VERBEKEN, A. (2005c). Fungal ecology and succession on *Phragmites australis* in a brackish tidal marsh II. Stems. Fungal Diversity 20 (accepted).

VAN RYCKEGEM, G. & VERBEKEN, A. (2005d). The complex fungal community structure associated with above ground plant parts of *Phragmites australis* in a brackish tidal marsh. (to be submitted to Mycol. Res.).

VAN RYCKEGEM, G., GESSNER, M.O. & VERBEKEN, A. (2005a). Fungi on leaf blades of *Phragmites australis* in a brackish tidal marsh: diversity, succession and leaf decomposition. (to be submitted to Mycologia).

VAN RYCKEGEM, G., VAN DRIESSCHE, G., VAN BEEUMEN, J.J. & VERBEKEN, A. (2005b). Fungal dynamics during decomposition of *Phragmites australis* leaf sheaths and stems in a brackish tidal marsh. (to be submitted to Microb. Ecol.).

VANDEN BOSSCHE, H. (1990). Importance of sterols in fungal membranes. *In* KUHN, P.J., TRINCI, A.P.J., JUNG, M.J., GOOSEY, M.W., & COPPING, L.G. (ED.) Biochemistry of cell walls and membranes in fungi. Springer-Verlag, Berlin. 135-157.

WATLING, R. (1997). Pulling the threads together: habitat diversity. Biodiv. Conserv. 6: 753-763.

WIRSEL, S.G.R. (2004). Homogenous stands of a wetland grass harbour diverse consortia of arbuscular mycorrhizal fungi. FEMS Microb. Ecol. 48: 129-138.

WIRSEL, S.G.R., LEIBINGER, W., ERNST, M. & MENDGEN, K. (2001). Genetic diversity of fungi closely associated with common reed. New Phytol. 149: 589-598.

PART 3

FUNGAL DIVERSITY: PERSONAL OBSERVATIONS AND WORLD-WIDE DIVERSITY OF REED FUNGI

Observed diversity

During the period 1998-2004 several wetlands with reed vegetations have been visited. Above ground reed parts were screened for fungal presence. This yielded a total species list of 214 taxa (134 genera). All those taxa are listed and described in the appendix and further illustrated on a website (http://biology.ugent.be/reedfungi). 59% of the taxa could be identified up to species level, at least 20 taxa are expected to be new to science (Table 1), 4 taxa have been described in mean time (Van Ryckegem & Verbeken, 2000; Van Ryckegem & Aptroot, 2001; Van Ryckegem et al., 2002).

The taxonomic position of the observed taxa is presented in Table 2. About equal taxa richness was observed for the ascomycetes and the anamorphic fungi. Within the anamorphic fungi, coelomycetes and hyphomycetes had equal share in taxa numbers. However, coelomycetes are by far the most abundant (see e.g. chapter 2). A relatively low percentage of the taxa belonged to the basidiomycetes.

Most representatives of the anamorphic taxa (asexual states) will have a teleomorph (sexual state) classified as an ascomycete. Unfortunately, the connection between the asexual and sexual stage remains unknown for most taxa. During our study we tried to unravel some of the anamorph-teleomorph connections by culturing taxa on corn meal agar (CMA) or on sterile reed parts in water agar. This way we hoped to retrieve the sexual or asexual stage. In total we tried to get 67 taxa in culture. Half of the cultures started from the sexual ascomycete stage (33 taxa), the remaining originated from the anamorphic stage (23 coelomycetes, 10 hyphomycetes). We also tried to grow *Coprinus kubickae* (basidiomycetes) but this culture got contaminated. Most cultures have been dried and are included in our herbarium collection (GENT). Which species were successfully cultured is indicated together with the species descriptions (see appendix). Four collections were deposited in MUCL* [*Massarina arundinacea* (MUCL 45115, 45116); *Massariosphaeria* sp. (MUCL 45118); *Halosphaeria hamata* (MUCL 45117)].

The success in discovering the ana-or teleomorph connection was low compared to the effort we invested in the culture project. We did not succeed in inducing the formation of sexual sporulation after plating conidia. Several anamorphs just formed a new conidial stage, a common phenomenon because those asexual stages are much easier retrieved from cultures (Arx, 1981). However several anamorphic taxa demonstrated to have more than one asexual stage – pleioanamorphism. For example several *Stagonospora* spp. (sensu Sutton, 1980; see appendix) also formed a *Phoma*-like anamorph. *Pseudoseptoria donacis* formed a hyphomycetes anamorph in culture very similar to a *Microdochium* sp. Growing them helped us to recognize and unite several of the formed anamorphic stadia. The more common practice, plating sexual spores and hoping to induce the asexual stage, remained also relatively unsuccessful. One ana-teleomorph connection has been discovered, namely those of the *Septoriella* sp(p). state of *Mycosphaerella lineolata* (see appendix for full description). However we kept the taxa separated

* Mycothèque de l'Université catholique de Louvain

in this Ph.D. because we could not readily recognize the variability of the *Septoriella* sp(p). on the reed host. Other already previously established connections were *Pleurophomopsis* state for *Lophiostoma semiliberum* and *L. appendiculatum*; *Hendersonia* (*Stagonospora* s.l.) state for *Phaeosphaeria* spp.

Table 1. Taxa listed which are considered to be new to science. Several other taxa could make the list but more taxonomic research is needed.

Taxa	**Divisio - form class**
Tremella spicifera Van Ryck., Van de Put & P. Roberts	Basidiomycota
Acrocordiopsis sp.	Ascomycota
Ceratosphaeria sp.	Ascomycota
Cryptodiscus sp.	Ascomycota
Discostroma sp.	Ascomycota
Lasiosphaeria sp.	Ascomycota
Massarina fluviatilis Aptroot & Van Ryck.	Ascomycota
Massariosphaeria sp.	Ascomycota
Phomatospora sp. III	Ascomycota
Phomatospora sp. IV	Ascomycota
Podospora sp. I	Ascomycota
Rosellinia musispora Van Ryck. & Verbeken	Ascomycota
Sphaerodes sp.	Ascomycota
Stictis sp.	Ascomycota
Stictis sp. II	Ascomycota
Wettsteinina moniliformis Van Ryck. & Aptroot	Ascomycota
Camarosporium sp.	Ascomycota – coelomycetes
Pseudorobillarda sp.	Ascomycota – coelomycetes
Stagonospora sp. I	Ascomycota – coelomycetes
Stagonospora sp. II	Ascomycota – coelomycetes

Table 2. Taxonomic position and diversity of fungi on *Phragmites australis* found during this Ph.D. study.

	Total no. of taxa	no. of genera	% of taxa in group
basidiomycetes	21	18	9.8
ascomycetes	99	55	46.3
anamorphic fungi	94	62	43.9
coelomycetes	48	26	22.4
hyphomycetes	46	36	21.5

References

ARX, VON, J.A. (1981). The genera of fungi sporulating in pure culture. Vaduz: J. Cramer, Stuttgart. 430 pp.

SUTTON, B.C. (1980). The Coelomycetes. Commonwealth Mycological Institute. 696 pp.

VAN RYCKEGEM, G. & APTROOT, A. (2001). A new *Massarina* and a new *Wettsteinina* (Ascomycota) from freshwater and tidal reeds. Nova Hedwigia 73: 161-166.

VAN RYCKEGEM, G., VAN DE PUT, K. & ROBERTS, P. (2002). *Tremella spicifera* sp. nov., a parasite of *Massarina arundinacea*. Mycotaxon 81: 185-189.

VAN RYCKEGEM G. & VERBEKEN A. (2000). *Rosellinia musispora* sp. nov., een nieuw tepelkogeltje op riet (*Phragmites australis*). Sterbeeckia 19: 3-5.

Fungal diversity on a single host plant: common reed (*Phragmites australis*)

Van Ryckegem, G. & Verbeken, A.
Manuscript in preparation

Abstract An overview of the known global fungal diversity on a single host plant, common reed (*Phragmites australis*), is given based on both personal surveys and available literature and an estimate is made on the potential real world-wide diversity. An exhaustive literature search and personal surveys have hitherto revealed 822 taxa (estimated 682 species) occurring on *Phragmites australis*. The number of species colonizing common reed in the world is conservatively estimated at 863-1345 of which between 78 and 269 species can be considered to be host specific. In order to estimate fungal diversity more accurately more detailed habitat research, including both spatial and temporal variables, is needed. Factors related to the high diversity of fungi on the host plant are discussed. Those factors are considered to be mainly related to plant structure, the wide ecological amplitude and the cosmopolitan distribution of the host creating huge habitat diversity for fungal colonizers. Factors related to host-specificity are discussed. Detailed studies focussing on a single host plant in various conditions could substantially improve the resolution of fungal diversity estimates. More efforts are needed by thorough research of a wide range of host species to reveal recurrent patterns in fungal diversity. Such future research is predicted to result in a higher ratio of 6:1 of specific fungal colonizers per host and hence stresses the underestimated fungal diversity based on this ratio.

Introduction

Fungal diversity is largely unexplored (May, 1991; Arnold et al., 2000; Hawksworth, 2001; Vandenkoornhuyse et al., 2002;). Only 5% of the world's fungi are currently known if the estimated number of 1.5 million fungal species is accepted (Hawksworth, 2001). One of the most important arguments for establishing the 1.5 million hypotheses is the ratio of 6 host specific fungi to a single vascular plant species (Hawksworth, 1991). However, the assumed ratio of 6:1 fungal species to a host plant is mainly derived from species lists versus native plants growing in a particular region (Hawksworth, 1991) without sound evidence from long-term studies of plants in different stages of growth and decay for the inventory of fungi on particular hosts. Such detailed studies could substantially improve the resolution of fungal diversity estimates and bear important consequences for medicine, industry, agriculture (Petrini et al., 1992; Swift & Anderson, 1993; Bills et al., 2002), ecosystem functioning (Schulze & Mooney, 1993; Schläpfer et al., 1999; Naeem et al., 2000; Cardinale et al., 2002; Gessner et al., 2004) inclusive global change research (Tresender & Allen, 2000) and conservation (Moore et al., 2001).

Phragmites australis (Cav.) Trin. ex Steud. is one of the best studied grass species with respect to fungal diversity (Van Ryckegem, 2005). It is a widespread species considered to be subcosmopolitan and indigenous to all continents, except Antarctica (Rodewald & Rudescu, 1974; Clevering & Lissner, 1999).

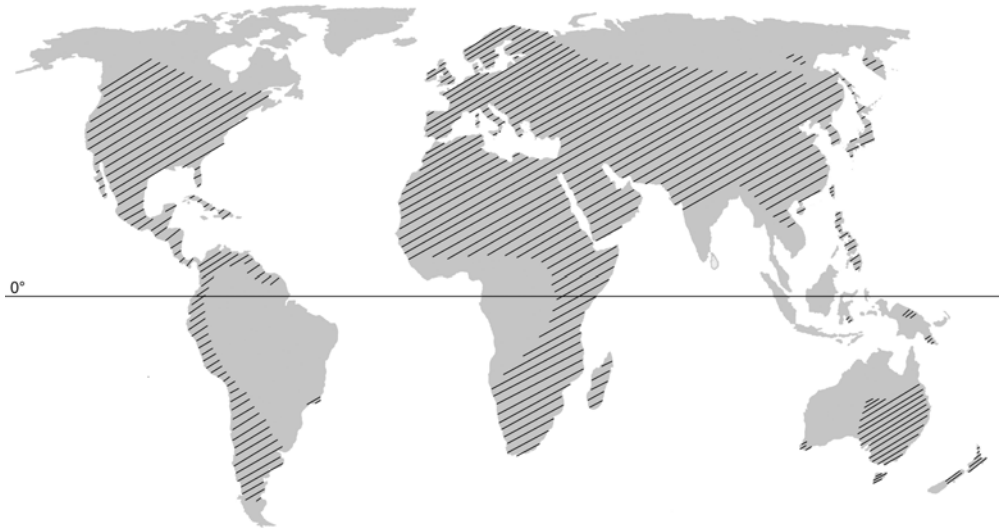


Figure 1. Word-wide distribution of *Phragmites australis* (based on van der Toorn, 1972; Rodewald & Rudescu, 1974 and Clevering & Lissner, 1999).

Common reed drew the attention of scientists because of a considerable industrial use for thatching or basket braiding (Rodewald & Rudescu, 1974), and its qualities and role in wastewater treatment and wetland ecology (Kadlec & Knight, 1996; Mitsch & Gosselink, 2000). In all these domains fungi play crucial roles such as in weakening and decomposing thatch (Haslam, 1989; Anthony, 1999), and as dominant components in carbon and nutrient cycles in reed vegetations (Komínková et al., 2000; Van Ryckegem et al., 2005a, b). Furthermore fungi could have potential for biocontrol of the recently expanding reed vegetations in North America (Evans, 1995; Tewksbury et al., 2002). The important functional role of fungi in reed vegetations seems to be reflected in a high diversity of fungi that are active during the entire life cycle of the reed plant. Several species cause diseases and are known as pathogens (Durska, 1969, 1970; Bán et al., 1996). A huge diversity of species living seemingly without showing symptoms of infection on the living reed tissues have been recorded (Peláez et al., 1998; Wirsel et al., 2001; Ernst et al., 2003). These endophytes (sensu Wilson, 1995) and a consortium of arbuscular mycorrhiza (Oliveira et al., 2001; Wirsel, 2004) probably have a substantial impact on the growth and fitness of the reed plant (Ernst et al., 2003; Wirsel, 2004). Once the reed plant dies several authors reported multiple species possibly involved in the decomposition process (e.g. Apinis et al., 1972a, b, 1975; Taligoola et al., 1972, 1975, Poon & Hyde 1998; Peláez et al., 1998; Wirsel et al., 2001; Wong & Hyde 2001).

In this paper known world-wide fungal diversity is summarized on a single host plant, *Phragmites australis* and by extrapolation we try to estimate global diversity. This approach starts from our own research on phragmiticolous fungi but is extended by reviewing all further available information on fungal diversity on the host plant. Fungal diversity is discussed, considering ecological and geographical influence (habitat diversity) and host characteristics.

Materials and methods

Fungal datasets used to estimate diversity

Reference site: a brackish tidal marsh in the Scheldt estuary on the Dutch-Belgian border in the Netherlands at the edge of Saeftinghe Marsh (51° 21' N, 4° 14' E) was intensively investigated with monthly fungi inventories on all above ground reed parts (leaf blades, leaf sheaths and stems) from May 2000 till April 2003. Two plots of reed were monitored with one growing season (year) in between. Plot 1 was monitored from May 2000-April 2002 and plot 2 from May 2001-April 2003. Both successive inventories proved comparable and show that diversity and community structure show distinct, recurrent seasonal patterns. Four microhabitats within each site were screened: litter layer, basal, middle and upper part of standing shoots and three plant parts were screened for sporulation structures during the living host state and during standing and fallen decay until nearly complete decomposition of the resource (Van Ryckegem & Verbeken, 2005d). For details on sampling, results and discussion the reader is referred to Van Ryckegem & Verbeken (2005d).

In the Scheldt estuary, 4 tidal marshes along the entire salinity gradient with *P. australis* vegetations were compared with each other on an equal sample scale to evaluate the influence of ecological gradients on species diversity. Samples originated from standing dead reeds and from the litter layer. Details on methodology and sampling are presented in Van Ryckegem & Verbeken (2005a).

Fallen litter from four Flemish, non tidal wetlands with a salinity range from fresh to mesohaline near the Scheldt estuary was compared with three tidal sites on an equal monthly sample scale during nine months (Van Ryckegem, 1999).

Literature database

A database compiling all available species data was realized by scrutinizing several hundreds of literature references. Important works were Saccardo's Sylloge Fungorum vol. I-XXVI (1882-1972), the Index of Fungi (1920-1999), checklists and recent databases available at the web (Farr et al., 2004). Many mycologists, both professionals and amateurs, contributed and kindly provided additional records.

Estimates

Fungal diversity in our reference site was estimated using EstimateS (Version 7.0, http://viceroy.eeb.uconn.edu/estimates) (Colwell, 2004). Rarefaction was used for graphing the estimates for our reference study. Two nonparametric estimators, which performed a minimal and a maximal estimate, were used to demonstrate the range of expected diversity. The Abundance-based Coverage Estimator (ACE) (minimal) and Chao1 (maximal) were used and plotted after 100 randomizations of records accumulation order.

Fungal taxa richness in the other systems was estimated more roughly by comparing those smaller scaled studies with our reference study site. This could be realized because the reference site was also monitored as one of the sites included in the small scaled multi-site inventories. The percentage of species recovered from our reference site in each of the smaller studies was used as a measure to evaluate our survey completeness (Com). The value is a fraction ranging between 0 and 1 with '0' no species in common with the reference study and '1' standing for a study that has a completely identical species list. The species numbers (S_{obs}) for each of the studies were divided by the fraction of completeness and summed for all sites i. This estimate was corrected with the average Marczewski-Steinhaus (M-S) distance (Pielou, 1984) of all possible site combinations (C_i). This distance is the complement of the more familiar Jaccard index of similarity. The Jaccard's index (JI) was used as the measure of similarity between sites screened on an equal sample scale.

$$JI = a/(a+b+c) \quad (1)$$

where a is the number of taxa occurring in both sites and b and c are the unique species to respectively one of the sites (Jaccard, 1912). The formula used to estimate the overall diversity in the sites studied is:

$$\left(\sum_{i}^{0} \frac{S_{obs(i)}}{Com}\right) * \left[1 - \left(\sum_{C_i} JI * C_i^{-1}\right)\right] \quad (2)$$

Results

Reference site: brackish tidal marsh

The survey conducted in our best studied site was considered to be substantially complete (however see below) and resulted in a list of 95 sporulating taxa on a total of 3975 fungal records.

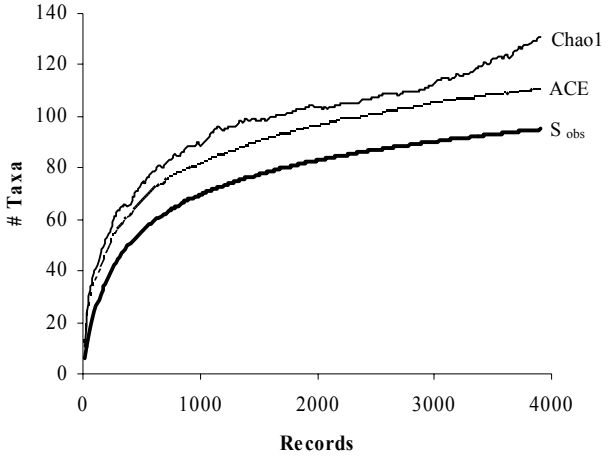


Figure 2. Record-based rarefaction curve of fungal taxa richness and two non-parametric estimators of fungal taxa richness on *Phragmites australis* in a brackish tidal marsh (reference site).

Based on the species data available in our best studied site we could estimate the number of taxa occurring on above ground parts of *Phragmites* in a brackish tidal marsh. ACE (minimal estimator) and Chao1 (maximal estimator) and the sample based rarefaction curve (S_{obs}) do not merge together at the end (Fig. 2), indicating that additional species/taxa may still be discovered in our sampling site. The estimators (ACE-Chao1) predict that after the three year study period on the screened plant organs and microhabitats still 17-38% of the species/taxa remain undiscovered. The predicted taxa richness on above ground reed in our two plots in the brackish tidal marsh (with the used method and the screened microhabitats in a reed stand) is 111 taxa (ACE) up to 131 (Chao1).

An estuary

Compared to a single brackish marsh the species diversity is much higher within the entire estuarine ecosystem with changing environmental gradients along the river continuum. A different fungal species composition was observed between sites with different salinity influence (Van Ryckegem & Verbeken, 2005a). The estuarine study yielded 114 different taxa on a total of 1321 fungal records. The environmental effect is demonstrated by the low Jaccard similarity between the four tidal marshes investigated. Over a distance of 50 km, fungal species composition changed substantially along the salinity gradient of the river with eventually less than 40% overall Jaccard similarity between sites (data from Van Ryckegem & Verbeken, 2005a). If we address the completeness of the species lists by comparing the reference study with the results obtained in this study for the same site included, we find that 56% of the litter layer fungi and 62% of the canopy fungi were recovered (Com). Assuming an equal survey efficiency and maintained similarity for the other sites, species richness is estimated to be about

180 taxa in the litter layer and 90 taxa for the whole reed canopy of the Scheldt estuary. Because of the natural decay pattern, characterized by falling of standing dead reeds in the litter layer, the probability to retrieve a canopy species in the litter layer is high. This results in a Jaccard index of 52% between canopy samples and litter layer samples calculated for the reference study. Using these figures one might conclude a fungal diversity within the estuary of about 140 taxa, a number even below the actual found diversity in the estuary. 166 taxa were observed during the nonsystematic survey of more sites besides the four permanent plots within the estuary (unpublished data) (Table 1). This suggests that the number of investigated sites and/or environmental heterogeneity within these sites is not large enough to produce a good estimate on fungal diversity within our estuarine ecosystem.

Flemish wetlands

Another study by Van Ryckegem (1999) focused on a larger environmental heterogeneity including both non-tidal and tidal wetlands in a common survey of litter layer fungi (Table 1). Contrasting both of those systems on an equal sample scale resulted in 128 taxa (1054 fungal records) and an overall Jaccard similarity of 46%, furthermore it showed that fungal diversity within a tidal estuarine system is impoverished compared to diversity in freshwater to mesohaline wetlands (Van Ryckegem & Verbeken, 2005a). The four non-tidal reed stands, with contrasting salinity, showed a higher Jaccard similarity of about 60%. This again suggests the important effect of environmental circumstances on single host diversity lists.

If we address the completeness of the species lists by comparing the reference study with the results obtained by Van Ryckegem (1999) for the same site we find a species list incorporating about 48% (Com) of the litter layer taxa that could retrieved in the reference site. Estimating taxa richness in the seven sites by equation (2) gives 403 taxa. The taxa are assumed to occur in the litter layer while it was estimated from our reference study that Jaccard similarity between canopy and litter layer fungi was 52%, meaning an estimated species richness of roughly 600 taxa is to be expected in Flemish wetlands on a single host species. Furthermore this estimate might be considered conservative as it is based on a limited number of habitats (see below).

Table 1. Summarized results of all personal fungal inventories on *Phragmites australis* and of the literature study.

Study		Geography	n sites	plant parts[1]	Rec.	S_{Obs}[2]	Jaccard (%)	Wetland type	S_{count}[3]
Personal Database	total	Western-Europe	32	L, Ls, St, Inflor.	9915	214			214
	total	Belgium	21	L, Ls, St	9726	201			
	estuary	Belgium	8	L, Ls, St	9066	166		Brackish till freshwater tidal marshes	
Reference study	total (4 microhabitats)	Border Belgium- The Netherlands	1	L, Ls, St	3975	95		Brackish tidal marsh	
Estuary	litter layer	Belgium	4	Ls, St	347	77	35.1 ± 6.9	Mesohaline till freshwater tidal marshes	
	standing	Belgium	2	Ls, St	947	71	46.5	Brackish and freshwater tidal marsh	
Estuary-wetlands compared[$]	litter layers estuarine	Belgium	3	L, Ls, St	414	128	45.8 ± 7.6	Brackish till freshwater tidal marshes	
	litter layers non-tidal	Belgium	4	L, Ls, St	640			4 different types of non-tidal wetlands with different salinity	
Literature database		Belgium[§]				19			19
		World[£]							589

[1] Plant parts investigated during study: L: leaves; Ls: leaf sheaths; St: stems; Inflor.: inflorescence.

[2] S_{obs} is the number of species observed during the study.

[3] S_{count} is the number of species incorporated to count the recorded diversity of phragmiticolous fungi.

[$] Data from Van Ryckegem (1999), species list corrected.

[§] Taxa not found during personal studies.

[£] Exclusive taxa from personal database or found in Belgium.

Personal database

Including all previous studies, several other wetlands with reed vegetations were visited during the period of 1998-2004 and screened for fungal presence, yielding a total species list of 214 taxa (134 genera) occurring on above ground parts of *P. australis* (Van Ryckegem, 2005). 59% could be determined to species level, at least 20 taxa (9%) are new to science, 4 taxa have been described in the meantime (Van Ryckegem & Verbeken, 2000; Van Ryckegem & Aptroot, 2001; Van Ryckegem et al., 2002) and of the 88 unknown taxa, about 16 taxa are ready to be published as new species while several taxa need more research.

Table 2. Taxonomic composition and diversity of fungi on *Phragmites australis* as revealed from the literature study.

	Total no. of taxa[1]	no. of genera	% of taxa in group	Type on *P. australis*[2]	% of types in group
ascomycetes	272	128	39	92	40
basidiomycetes	81	47	11	35	15
anamorphic fungi	339	176	48	104	45
coelomycetes	134	56	19	69	30
hyphomycetes	205	120	29	35	15
oomycetes	4	2	< 1%	0	0
zygomycetes	2	2	< 1%	0	0
glomeromycetes*	3	2	< 1%	1	0
Total	701	357		232	

[1] Corrected for synonyms.

[2] Uncorrected for synonyms.

* With a much higher diversity of unidentified species (Wirsel, 2004).

Literature review

An extensive literature survey, excluding synonyms and taxa only determined up to genus level (100 taxa), revealed 701 fungal records named up to species level. These taxa belong to 357 genera ever recorded on *P. australis* (Table 2).

Discussion

The results of the combined surveys and the literature study demonstrate a huge fungal diversity on *P. australis*. This high diversity is probably caused by multiple internal and external factors influencing the host's characteristics and its potential (local and global) area occupation. Realized fungal niche on the host and fungal geographic distributions behave in close relation with evolutionary processes (Webb et al., 2002). Those different aspects are obviously connected but difficult to unravel. The following discussion proposes some clues for possible causes of the high fungal diversity on a single host plant *P. australis*.

Habitat diversity for fungal colonizers

Fungal niche differentiation is considered to be influenced by two types of variables: external variables and internal, host related, variables (Cooke & Rayner, 1984; Gessner et al., 1997). The possible effect of those variables and the interconnectness of them is discussed below.

External variables – environmental impact

The host's growth place – on an environmental and geographical scale – creates a typical environmental habitat which can be very diverse and which will impact fungal colonization and growth (Hawker, 1966; Petrini, 1996; Saikkonen et al., 1998). The environmental influence acts on various scales, ranging from micro- to macro-environmental conditions. Micro-environmental variables are considered to act within reed stands, resulting in a range of microhabitats in close association with the host plant characteristics and phenotype. Microclimates are created by a specific plant size and density within a single site influencing temperature regimes and the humidity in a reed stand (Rodewald & Rudescu, 1974) which could be key determinants of overall infection level of (endophytic) fungi (Carroll, 1986; Rodrigue, 1994). Reed habitats are above all characterized by ideal habitat conditions for fungi in the lower canopy and in the litter layer: high moisture and sheltered conditions will promote spore germination and fungal growth, possibly one of the factors responsible for the high number species on *P. australis*. Further evidence of the importance of within stand micro-environmental variables is provided by comparing the studies of Poon & Hyde (1998) and Wong & Hyde (2000) sampling within the same site. However, Poon & Hyde (l.c.) sampled tidal water reeds while Wong & Hyde (l.c.) sampled near the land site. This small topographical difference in a same site resulted in an approximated Jaccard similarity index of less than 20% between both studies.

Macro-environmental conditions have substantial impact on fungal species composition on plant species (e.g. Cook & Rayner, 1984). Specifically for reed stands an important effect on diversity and species composition was noticed among sites which differ in salinity, tidal or non-tidal, flooding frequency, water table etc. (Taligoola, 1969; Wirsel, et al., 2001; Van Ryckegem & Verbeken, 2005a).

Ultimately, at the largest scale, differences in fungal species composition between remote plant populations could be caused by climatic conditions (e.g. temperate versus tropical) a phenomenon unstudied in detail for a single plant species. Some indications for an influence of climate might be seen in the different species composition between temperate and subtropical reeds (see biogeography).

Internal variables

The internal resource quality is ultimately determined by external conditions, but also by the host genotype (Haslam, 1972; Lissner & Schierup, 1997; Clevering & Lissner, 1999) together resulting in a huge phenotypic variability of *Phragmites* among sites and clones (Haslam, 1972; Clevering & Lissner, 1999). On his turn morphological variance creates specific microhabitats in reed belts characterized by specific micro-environmental conditions (see above) and subtle differences in interal resource quality along the plants axis (see Van Ryckegem & Verbeken, 2005d). Between habitats, reeds most probably differ by a set of physical (anatomical) and chemical characteristics (some indications in Čižková-Končalová et al., 1992).

Biotic interactions such as herbivory, detritivory and fungivory are changing and influenced by abiotic variables and hence the habitat types and the geographical context of *P. australis*, could have a considerable impact. Several studies demonstrate the complex interactions and the potential impact of biotic interference on fungal communities and the outcome could be positive, negative or neutral for fungal species richness found on the host (Saw, 1992; Bärlocher, 1992; McLean et al., 1996; Gessner et al., 1997; Hatcher & Paul, 2000). A complete discussion of possible effects of biotic interactions is beyond the scope of this paper but should be considered as a potential factor influencing fungal diversity on a plant host.

Host growth characteristics

Furthermore the rhizomatous, perennial way of growing could have positive effects on the fungal diversity associated with a host (Clay, 1995). Probably part of the rich mycoflora on reed is related to the predictability and appearance of large and ancient reed stands (cf. phytophagous insects Tscharntke, 1999). Above all, *P. australis* is a very productive plant species (e.g. Květ & Westláke, 1998; Mitsch & Gosselink, 2000) resulting in huge amounts (~fungal habitat space) of plant material available for fungal colonization.

Perhaps *P. australis* could accumulate endophytic species over its lifespan. The latter would strictly mean that endophytes withdraw or survive in the below ground parts once the above ground plant parts die and more endophytes could accumulate in older reed clones. The importance of this phenomenon is unknown for perennials but it may be an important source for endophytic colonization of above ground parts besides the yearly colonization and seasonal accumulation of the horizontally transmitted endophytes. Furthermore, perennial plant species could be associated with more short-cycle endophytes compared to annual plant species as those fungal symbionts could represent a response of long-lived plants to the challenge presented by short-cycled pests (Carroll, 1986; Cabral et al. 1993). Although no solid prove for this hypothesis is known, it was observed that an annual *Juncus* species had fewer endophytes with high infection frequency compared to perennial representatives of the same genus which could benefit more from the mutualistic fungi active against short-cycle insects or other pathogenic fungi (Cabral et al. 1993).

Biogeography

Comparing the few studies available on phragmiticolous fungi we could evaluate some of the large distance patterns of fungi occurring on *P. australis*. Nearby Belgium, in England, several types of wetlands were screened for phragmiticolous fungi by Taligoola (1969). Only 37% of the species fully named by Taligoola (1969) (i.e. 36 of the 96 taxa) were recovered during our surveys. Probably these differences do not solely represent geographical differences in mycoflora between Belgium and England because both countries are nearby and show a relatively comparable mycoflora. Furthermore, the real number of taxa in common is probably

higher as during both studies many species could not be identified and thorough comparison is impossible as no taxonomic descriptions are available for most of the taxa mentioned by Taligoola (1969). However, it should be noticed that the methods used makes it hard to compare both studies: plating and moist chamber incubation of reed parts by Taligoola versus direct observations in our studies. Furthermore, ecological differences between the sites investigated by Taligoola (Apinis et al., 1972a, b, 1975; Taligoola et al., 1972) are causing additional divergence between species lists.

Of the 31 taxa (29 with complete species name) mentioned by Farr et al. (1989), 24 taxa (83%) are found on *P. australis* in Europe. This restricted species list of fungi occurring on *P. australis* in North America demonstrates that phragmiticolous fungi are undersampled in this area. The list mainly contains common, widespread taxa occurring on multiple hosts possibly overemphasizing similarity. However, a pattern of closely resembling fungal colonizers seems roughly consistent for the entire northern hemisphere as further suggested by Eastern European and Soviet publications (Tepla, 1963; Durska, 1969; Bán et al., 2000) and by other species comparisons between American and European collections of a single host plant species with a wide distribution (e.g. Chapela & Boddy, 1988; Chapela, 1989).

Data from tropical and subtropical areas are scarce, but recent detailed studies from subtropical Hong Kong (Poon & Hyde, 1998; Wong & Hyde, 2001; Lu et al., 2000) allow some comparison. Less than 20% of the species recorded on *P. australis* in Hong Kong (direct observation and moist chamber incubation) were found in Europe, demonstrating a distinct difference in species composition between temperate and (sub)tropical phragmiticolous fungi. The majority of those species in common appear to the plurivorous species. However, the limited information present suggests that several genera have both dominant representatives in Europe and subtropics (Table 3). This low species similarity may indicate a separate speciation of taxa within the same dominant genera in different climatic zones. However no *Mycosphaerella* and few *Phaeosphaeria* species were found in (sub)tropical Hong Kong. *Phaeosphaeria* seems to be more species rich in temperate climates in contrast to *Aniptodera* and *Phragmitensis*, exclusively collected from (sub)tropical areas. The most common anamorphic (all coelomycetes) genera are presented in Table 3. Also within in this group there seem to be several genera in common between both climatic zones such as *Phoma*, *Stagonospora* and *Septoriella*. However *Phomopsis*, *Colletotrichum* and *Septoria* seem to be restricted to (sub)tropical reeds. The latter genus could be a result of taxonomic instability as genera such as *Stagonospora*, *Septoriella*, *Hendersonia* and *Septoria* got different taxonomic interpretation depending on authors and could be united into *Septoria* s.l.

Table 3. Dominant* genera on *P. australis* selected from temperate European and subtropical Hong Kong studies

	# taxa known from temperate Europe on *P. australis*[1]	# taxa known from subtropical Hong Kong on *P. australis*[2]	# taxa known from both climatic zones on *P. australis*
ascomycetes			
Mycosphaerella	1	0	0
Phaeosphaeria	14 (3)	1 (1)	0
Massarina+ *Lophiostoma*	4 (1) + 4 (2)	2 (1) + 0	1?
Phomatospora	7 (1)	1 (1)	1
Aniptodera	0	3 (2)	0
Phragmitensis	0	2 (2)	0
Lignicola	0	1	0
coelomycetes			
Phoma	5 (2)	?2[&] (?)	0[£]
Stagonospora	9 (4)	1 (0)	0 or 1[£]
Septoriella	2 (2)	1 (1)	0 or 1[£]
Hendersonia	4 (2)	0	0
Phialophorophoma	1 (1)	0	0
Phomopsis	0	?2[&]	0
Colletotrichum	0	3 (?)	0
Septoria	1 (0)	7 (?)	0 or 1[£]

[1] Personal data.

[2] Poon & Hyde (1998) Hyde & Wong (2001); Lu et al., (2002).

[*] There are no dominant genera classified within the basidiomycetes or within the hyphomycetes; between brackets is the number of frequently occurring species within the genus on *P. australis* in the studies.

[£] Impossible to check for common species between inventories as there are no identifications up to species level.

[&] Personal communication (Hyde, K.D.) expected for these numbers.

Do widespread species have a higher local diversity?

The positive correlation of a large geographical range of a host plant with high fungal species richness independent of sample effort was demonstrated by Clay (1995). This observation seems to be a direct area effect i.e. widespread plant species would have more chance to get colonized by local non-specific fungi. Species richness of non-specific fungi between remote sites on our host might depend on the regional species pool available from other (related) hosts (Cornell & Lawton, 1992). But what about the local situation i.e. have widespread plant species higher fungal richness on a local scale contrasted to another plant species (similar plant quality, architecture,… i.e. having potentially equal fungal habitat quality and quantity) in the same habitat but with a more restricted distribution? Such questions could

be explained in the framework of MacArthur & Wilson's (1967) theory of island biogeography. Therefore we could compare widespread plant species with large islands and local endemic plant species to small islands. We hypothesize that the large island effect will be important for host specific species. Widespread, common plant species behave as stable resources with lower species extinction potential and more opportunities for close relationships with host-specific or host recurrent fungi and hence will have a higher number of specific fungal colonizers.
Furthermore, as with herbivorous insects (cf. phytophagous insects on *Pteridium aquilinum*, Lawton, 1984) it is suspected that the physical area occupation or abundance of the host in a certain geographic region influences the specific fungal diversity. In regions with sporadic occurrence of the host plant, specific taxa are more likely to go locally extinct because the chance of recolonization each growth season is smaller than in regions with dense and old vegetations with high productivity.

Is host specificity high on Phragmites australis?

232 taxa have been originally described from *P. australis*, with several identical species described independently from each other under a different name (Table 2). Of these, 9 taxa are rusts and smuts (number adjusted for synonyms) and therefore likely to be host specific. Moreover a high diversity of endophytic taxa is known (Peláez et al., 1998; Wirsel et al., 2001; Van Ryckegem & Verbeken, 2005d). Endophytes are considered to be specific for a host if they have a large number of isolates (dominant species) on the host (or part of a host) and current evidence suggests that these fungi do not colonize hosts in other plant families but their occurrence is often limited to only one or a few taxonomically closely related hosts (Petrini, 1986). Moreover, Petrini et al. (1992) state that for endophytic fungi proven not to be host specific, there is evidence suggesting host-specific strain formation within the same species. Within the literature database we could not find other host plants for about 150 fungal taxa. However, such numbers might be of less value as they depend on the poor knowledge of fungal occurrence on other grasses.

The proportion of host specific species occurring on *P. australis* from our personal dataset was determined relying on experience and by comparison with all available specialized literature cross checked with Farr et al. (2004). Although we consider 19 taxa from our dataset to be host specific (Table 4) it was clear that several other taxa had a strong host recurrence (*sensu* Zhou & Hyde, 2001) which suggests a close relationship of those fungi with *P. australis* based on taxa frequency (see Lodge, 1997). Those taxa show only a sporadic occurrence on other hosts. However, they are not considered in our host specific list.

Based on above data we can generalize a host specific ratio. This ratio gives the number of host specific fungi on a complete survey list. It is estimated between 1:5 and 1:11. Thus, one species is probably host specific in a species list of five to eleven taxa. The lower ratio is obtained by our personal dataset i.e. ~214/19; the higher ratio, is obtained by considering the literature dataset (excluding synonyms) as the pool for assigning host specificity i.e. ~701/150.

Table 4. List of host specific taxa considered to occur on *Phragmites australis* in Flanders (Belgium).

Group	Host specific fungi considered
A	*Keissleriella linearis* E. Müll.
A	*Lophiostoma arundinis* (Pers.) Ces. & De Not.
A	*Massarina arundinacea* (Sowerby: Fr.) Leuchtm.
A	*Massarina fluviatilis* Aptroot & Van Ryck.
A	*Mollisia retincola* (Rabenh.) P. Karst.
A	*Phaeosphaeria phragmiticola* Leuchtm.
A	*Phaeosphaeria phragmitis* (Hollós) Leuchtm.
A	*Phragmiticola rhopalospermum* (Kirschst.) Sherwood
A	*Stictis* sp.*
B	*Puccinia magnusiana* Körn.
B	*Puccinia phragmitis* (Schumach.) Körn.
B	*Ustilago grandis* Fr.
C	*Cytoplacosphaeria rimosa* (Oudem.) Petrak
C	*Neottiosporina australiensis* B. Sutton & Alcorn
C	*Phoma* sp. III (cf. *Phoma rimosa* Oudem.)
C	*Pseudorobillarda phragmitis* (Cunnell) M. Morelet
C	*Stagonospora cylindrica* Cunnell
C	*Stagonospora elegans* (Berk.) Sacc. & Traverso
H	*Deightoniella roumegueri* (Cavara) Constant.

* Species to be described, abundant endophytic taxon.

Estimated global diversity

Extrapolation of the results from the surveys by comparison with a reference study, and an exhaustive literature study enables us to estimate global fungal diversity on a single host *P. australis*.

The total number of taxa, summing personal data and literature records, on *P. australis* is 822 (Table 1) (uncorrected for doubly counted taxa with their anamorph and teleomorph on *P. australis*). Even this huge compilation is expected to be highly incomplete because all studies and observations of species rely on incomplete surveys with few detailed studies from all over the world.

Indications that even a list of 822 taxa is probably incomplete are given by our detailed reference study. Taxa richness in this one site is estimated between 111 and 131 taxa based on nonparametric estimators considered as being minimal estimators for species richness (Colwell, 2004) and multivariate analysis of this inventory demonstrated the importance of micro-environmental conditions and the necessity to consider the different successional phases during decay. For example, it was demonstrated that species succession follows three phases in all four microhabitats screened (Van Ryckegem & Verbeken, 2005b, c; Van Ryckegem et al.

2005a) and on the different plant parts (Van Ryckegem & Verbeken, 2005d). Hence, any study attempting to make a substantially complete inventory on fungal diversity on a specific host should consider the spatial heterogeneity on a single plant species (in relation to its architecture) and include all of the studied niches sufficiently long in time to include all successional phases. The study, in a single site, revealed seventeen subcommunities, all of them contributing differently to the total fungal diversity picture (Van Ryckegem & Verbeken, 2005d). For example, a mature fungal community (phase II in succession) contributes more to the total species richness compared to the species poorer last phase of decay. Furthermore, our inventory might be imprecise as taxa richness is not reduced for anamorphs with known corresponding teleomorphs (see below). On the other hand, additionally monitoring inflorescences together with supplementary methods such as genetic techniques and indirect screening would yield a substantially higher fungal richness (Van Ryckegem & Verbeken, 2005a). Furthermore, our survey missed (endophytic) species which show host expression specificity (Petrini, 1996), other species were sporulation was prevented by environmental constrains, or species with ephemeral fruit bodies (Van Ryckegem & Verbeken, 2005a). Conclusive it can be stated that our inventory and hence the estimated diversity of species colonizing above ground parts of common reed in our reference site is still at the lower end of the real number of species involved. Thus, actual percentage of undiscovered species might be closer to the higher range mentioned (17-38% undiscovered taxa) (Estimate A & B, Table 5).

In expanding estimation to Flemish wetlands, the number of species was conservatively estimated to be about 600 taxa (Estimate C, Table 5, see results). However, based on a comparison with Taligoola's (1969) results (see above) a species dissimilarity was assumed to be between 63-45% and a factor of 50% was taken to calculate a maximal diversity estimate of phragmiticolous fungi in the Flanders. Such a higher estimation should account for the potential to retrieve additional taxa if more habitats were screened and other techniques (plating, incubating or molecular work) were used. Ultimately, this gives a maximal diversity estimate of 900 taxa possibly occurring on *P. australis* in the Flanders (Belgium) (Estimate D, Table 5).

Estimated fungal phragmiticolous diversity in Northern, temperate hemisphere was assumed not to be substantially different from the Flemish – European situation (see above) and the estimates were conservatively considered as a good estimate for the Northern temperate hemisphere.

Table 5. Estimates of the total numbers of taxa occurring on *Phragmites australis* in different geographic regions of the world derived by estimation by nonparametric estimators (estimate A & B) or by extrapolation after comparison (remaining estimates) (see text for further explanation).

Region		Region estimate		Taxa estimate	
Reference study	Min.	111	(A)		
	Max.	131	(B)		
Flemish wetlands	Min.	600	(C)		
	Max.	900	(D)		
Northern temperate hemisphere	Min.	600	(E)	600	(1)
	Max.	900	(F)	900	(2)
Tropical and subtropical zone	Min.	600	(G)	480	(3)
	Max.	900	(H)	720	(4)
Southern temperate hemisphere	Min.	?	(I)	?	(5)
	Max.	?	(J)	?	(6)
World total				1040-1620	
World total minus allowance for anamorphs having a teleomorphic state on *P. australis*				863-1345	

Data from (sub)tropical areas indicate at least an equal fungal diversity on *P. australis*. At present more than 120 taxa are known from (sub)tropical areas, mainly based on two small studies (Poon & Hyde, 1998; Wong & Hyde, 2001) and many species are undescribed and new to science (Poon & Hyde, 1998; Wong & Hyde, 2001). The latter stresses, together with the low similarity in species composition with our studies, that (sub)tropical reeds differ in fungal taxa composition compared to northern temperate regions. Based on a low similarity of 20% (see above) we estimated taxa typical for (sub)tropical regions to be between 480-720 (Estimate 3, 4, Table 5). This is assuming an equal taxa richness in (sub)tropical areas (Estimate G, H, Table 5), an assumption which might be considered conservative as fungal species richness is often assessed as being higher in tropical regions (Isaac et al., 1993; Lodge & Cantrell, 1995; Hyde, 1997; cf. Stevens, 1989; Gaston, 2000 but see Clay, 1995). On the other hand the area (ha) occupied by reed vegetations in (sub)tropical regions is far less than those in northern temperate wetlands (Mitsch & Gosselinck, 2000; see Fig. 1).

Only sporadic records are available from southern, temperate hemisphere and those make it impossible to guess if species composition differs between other regions. However, there are no indications to assume a lower diversity of phragmiticolous fungi in the southern hemisphere and probably many unique taxa for this region are waiting to be discovered

(Estimate 5, 6, Table 5). No estimates could be made for the southern temperate hemisphere and hence they are not incorporated in our global diversity estimate of phragmiticolous fungi.

By adding estimate (1) + (3) we get a minimal estimate (1040 taxa) and adding estimate (2) + (4) gives the maximal estimate (1620 taxa) for global diversity of fungal taxa possibly occurring on *P. australis* in the field.

Note that the preceding estimates do not allow for anamorphs where the corresponding teleomorphs are also recorded on *P. australis*. Establishing such a ratio for fungal pleiomorphism is difficult because connections between anamorphic state and teleomorphic state remains to be sorted out for most taxa. However, for some genera there are strong indications they occur with both their anamorphs and teleomorphs on *P. australis,* for example *Phaeosphaeria* and its '*Stagonospora*' anamorphs. A professional judgement is therefore the only possible option to establish such a fraction, and a 1/3 ratio was used to correct for the pleiomorphic states of fungi. This ratio is equal to the correction for anamorphs made by Bisby & Ainsworth (1943) and Hawksworth (1991) to estimate world-wide fungal diversity and might therefore be considered low for a reduction of the total species number on a single host. However, our knowledge is still to fragmentary to give a better correction factor. Previous research (Van Ryckegem & Verbeken, 2005d) showed that conidial fungi represent about 50% of the total, reducing the mentioned figures by about 17% (Table 5).

This number of 1345 species, although very high, will nevertheless prove to be conservative for several reasons: (i) estimates were based on similarity of smaller studies with our reference site which was considered too be substantially complete. However, as demonstrated above, this might not be the case. (ii) Projection of our Flemish data on the entire northern temperate hemisphere is probably to Eurocentric and there might well be separate speciation in northern America or eastern Asia of host specific species. Furthermore, non-host specific colonizers might differ as the surrounding species pool differs. (iii) Reeds in the southern temperate hemisphere were not assumed to harbour different taxa compared to the other regions. This assumption is conservative as many sporadic reed colonizers might be different by chance as the surrounding potential colonizers pool differs and hence it is not unlikely some different host-specific species occur on southern *P. australis*. (iiii) Comparing our estimate numbers with the current knowledge of 682 species ever found on *P. australis* (corrected for double counted anamorphs) would mean that already 51-79% of the phragmiticolous flora is described, an unlikely high percentage as there were very few detailed studies so far.

Based on our host-specificity ratio (see above) we can estimate the number of world-wide host specific phragmiticolous taxa to be between 78 and 269 species.

Implications for worldwide diversity estimates

The estimated ratio of specific fungi on a single plant species of 78-269:1 is much higher than the proposed ratio of 6:1 by Hawksworth (1991; 2001) for estimating global fungal diversity. However, this ratio is considered to be an extreme and valid for plant species with a

wide distribution occurring in a large diversity of habitats. This is the case for several emergent macrophytes with *P. australis* one of the most extreme examples (Santamaria, 2002). Data from *P. australis* suggests the potential increase and sensitivity of such host specificity ratio's to the extent of the study – which habitats are screened – and the host it is based on. The importance of a fine scaled habitat research, both spatially and temporally, is stressed. Such an experimental design is predicted to yield a species specific ratio higher than 6:1 even for plant species with a limited geographical and ecological niche and will markedly increase the current estimated global species richness of 1.5 x 10^6. Unfortunately host specificity, the anchor point of the diversity estimate, will remain to have little credibility towards policy, as long mycologists struggle with their limited knowledge of most fungi combined with the huge number of unscreened potential host species (plants, insects and other organisms). Hence, any fungal diversity estimate based on the ratio of specific fungi on a small group of peculiar hosts will remain a wild guess unless mycologists engage by thorough research of a wide range of host species and search for recurrent patterns in fungal diversity.

Acknowledgements

We are grateful to the many mycologists that contributed to the fungal database of phragmiticolous fungi or assisted in determination of several taxa: Aptroot, A., Baral, H.O., Declercq, B., Hyde, K.D., Leuchtmann, A., Læssøe, T., Moreau, P.A., Spooner, B.M., Vesterholt, J. and Walleyn, R. G.V.R. acknowledges the financial support of the Institute for the Promotion of Innovation by Science and Technology in Flanders, Belgium.

References

ANTHONY, P.A. (1999). The macrofungi and decay of roofs thatched with water reed, *Phragmites australis*. Mycol. Res. 103: 1346-1352.

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1972a). Colonisation of *Phragmites communis* leaves by fungi. Nova Hedwigia 23: 113-124.

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1972b). Microfungi colonizing submerged standing culms of *Phragmites communis* Trin. Nova Hedwigia 23: 473-480.

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1975). Microfungi colonizing nodes and internodes of aerial standing culms of *Phragmites communis* Trin. Nova Hedwigia 26: 495-507.

ARNOLD, A.E., MAYNARD, Z., GILBERT, G.S., COLEY, P.D. & KURSAR, T.A. (2000). Are tropical fungal endophytes hyperdiverse?. Ecol. Letters 3: 267-274.

BÁN, R., ENGLONER, A.I. & VIRANYI, F. (2000). Occurrence of fungal pathogens in relation to reed quality. Z. Pflanzenkrankh. Pflanzenschutz. 107: 258-266.

BÁN, R., FISCHL, G. & VIRANYI, F. (1996). A spatio-temporal analysis of fungal pathogens on reed in natural habitats. Acta Phytopathol. Entomycol. Hung. 31: 219-227.

BÄRLOCHER, F. (ED.) (1992). The ecology of aquatic hyphomycetes. Springer-Verlag, Berlin.

BEYER, W. (1997). Ascomyceten und einige Basidiomyceten an *Typha latifolia* L. and *Phragmites australis* (Cav.) Trin.. Beitr. Kenntn. Pilze Mitteleuropas 11: 49-113.

BILLS, G., DOMBROWSKI, A., PELÁEZ, F., POLISHOOK, J. & ZHIQIANG, AN (2002). Recent and Future Discoveries of Pharmacologically Active Metabolites from Tropical Fungi. *In* WATLING, R., FRANKLAND, J.C., AINSWORTH, A.M., ISAAC, S. & ROBINSON, C.H. Tropical Mycology: Volume 2, Micromycetes. CABI Publishing, Wallingford, UK. 165-194.

CABRAL, D., STONE, J.K. & CARROLL, G.C. (1993). The internal mycobiota of *Juncus* spp. microscopic and cultural observations of infection patterns. Mycol. Res. 97: 367-376.

CARDINALE, B.J., PALMER, M.A. & COLLINS, S.L. (2002). Species diversity enhances ecosytem functioning through interspecific facilitation. Nature 415: 426-429.

CARROLL, G.C. (1986). The biology of endophytism in plants with particular reference to woody perennials. *In* FOKKEMA, N.J. & VAN DEN HEUVEL, J. (EDS.) Microbiology of the Phyllosphere. Cambridge University Press, Cambridge, UK. 205-222.

CHAPELA, I.H. (1989). Fungi in healthy stems and branches of American beech and aspen: a comparative study. New Phytol. 113: 65-75.

CHAPELA, I.H. & BODDY, L. (1988). Fungal colonization of attached beech branches. II. Spatial and temporal organization of communities arising from latent invaders in bark and functional sapwood under different moisture regimes. New Phytol. 110: 47-57.

ČIŽKOVÁ-KONČALOVÁ, H., KVĚT, J. & THOMPSON, K. (1992). Carbon starvation: a key to reed decline in eutrophic lakes. Aquatic Bot. 43: 105-113.

CLAY, K. (1995). Correlates of pathogen species richness in the grass family. Canad. J. Bot. 73: 42-49.

CLEVERING, O. A. & LISSNER, J. (1999). Taxonomy, chromosome numbers, clonal diversity and population dynamics of *Phragmites australis*. Aquatic Bot. 64: 185-208.

COLWELL, R.K. (2004). EstimateS. Statistical estimation of species richness and shared species from samples. Version 7.0. University of Connecticut.

CORNELL, H.V. & LAWTON, J.H. (1992). Species interactions, local and regional processes, and limits to the richness of ecological communities: a theoretical perspective. J. Animal Ecol. 61: 1-12.

DURSKA, B. (1969). Distribution of some parasitic fungi on *Phragmites communis* Trin. in Poland. Acta Mycol. 5: 117-133.

DURSKA, B. (1970). Changes in the reed (*Phragmites communis* Trin.) condition caused by diseases of fungal and animal origin. Polsk. Arch. Hydrobiol. 17: 373-396.

ERNST, M., MENDGEN, K.W. & WIRSEL, S.G.R. (2003). Endophytic fungal mutualists: seed-borne *Stagonospora* spp. enhance reed biomass production in axenic microcosms. Molec. Pl.-Microbe Interact. 16: 580-587.

EVANS, H.C. (1995). Fungi as biocontrol agents of weeds: a tropical perspective. Canad. J. Bot. 73 (Suppl. 1): 58-64.

FARR, D.F, BILLS, G.F., CHAMURIS, G.P. & ROSSMAN, A. Y. (1989). Fungi on plants and plant products in the United States. APS Press, St. Paul, Minnesota USA. 1252 pp.

FARR, D.F., ROSSMAN, A.Y., PALM, M.E., & MCCRAY, E.B. (2004). Fungal Databases, Systematic Botany & Mycology Laboratory, ARS, USDA. http://nt.ars-grin.gov/fungaldatabases/.

GASTON, K.J. (2000). Global patterns in biodiversity. Nature 405: 220-227.

GESSNER, M.O., INCHAUSTI, P., PERSSON, L., RAFFAELLI, D.G. & GILLER, P.S. (2004). Biodiversity effects on ecosytem functioning: insights from aquatic systems. Oikos 104: 419-422.

GESSNER, M.O., SUBERKROPP, K. & CHAUVET, E. (1997). Decomposition of plant litter by fungi in marine and freshwater ecosystems. *In* WICKLOW, D.C. & SÖDERSTRÖM, B. (EDS.) The Mycota IV, Environmental and microbial relationships. Springer-Verlag, Berlin Heidelberg. 303-322.

HASLAM, S.M. (1972). Biological flora of the British Isles no. 128, *Phragmites communis* Trin. J. Ecol. 60: 585-610.

HASLAM, S.M. (1989). Early decay of *Phragmites* thatch: an outline of the problem. Aquatic Bot. 35: 129-135.

HATCHER, P.E. & PAUL, N.D. (2000). Beetle grazing reduces natural infection of *Rumex obtusifolius* by fungal pathogens. New Phytol. 146: 325-333.

HAWKER, L.E. (1966). Environmental influences on reproduction. *In* AINSWORTH, G.C. & SUSSMAN, A.S. (EDS.) The Fungi. Vol. 2. Academic Press, New York. 435-469.

HAWKSWORTH, D.L. (1991). The fungal dimension of biodiversity: magnitude, significance and conservation. Mycol. Res. 95: 641-655.

HAWKSWORTH, D.L. (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. 105: 1422-1432.

HYDE, K.D. (ED.) (1997). Biodiversity of tropical microfungi. Hong Kong University Press, Hong Kong.

ISAAC, S., FRANKLAND, J.C., WATLING, R. & WHALLEY, A.J.S. (EDS.) (1993). Aspects of tropical mycology. Cambridge University Press, Cambridge.

JACCARD, P. (1912). The distribution of flora in the alpine zone. New Phytol. 11: 37-50.

KADLEC, R.H. & KNIGHT, R.L. (1996). Treatment wetlands. CRC Press, Boca Raton. 293 p.

KOMÍNKOVÁ, D., KUEHN, K.A., BÜSING, N., STEINER, D. & GESSNER, M.O. (2000). Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of a large lake. Aquatic Microb. Ecol. 22: 271-282.

KVĚT, J. & WESTLAKE, D.F. (1998). Primary production in wetlands. *In* WESTLAKE, D.F., KVĚT, J. & SZCZEPAŃSKI, A. The production ecology of wetlands. Cambridge university press, Cambridge. 78-168.

LAWTON, J.H. (1984). Non-competitive populations, non-convergent communities, and vacant niches: the herbivores of bracken. *In* STRONG, D.R., SIMBERLOFF, D., ABELE, L.G. & THISTLE, A.B. (EDS.) Ecological communities conceptual issues and the evidence. Princeton University Press, Princeton. 67-100.

LISSNER, J. & SCHIERUP, H-H. (1997). Effects of salinity on the growth of *Phragmites australis*. Aquatic Bot. 55: 247-260.

LODGE, D.J. (1997). Factors related to diversity of decomposer fungi in tropical forests. Biodiv. Conservation 6: 681-688.

LODGE, D.J. & CANTRELL, S. (1995). Fungal communities in wet tropical forests: variation in time and space. Canad. J. Bot. 73 (Suppl. 1): 1391-1398.

LU, B., HYDE, K.D., HO, W.H., TSUI, K.M., TAYLOR, J.E., WONG, K.M., YANNA, & D.Q. ZHOU (2000). Checklist of Hong Kong Fungi. Fungal Diversity Press, Hong Kong. 207 pp.

MACARTHUR, R.H. & WILSON, E.O. (1967). The theory of Island Biogeography. Princeton University Press, Princeton, New York.

MAY, R.M. (1991). A fondness for Fungi. Nature 352: 475-476.

MCLEAN, M.A., KANEKO, N. & PARKINSON, D. (1996). Does selective grazing by mites and collembola affect litter fungal community structure?. Pedobiol. 40: 97-105.

MITSCH, W.J. & GOSSELINK, J.G. (2000). Wetlands (3rd edition). Van Nostrand Reinhold, New York. 920 pp.

MOORE, D., NAUTA, M.M., EVANS, S.E. & ROTHEROE, M. (2001). Fungal conservation issues: recognizing the problem, finding solutions. *In* MOORE, D., NAUTA, M.M., EVANS, S.E. & ROTHEROE, M. (EDS.) Fungal Conservation. Issues and Solutions. Cambridge University Press, Cambridge. 1-6.

NAEEM, S., HAHN, D.R. & SCHUURMAN, G. (2000). Producer decomposer co-dependency influences biodiversity effects. Nature 403: 762-764.

OLIVEIRA, R.S., DODD, J.C. & CASTRO, P.M.L. (2001). The mycorrhizal status of *Phragmites australis* in several polluted soils and sediments of an industrialised region of Northern Portugal. Mycorrhiza 10: 241-247.

PELÁEZ, P., COLLADO, J., ARENAL, F., BASILIO, A., CABELLO, A., DIEZ MATAS, M.T., GARCIA, J.B., GONZÁLEZ DEL VAL, A., GONZÁLEZ, V., GORROCHATEGUI, J., HERNÁNDEZ, P., MARTIN, I., PLATAS, G. & VICENTE, F. (1998). Endophytic fungi from plants living on gypsum soils as a source of secondary metabolites with antimicrobial activity. Mycol. Res. 102: 755-761.

PETRINI, O. (1986). Taxonomy of endophytic fungi of aerial plant tissues. *In* FOKKEMA, N.J. & VAN DEN HEUVEL, J. (EDS.) Microbiology of the Phyllosphere. Cambridge University Press, Cambridge, UK. 175-187.

PETRINI, O. (1996). Ecological and physiological aspect of host specificity in endophytic fungi. *In* REDLIN, S.C. & CARRIS, L.M. (EDS.) Endophytic Fungi in Grasses and Woody Plants. American Phytopathological Press, St.-Paul, MN. 87-100.

PETRINI, O., SIEBER, T.N., TOTI, L. & VIVET, O. (1992). Ecology, metabolite production, and substrate utilisation in endophytic fungi. Natural Toxins 1: 185-196.

PIELOU, E.C. (1984). The interpretation of ecological data. John Wiley & Sons, New York. 263 pp.

POON, M.O.K. & HYDE, K.D. (1998). Biodiversity of intertidal estuarine fungi on Phragmites at Mai Po marshes, Hong Kong. Bot. Mar. 41: 141-155.

RODEWALD, L. & RUDESCU, L. (1974). Das Schilfrohr. Die Binnengewässer Band XXVII: 1-302.

RODRIGUES, K.F. (1994). The foliar fungal endophytes of the Amazonian palm *Euterpe oleracea*. Mycologia 86: 376-385.

SAIKKONEN, K., FAETH, S.H., HELANDER, M. & SULLIVAN, T.J. (1998). Fungal endophytes: a continuum of interactions with host plants. Rev. Ecol. Syst. 29: 319-343.

SANTAMARIA, L. (2002). Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. Acta Oecol. 23: 137-154.

SAW, P.J.A. (1992). Fungi, fungivores, and fungal food webs. *In* Caroll G.C. & Wicklow, D.T. (Eds.). The fungal community. Its organisation and role in the ecosystem. 2de ed. Marcel Dekker, New York. pp. 295-310.

SCHLÄPFER, F., SCHMID, B. & SEIDL, I. (1999). Expert estimates about effects of biodiversity on ecosystem processes and services. Oikos 84: 346-352.

SCHULZE, E.-D. & MOONEY, H.A. (EDS.) (1993). Biodiversity and ecosystem function. Springer-Verlag, Berlin.

STEVENS, G.C. (1989). The latitudinal gradient in geographical range: how many species coexist in the tropics. Am. Nat. 133: 240-256.

SWIFT, M.J. & ANDERSON, J.M. (1993). Biodiversity and ecosytem function in agricultural systems. *In* SCHULTZE, E.-D. & MOONEY, H.A. (EDS.) Biodiversity and Ecosystem function. Ecological Studies 99. Springer-Verlag, Berlin. 15-41.

TALIGOOLA, H.K. (1969). Stages in the colonization of *Phragmites communis* Trin. by microfungi. unpublished doctoral thesis, Nottingham. 153 pp.

TALIGOOLA, H.K., APINIS, A.E. & CHESTERS, C.G.C. (1972). Microfungi colonizing collapsed aerial parts of *Phragmites communis* Trin. in water. Nova Hedwigia 23: 465-472.

TALIGOOLA, H.K., APINIS, A.E. & CHESTERS, C.G.C. (1975). Microfungi colonizing the inflorescences of *Phragmites communis* Trin. Nova Hedwigia 26: 483-494.

TEPLA, V.F. (1963). Die pilzflora auf *Phragmites communis* Trin.. Bot. J. URSS 48: 1686-1692.

TEWKSBURY, L., CASAGRANDE, R., BLOSSEY, R., HAFLIGER, P. & SCHWARZLANDER, M. (2002). Potential for biological control of *Phragmites australis* in North America. Biol. Control 23: 191-212.

TRESEDER, K.K. & ALLEN, M.F. (2000). Black boxes and missing sinks: fungi in global change research. Mycol. Res. 104: 1282-1283.

TSCHARNTKE, T. (1999). Insects on common reed (*Phragmites australis*): community structure and the impact of herbivory on shoot growth. Aquatic Bot. 64: 399-410.

VAN DER TOORN, J. (1972). Variability of *Phragmites australis* (Cav.) Trin. ex Steudel in relation to the environment. Doctoral thesis. Rijksuniversiteit Groningen (The Netherlands), Groningen.

VAN RYCKEGEM, G. (1999). Survey and ecological preferences of fungi on *Phragmites australis* in Flanders. Unpublished master thesis (in Dutch). Ghent University, Belgium, 163 pp.

VAN RYCKEGEM, G. (2005). Fungi on common reed (*Phragmites australis*): fungal diversity, community structure and decomposition processes. Ph.D. thesis. Ghent University, Ghent.

VAN RYCKEGEM, G. & APTROOT, A. (2001). A new *Massarina* and a new *Wettsteinina* (Ascomycota) from freshwater and tidal reeds. Nova Hedwigia 73: 161-166.

VAN RYCKEGEM, G., VAN DE PUT, K. & ROBERTS, P. (2002). *Tremella spicifera* sp. nov., a parasite of *Massarina arundinacea*. Mycotaxon 81: 185-189.

VAN RYCKEGEM G. & VERBEKEN A. (2000). *Rosellinia musispora* sp. nov., een nieuw tepelkogeltje op riet (*Phragmites australis*). Sterbeeckia 19: 3-5.

VAN RYCKEGEM, G. & VERBEKEN, A. (2005a). Fungal diversity and community structure on *Phragmites australis* (Poaceae) along a salinity gradient in the Scheldt estuary (Belgium). Nova Hedwigia 80: 173-197.

VAN RYCKEGEM, G. & VERBEKEN, A. (2005b). Fungal ecology and succession on *Phragmites australis* in a brackish tidal marsh I. Leaf sheaths. Fungal Diversity 19 (in print).

VAN RYCKEGEM, G. & VERBEKEN, A. (2005c). Fungal ecology and succession on *Phragmites australis* in a brackish tidal marsh II. Stems. Fungal Diversity 20 (accepted).

VAN RYCKEGEM, G. & VERBEKEN, A. (2005d). The complex fungal community structure associated with above ground plant parts of *Phragmites australis* in a brackish tidal marsh. (to be submitted to Mycol. Res.).

VAN RYCKEGEM, G., GESSNER, M.O. & VERBEKEN, A. (2005a). Fungi on leaf blades of *Phragmites australis* in a brackish tidal marsh: diversity, succession and leaf decomposition. (to be submitted to Mycologia).

VAN RYCKEGEM, G. , VAN DRIESSCHE, G., VAN BEEUMEN, J.J. & VERBEKEN, A. (2005b). Fungal dynamics during decomposition of *Phragmites australis* leaf sheaths and stems in a brackish tidal marsh. (to be submitted to Microb. Ecol.).

VANDENKOORNHUYSE, P., BALDAUF, S.L., LEYVAL, C., STRACZEK, J., YOUNG, J.P.W. (2002). Extensive fungal diversity in plant roots. Science 295: 2051.

WEBB, C.O., ACKERLY, D.D., MCPEEK, M.A. & DONOGHUE, M.J. (2002). Phylogenies and community ecology. Annu. Rev. Ecol. Syst. 33: 475-505.

WILSON, D. (1995). Endophyte - the evolution of a term, a clarification of its use and definition. Oikos 73: 274-276.

WIRSEL, S.G.R. (2004). Homogenous stands of a wetland grass harbour diverse consortia of arbuscular mycorrhizal fungi. FEMS Micorbiology Ecology 48: 129-138.

WIRSEL, S.G.R., LEIBINGER, W., ERNST, M. & MENDGEN, K. (2001). Genetic diversity of fungi closely associated with common reed. New Phytol. 149: 589-598.

WONG, M.K.M. & HYDE, K.D. (2001). Diversity of fungi on six species of Gramineae and one species of Cyperaceae in Hong Kong. Mycol. Res. 105: 1485-1491.

ZHOU, D. & HYDE, K.D. (2001). Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi. Mycol. Res. 105: 1449-1457.

Summary – general discussion Conclusions and future perspectives

The important role of fungi in ecosystem functioning is well recognized (Christensen, 1989; Dix & Webster, 1995) but fungal research is only sparsely implemented in ecosystem studies and the exact impact of their presence and their diversity remains undocumented in most habitats.

In this thesis we investigate several aspects of fungi associated with a single host plant, common reed (*Phragmites australis*). This grass species is not only the dominant macrophyte in our study area, the Scheldt estuary (The Netherlands - Belgium), it is also a highly productive plant species in many (sub)tropical and temperate wetlands (Mitsch & Gosselink, 2000). In those wetlands, common reed is often a key species and knowledge about fungal interactions with this plant could prove to be important in order to understand the plant's ecology, the exact fate of their produced organic matter and wetland functioning.

The specific aims of this study were (1) describing fungal biodiversity on common reed based on critical taxonomic observations, (2) describing fungal ecology: community structure in detail in one site and ecological patterns along changing environmental gradients in an estuary. Both based on direct observation in the field using our taxonomic knowledge, (3) the establishment of fungal importance during decomposition processes by the monitoring of fungal biomass, heavy metal, carbon and nutrient dynamics.

The majority of our research was conducted in the Scheldt estuary (The Netherlands - Belgium). This ecosystem provides an excellent study area to investigate species responses to changing environmental gradients prevalent on the tidal marshes. A single tidal marsh ('Saeftinghe') was used as experimental site with several experimental plots established. Here we monitored the fungal community and the decomposition of reed (see framework).

1. Fungal biodiversity

A first step in our study was the fundamental step to establish taxonomic knowledge. In order to study the ecology of a group of organisms one has to possess a sound taxonomic base to identify the taxa involved, a point which is not evident in mycology.

Detailed taxonomic information is presented in the **appendix**. All 214 taxa (134 genera) recognized on *P. australis* during this study are documented by a description and illustrations for most taxa. The taxonomic database is made accessible on the following URL: http://biology.ugent.be/reedfungi. 59% of the taxa could be identified up to species level while at least 20 taxa (9%) are considered to be new to science. Four were already described in short publications and at least 16 other taxa are waiting for publication. Other unidentified species belong to species complexes that are in need of revision or insufficient material was available to draw conclusions. Ascomycetes are the dominant group of reed colonizers with

equal shares of sexual and asexual states, unlike few basidiomycetes were recorded. This taxonomic balance is comparable to previous studies investigating fungal diversity on herbaceous plants and grasses inclusive *P. australis* (e.g. Webster, 1956; Yadav & Madelin, 1968; Apinis et al., 1972; Gessner, 1977; Wong & Hyde, 2001). However the number of hyphomycetes was lower compared to other studies (e.g. Apinis et al., 1972). This difference is probably due to the methods used and to the specific study area. The specific habitat conditions in tidal marshes could be unfavourable for hyphomycetes (chapter 8). Moreover I did not incubate plant parts in moist chamber nor did I plate plant parts on agar after surface sterilization, two techniques generally favourable to induce hyphomycete sporulation. I only recorded species sporulating in the field on reeds after a maximal incubation of two weeks at 4°C in the refrigerator.

In **Chapter 10 and 11** we summarize and extrapolate all available data on fungal diversity on common reed. Our data show a ***'hyper-diversity'*** of fungi on common reed. An exhaustive literature search and multiple personal surveys revealed 822 taxa (estimated 682 species) occurring on *Phragmites australis* hitherto. From the available data the diversity of species colonizing common reed in the world is conservatively estimated at 863-1345 of which 78 till 269 species are considered to be host specific. Such an estimate is far more than the average assumed ratio of 6 host specific fungi per plant species (Hawksworth, 2001). Factors related to such a high world-wide diversity are discussed. Those factors are considered to be mainly related to plant structure, the wide ecological amplitude and the cosmopolitan distribution of the host, creating huge habitat diversity for fungi. In a single site (Saeftinghe, The Netherlands) we found equally well a high diversity (95 sporulating taxa) (chapter 2). Probably, the high diversity in a single site originates from the diverse niches available on a reed plant each of those niches influenced by a variety of internal and external conditions. Eventually this range of conditions will select on the fungal component present in the surroundings which is both diverse and each unique in life history, not surprisingly resulting in a wide spectrum of taxa and decay patterns even for a single plant resource.

We stress the importance of detailed habitat research, including both spatial and temporal parameters, to estimate fungal diversity on a wider geographical and ecological scale. Our study shows that detailed inventories focusing on a single host plant in various conditions could substantially improve the resolution of fungal diversity estimates (see Hawksworth, 1991, 2001); moreover such detailed studies will demonstrate that the diversity estimate of Hawksworth (l.c.) is a conservative one.

However, how many fungal taxa are required for a functional reed decay-ecosystem? This answer can only be guessed for. We presume (as other authors suggested before e.g. Swift & Anderson, 1993) that the decomposer system is very resilient. This would mean that not all of the present species are be indispensable to have an equally well functional decay system. However, the importance of each taxon will be different both in space and time in a reed decay system. Indications for this are (1) a reed plant is colonized by several fungal subcommunities

separated in space that undergo a species succession (see part 1) and (2) the large differences in species composition observed between ecologically different reed sites (see chapter 8).

Few species are dominant in a study site (i.e. 11 taxa make 60% of all observations in the brackish tidal marsh, Saeftinghe). This is a common observation (e.g. Sieber, 1985, Kowalski & Kehr, 1996). Even less species are both dominant and are found in all reed belts visited (e.g. *Phoma* sp. III, *Massarina arundinacea*, and several species belonging to *Lophiostoma* and *Phaeosphaeria* (and anamorphs)). Those taxa might be general-host-specific endophytes (and most of them become primary saprotrophs) but more data is needed to verify this statement. Petrini (1996) considers a dominant endophyte to be host specific. However, dominance depends on the scale of screening a host and the size of the unit community should be considered carefully because nonsystemic endophytes could be dominant on a host only in small niches. Therefore, if a host plant is screened with a large grid, a species could appear rare (or even overlooked) while in fact it could be dominant at smaller scale in a specific microhabitat. What about the many other taxa? We should stress that we recorded only sporulation structures. Therefore, a species sporulating rarely could be a common mycelial inhabitant of a plant (Petrini, 1996). Molecular data or plating of surface sterile material is needed to revail this. The less dominant taxa are probably able to secure their resource from invasion by the dominant species (small patch) and/or they are better adapted to some specific conditions were dominant taxa are out competed.

2. Fungal community structure and ecology

Variability in species associated with common reed was investigated at two levels. First we focused in **chapters 2-5** on the species composition during reed growth and decay in a single, brackish tidal marsh. Additionally we assessed the species composition between ecologically different sites in **chapter 8**.

In **chapter 2** species data are analyzed all together (3976 records) and the complex fungal community is spatially and temporally examined. Our data show that the ***sporulating fungi on reeds are well ordered in space and time***. Significant differences were found in species composition between plant parts (leaf blade, leaf sheath and stems) and plant part specificity or recurrence of species is demonstrated. Multivariate analysis demonstrated a vertical distribution pattern with different subcommunities along the vertical axis of standing reed shoots and a different subcommunity in the litter layer. These subcommunities change temporally due to successional events during the growth and decay of the reed plant. At least eleven species groups could be recognized on living and decaying above ground plant material and each group was characterized by indicator species. A high level of fungal specificity was noticed with several species restricted to a particular plant part or to a well-defined successional phase. All observations were repeatable between years. The latter is of importance to demonstrate

that the observed species patterns are specific for our study site and not due to random fungal colonization.

Subsequently we investigated in detail the temporal aspects in fungal communities during decomposition and describe succession of fungal sporulation structures in **Chapters 3, 4** and **5** respectively for leaf sheaths, stems and leaf blades.

On each plant part we could recognize a high fungal diversity (77 taxa on leaf sheaths, 49 taxa on stems and 35 taxa on leaf blades). All plant parts were followed during standing decay at several heights in the canopy and in the litter layer almost until decomposition was complete. Detrended correspondence analysis (DCA) of samples suggests the importance of a spatial separation (microhabitat) in explaining species variation between samples. Within each of those microhabitats DCA indicated a specific temporal pattern (succession). Fungal succession (community development) was described by a sequence of *three phases in fungal sporulation*. This three-step-succession of fungal community development is comparable to the observations by Apinis et al. (1972), Gessner et al. (1993) and the general patterns described by Dix and Webster (1995) and Cooke and Rayner (1984). We recognize an initial pioneer community (Phase I), which is relatively open for fungal invasion and usually characterized by low species diversity, with few stress tolerant or ruderal species having a high abundance. Pathogens and quick sporulators after damage are included in this stage. The second stage (Phase II) is a more closed, mature community with high diversity of more combative species. Endophytes (as primary saprophytes) are considered to form fruit bodies during this stage. This metamorphose from endophyte to saprophyte was suggested before (by e.g. Kowalski & Kehr, 1996; Parbery, 1996, Zhou & Hyde, 2001) and is probably a common phenomenon. Gradually secondary saprophytes start to colonize and a following stage is announced. The third phase (Phase III) is characterized as an impoverished community dominated by few species with low sporulation frequency typified as stress tolerant and/or highly combative taxa. Plant parts could undergo (no fungi were found sporulating during this stage but biomass remained considerable) a final fourth stage (Phase IV) in the litter layer characterized by a colonisation by common soil fungi until the substrate is almost entirely decomposed in favourable conditions (aerated, not too much fragmentation) and eventually bacteria take over the end phase of decay (e.g. Benner et al., 1986; Sinsabaugh & Findlay, 1995).

The effect of seasonality and host's physiology on fungal succession is discussed (chapter 3). A striking observation is the ***close association between sporulation and the host's life cycle***. Obviously many (endophytic) species may delay their sporulation on leaf sheaths until new leaf blades appear next season. Such a conspicuous association between fungal sporulation and host life cycle is probably not rare (Parbery, 1996; Saikkonen et al., 1998) and was recently observed for oak-endophytes (Verkley, G.J.M., pers. comm.). This observation could one of

the explanations in the origination of hostspecificity for certain taxa well adapted to the timing of their host.

The suggested importance of fungi during standing decay (Kuehn et al., 2004) is confirmed during our research both in diversity and in biomass development on plant parts in standing positions. Specifically the effect of fungi colonizing standing stems is discussed in chapter 4 and 6.

In **chapter 8** we examined fungal communities on stems and leaf sheaths of common reed along a salinity gradient (mesohaline to freshwater) present in the Scheldt estuary (The Netherlands - Belgium). This was done in order to get an idea of the fungal (community) sensitivity towards a broader spectrum of changing ecological variables among sites. Species distribution on reed litter, investigated by multivariate analysis, was strongly correlated with flooding frequency and salinity. Influences of salinity on the fungal community are discussed and tested by a culture experiment. Taxa were divided into three basic distributional types: taxa present only in the saline-brackish water zone, species present only in the freshwater zone, and a group of eurytopic species found over the entire salinity gradient sampled. Indicator species for each group were determined. In two sites, mesohaline brackish and freshwater, the vertical distribution of mycota was analysed. Higher similarities between the same vertical zones, especially in middle and apical parts of the culms, indicated that aerial canopy fungi (no direct contact with tidal exchange water) were less influenced by site-specific characteristics. However, in both sites, a few common indicator species for standing *Phragmites* culms could be identified. Flooding height (and frequency) is demonstrated to influence vertical species distribution. Fungal diversity in tidal compared to non-tidal marshes is lower in non-tidal reed marshes. This is obvious from the low number of Agaricales, discomycetes and hyphomycetes retrieved from tidal marshes.

3. Fungi and decomposition processes

Fungi are well known to colonize and decompose plant tissues in various environments, but information on fungal communities on wetland plants, their relation to microhabitat conditions and link to decomposition processes is scant (Gessner et al., 1997; Kuehn et al., 2004). Therefore in **chapter 5** and **6,** we examined fungal diversity and succession on leaf blades, leaf sheaths and stems of *Phragmites australis* in naturally decay situations in combination with fungal biomass (ergosterol content) and decomposition variables. We analysed nitrogen dynamics (and additionally phosphorus and carbon dynamics in chapter 6 for leaf sheaths and stems) and litter mass loss on the sediment surface of the marsh.

Our results show a ***high fungal biomass development in decaying plant parts during decomposition in the canopy and in the litter layer***. These results agree with previous

studies highlighting the important function of fungi during standing reed decay (Gessner, 2001; Kuehn et al., 2004) in the litter layer (Findlay et al., 2002) and in submerged conditions in wetlands (Komínková et al., 2000). This high biomass development was found to depend on a specific fungal community present in a specific microhabitat as a change in position from the canopy to the litter layer for dead leaves resulted in a striking mass extinction that involved species replacements and a sharp decline in fungal biomass. This transition phase clearly illustrates that ***fungal communities are adapted to certain microhabitats*** and cannot sporulate and most taxa won't survive in other situations (cf. Newell et al., 1989; Tanaka, 1991, 1993).

All plant parts had a decomposition rate at the upper range compared to previous studies in other study areas (Gessner, 2000). Estuaria could be favourable places for a fast breakdown with regular flooding if flooding frequency is not to high (Lee, 1990; Halupa & Howes, 1995) (see chapter 1). Leaves (blades slightly slower than sheaths) lost almost 50% of their initial mass in six months. Stem tissue decomposes much slower with negligible mass loss during the first six months but a steady decay afterwards once fungal colonization was established. Stem tissue, in contrast to leaf tissues, gets colonized slowly by fungi and this delay period should be considered in experimental studies.

Specifically in **chapter 6** we tried to reveal the most important ecological variables in structuring fungal community. Therefore we used multivariate analysis to investigate which environmental variables had most power in explaining fungal succession on the decaying culms. Results of multivariate analysis point cellulose to be the variable best explaining the observed fungal succession during decay.

After one year of standing decay fungal colonization was assessed in a standing position and the fungal biomass pattern along the vertical axis of standing dead stems was screened. Substantial fungal colonization was found. A vertical pattern in ergosterol content was noticed with minimal fungal colonization in the lowest internode but increased steeply a few centimetres higher up the stem. Fungal biomass decreased again towards the middle height of the standing stems and subsequently increased to reach maximal fungal crop near the apex. Possible causes of such vertical patterns are discussed. For example this could be related to plant physiology, anatomy or because of external humidity conditions. These results show that a vertical pattern in CO_2 efflux from a wetland ecosystem should be considered (cf. Kuehn et al., 2004).

Fungal colonizers are considered to ***contain an important fraction of nutrients*** within the decaying plant matter within their biomass. Fungal biomass accounted for up to 34 ± 7% of the total nitrogen in dead leaf blades attached to shoots. This proportion of N retention reduced to only ± 12% in the litter layer. These data suggest that fungi are instrumental in N retention and leaf mass loss during leaf senescence and early aerial decay but that their

importance in N retention diminishes during decomposition on the marsh surface particularly for leaf blades (chapter 5).

In December when the standing stems are considered to be senescent, fungal biomass accounted for 104 ± 14% of total nitrogen and for 94 ± 14% of total phosphorus in the leaf sheaths. While at this moment stems were scantly colonized by fungi which contained less than 10% of the total nitrogen present. Fungal N incorporation was estimated to be 64 ± 13% and 102 ± 15% of total N pool respectively for leaf sheaths and stems during the decay process. Fungal P incorporation was estimated to be 37 ± 9% and 52 ± 15% of total P respectively for leaf sheaths and stems during the decay process. These data suggest that fungi are important in N retention during senescence and aerial decay for leaf sheaths but not for stems and during decomposition on the marsh surface for leaf sheaths and stems. Furthermore, within the stem tissue, fungi are suggested to become active immobilizers of nutrients from the external environment (chapter 6).

From our studies it should be stressed that different plant parts should be studied separately, as already noticed by Gessner (2000). Different plant parts not only possess a specific microbial community; they show distinct decay patterns as they have a different chemical and physical composition (see chapters 2, 6), a feature ignored in a recent study by Findlay et al. (2002) merging stems and leaf sheaths. Our study demonstrates that sufficient replication of homogenous samples (unit communities) in a stable environment yields an overall, characteristic fungal community which could be typified by indicator species forming together specific ecological species groups. Those fungal communities are considered to be much smaller than the better known plant communities, aspects stressed since Swift (1976). Furthermore the importance and advantages of screening host plants *in situ* and the use of indicator species analysis for fungal communities is addressed (see chapter 2, 3 and 8).

In **chapter 7** we further investigated the possible interactions of fungi in wetland ecosystems and tested the hypothesis that fungi could be involved in metal retention. This is a relevant topic as many marshes (including Scheldt marshes) are contaminated with metals and a better understanding of the influences of vegetation on metal retention is needed (Catallo, 1993). Metal contents of decomposing leaf blades, stems and sheaths of reed plants (*Phragmites australis*) were monitored. Most metal contents in reed litter increased considerably during decomposition. As reed biomass turnover is also very high, metal accumulation by litter could be a very important parameter to monitor and take into account when discussing metal transfer at the base of the food chain in intertidal reed beds***. There are indications that fungal activity is an important factor determining metal accumulation*** in decomposing stem tissue. This could also be the case for leaf blades, but for this tissue type the effect of fungal activity on metal concentrations is found to be overruled by passive metal sorption and trapping of sediment particles and associated metals. Both factors seem to be of intermediate importance for leaf sheath tissue.

Chapter 9 is a concluding chapter and summarizes the **established and potential impact of fungi in the Scheldt ecosystem**. A ***huge diversity*** of 166 fungal taxa is recorded on *Phragmites australis* in the Scheldt estuary during this study. Besides substantial diversity, fungi sustain a ***large biomass*** and within a single sample fungi could contain up to 10% of reed carbon. Moreover within decaying stems and leaf sheaths fungi contain the bulk of nutrients (N and P) present in the canopy and generally more than half of the nutrient stock in the litter layer. Fungi were found to ***incorporate a considerable proportion of nutrients*** (N) (~37%) in leaf blades in the canopy and on average 14% of leaf blade N in the litter layer. The mycelial decomposers are thought to immobilize external nutrients and heavy metals potentially raising the sink function of a tidal marsh. Furthermore extrapolation predicts that fungi sustain a high biomass on a unit marsh base in the canopy in for example in 1) leaves (blades and sheaths) before shedding of ≈ 45 mg fungal biomass m^{-2} and 2) stems ≈ 10.8 and 11.2 mg fungal biomass m^{-2} after one year decay respectively in the litter layer and in the canopy. The potential importance of fungi as a food source for detritus feeding invertebrates and the potential importance of fungi for plants vigour is briefly discussed.

4. Conclusions and future perspectives

Our study demonstrates a huge fungal diversity on a single host plant, common reed (*Phragmites australis*). This diversity is described by direct observation of sporulation structures in the field and proved to be highly organized on the reed plant both in space and time. We noticed and stress the importance of detailed sampling in regard to microhabitat, tissue type and ecology of the sites. The multivariate analysis showed that a reed plant is colonized by several recurrent subcommunities of fungi.

The monitoring of the decomposition of *P. australis* showed, by measuring ergosterol concentrations, a high fungal biomass present during early death and decay. Saprophytic fungi are thought to be of major importance in the carbon cycle and are demonstrated to incorporate considerable amounts of nutrients present in the plant resource. Furthermore our research indicates that fungi are able to immobilize nutrients from the external environment and could be involved in the retention of metals within the plant during the decay process.

We would like to stress the need to include fungi in wetland ecosystem studies. Such research is trivial to understand the ecosystem functioning and are above all closely associated with the plant and plant's life cycle. You cannot study a plant without taking the fungus in consideration. However, in order to fully appreciate the role of fungi in wetlands much research needs to be done.

The scanty knowledge about the functional ecology of fungi in wetland ecosystems and the unknown environmental impact on fungal performance offers many exciting pathways for future research. For example the increased interest during past two decades for climatic

changes offers opportunities for experimental studies. Those studies could now profit from the insights gathered in this thesis as a background for experimental design. One of the questions could be: do fungal communities change in composition, activity, … due to global change (some of those questions are presently investigated by M.O. Gessner (pers. comm.)).

Molecular work seems indispensable to elaborate our understanding of fungal ecology on the host. Is the patchy sporulation consistent with the internal growth of the taxa in the substrate? Fingerprinting the fungal community would result in first hand information and would be a tremendous surplus to the understanding of a community and the behaviour of fungal mycelia. Additionally it is the best tool (in combination with plating techniques and direct observation) to focus on the endophytic community and the impact of these fungi on the plant's physiology. The latter is another exciting multidisciplinary field to explore: which are the mechanisms behind an improved growth of reeds associated with *Phaeosphaeria* endophytes (cf. Ernst et al. 2003)? What is the ecological significance of this type of interactions?

More research is needed to describe mycological diversity (taxonomic work!) and understand fungal biodiversity patterns. We know little about fungal biogeography and have few clues on host specificity. A world-wide sampling design could offer very interesting insights. Detailed local comparisons between plant species screened for fungal colonizers would be of interest and could offer clues for the observed discrepancy in fungal species richness on different (~comparable in morphology, structure, etc.) plant species.

Interesting work could be done to explore deeper the importance and energy flow from fungi in the food chain. How do fungi interact with invertebrate grazers (in the Scheldt estuary very common), a domain presently explored for hyphomycetes on leaves in lotic streams (e.g. Bärlocher, 1992) and for ascomycetes colonizing *Spartina* in salt marshes (e.g. Graça et al., 2000).

References

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1972). Colonisation of *Phragmites communis* leaves by fungi. Nova Hedwigia 23: 113-124.

BÄRLOCHER, F. (ED.) (1992). The Ecology of Aquatic Hyphomycetes. Springer-Verlag, Berlin.

BENNER, R., MORAN, M.A. & HUDSON, R.E. (1986). Biogeochemical cycling of lignocellulosic carbon in marine and freshwater ecosystems: relative contributions of prokaryotes and eukaryotes. Limnol. Oceanogr. 31: 89-100.

CATALLO, W.J. (1993). Ecotoxicology and wetland ecosystems: current understanding and future needs. Environ. Toxicol. Chem. 12: 2209-2224.

CHRISTENSEN, M. (1989). A view of fungal ecology. Mycologia 81: 1-19.

COOKE, R.C. & RAYNER, A.D.M. (1984). Ecology of saprotrophic fungi. Longman Group Limited, London.

DIX, N.J. & WEBSTER, J. (1995). Fungal Ecology. Chapman & Hall, London.

ERNST, M., MENDGEN, K.W. & WIRSEL, S.G.R. (2003). Endophytic fungal mutualists: seed-borne *Stagonospora* spp. enhance reed biomass production in axenic microcosms. Molec. Pl.-Microbe Interact. 16: 580-587.

FINDLAY, S.E.G., DYE, S. & KUEHN, K.A. (2002). Microbial growth and nitrogen retention in litter of *Phragmites australis* compared to *Typha angustifolia*. Wetlands 22: 616-625.

GESSNER, M.O. (2000). Breakdown and nutrient dynamics of submerged *Phragmites* shoots in the littoral zone of a temperate hardwater lake. Aquatic Bot. 66: 9-20.

GESSNER, M.O. (2001). Mass loss, fungal colonisation and nutrient dynamics of *Phragmites australis* leaves during senescence and early decay. Aquatic Bot. 69: 325-339.

GESSNER, M.O., THOMAS, M., JEAN-LOUIS, A.M. & CHAUVET, E. (1993). Stable successional patterns of aquatic hyphomycetes on leaves decaying in a summer cool stream. Mycol. Res. 97: 163-172.

GESSNER, R.V. (1977). Seasonal occurrence and distribution of fungi associated with *Spartina alterniflora* from a Rhode Island estuary. Mycologia 69: 477-491.

GRAÇA, M.A., NEWELL S.Y., KNEIB, R.T. (2000). Grazing rates of organic matter and living fungal biomass of decaying *Spartina alterniflora* by three species of salt-marsh invertebrates. Mar. Biol. 136: 281-289.

HALUPA, P.J. & HOWES, B.L. (1995). Effects of tidally mediated litter moisture content on decomposition of *Spartina alterniflora* and *S. patens*. Mar. Biol. 123: 379-391.

HAWKSWORTH, D.L. (1991). The fungal dimension of biodiversity: magnitude, significance and conservation. Mycol. Res. 95: 641-655.

HAWKSWORTH, D.L. (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. 105: 1422-1432.

KOMÍNKOVÁ, D., KUEHN, K.A., BÜSING, N., STEINER, D. & GESSNER, M.O. (2000). Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of a large lake. Aquat. Microb. Ecol. 22: 271-282.

KOWALSKI, T. & KEHR, R.D. (1996). Fungal endophytes of living branch bases in several European tree species. *In* REDLIN, S.C. & CARRIS, L.M. (EDS.) Endophytic Fungi in Grasses and Woody Plants. American Phytopathological Press, St.-Paul, MN. 67-86.

KUEHN, K.A., STEINER, D. & GESSNER, M.O. (2004). Diel mineralization patterns of standing-dead plant litter: implications for CO_2 flux from wetlands. Ecology 85: 2504-2518.

LEE,S.Y. (1990). Net aerial primary productivity, litter production and decomposition of the reed *Phragmites communis* in a nature reserve in Hong Kong: management implications. Mar. Ecol. Prog. Ser. 66: 161-173.

MITSCH, W.J. & GOSSELINK, J.G. (2000). Wetlands (3rd edition). Van Nostrand Reinhold, New York.

NEWELL, S.Y., FALLON, R.D. & MILLER, J.D. (1989). Decomposition and microbial dynamics for standing, naturally positioned leaves of the salt-marsh grass *Spartina alterniflora*. Mar. Biol. 101: 471-481.

PARBERY, D.G. (1996). Trophism and ecology of fungi associated with plants. Biological Reviews of the Cambridge Phylosophical Society 71: 473-527.

PETRINI, O. (1996). Ecological and physiological aspect of host specificity in endophytic fungi. *In* REDLIN, S.C. & CARRIS, L.M. (EDS.) Endophytic Fungi in Grasses and Woody Plants. American Phytopathological Press, St.-Paul, MN. 87-100.

SAIKKONEN, K., FAETH, S.H., HELANDER, M. & SULLIVAN, T.J. (1998). Fungal endophytes: a continuum of interactions with host plants. Rev. Ecol. Syst. 29: 319-343.

SIEBER, T.N. (1985). Endophytische Pilze von Winterweizen (*Triticum aestivum* L.). Dissertation ETH Nr. 7725. Swiss Federal Institute of Technology, Zürich, Switzerland.

SINSABAUGH, R.L. & FINDLAY, S. (1995). Microbial production, enzyme activity, and carbon turnover in surface sediments of the Hudson River estuary. Microb. Ecol. 30: 127-141.

SWIFT, M.J. & ANDERSON, J.M. (1993). Biodiversity and ecosytem function in agricultural systems. *In* SCHULTZE, E.-D. & MOONEY, H.A. (EDS.) Biodiversity and Ecosystem Function. Ecological Studies 99. Springer-Verlag, Berlin. 15-41.

TANAKA, Y. (1991). Microbial decomposition of reed (*Phragmites communis*) leaves in a saline lake. Hydrobiologia 220: 119-129.

TANAKA, Y. (1993). Aerobic cellulolytic bacterial-flora associated with decomposing *Phragmites* leaf-litter in a seawater lake. Hydrobiologia 263: 145-154

WEBSTER, J. (1956). Succession of fungi on decaying cocksfoot culms. I. J. Ecol. 44: 517-544.

WONG, M.K.M. & HYDE, K.D. (2001). Diversity of fungi on six species of Gramineae and one species of Cyperaceae in Hong Kong. Mycol. Res. 105: 1485-1491.

YADAV, A.S. & MADELIN, M.F. (1968). The ecology of microfungi on decaying stems of *Urtica dioica*. Trans. Brit. Mycol. Soc. 51: 249-259.

ZHOU, D. & HYDE, K.D. (2001). Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi. Mycol. Res. 105: 1449-1457.

Samenvatting – algemene discussie

De belangrijke rol van zwammen in het functioneren van ecosystemen is algemeen aanvaard en vaak aangehaald (Christensen, 1989; Dix & Webster, 1995); nochtans worden zwammen als studieobject zelden geïntegreerd in ecosysteemstudies waardoor de exacte impact vaak onbekend is.

In dit proefschrift onderzoeken we verschillende aspecten van zwammen die voorkomen op riet (*Phragmites australis*). Deze grassoort is niet alleen dominant in ons studiegebied (het Schelde estuarium, België - Nederland), maar het is ook een uiterst productieve plant in vele vochtige gebieden zowel in (sub)tropische als meer gematigde streken (Mitsch & Gosselink, 2000). Omwille van de hoge productiviteit wordt deze oeverplant vaak aangeduid als sleutelsoort in ecosysteemprocessen. Informatie over de interacties tussen zwammen en deze plant kunnen onze kennis en inzicht in de ecologie van riet, wetlands in het algemeen en afbraakprocessen specifiek, aanzienlijk verhogen.

De specifieke doelstellingen van deze studie waren (1) het beschrijven van de mycodiversiteit op riet gebaseerd op kritische taxonomische beschrijvingen, (2) het beschrijven van de ecologie van de soorten: de gemeenschapsstructuur in detail in één enkele site en de invloed van veranderende milieuvariabelen op de soortensamenstelling langsheen een estuariene (saliniteits)gradient. Beide deelaspecten zijn gebaseerd op de taxonomische kennis bekomen in (1). (3) Tenslotte het onderzoeken van het belang van fungi gedurende afbraakprocessen door het opvolgen van zwam-biomassa, zware-metalen concentraties, koolstof- en nutriënt-veranderingen.

Het grootste gedeelte van ons onderzoek werd uitgevoerd in het Schelde estuarium (Nederland - België). Dit ecosysteem is geschikt om veranderingen in soortensamenstellingen te bestuderen, doordat het een verscheidenheid vertoont van natuurlijke milieugradiënten (b.v. saliniteit). Een brakwater schor ('Saeftinghe') werd uitgekozen als experimentele site. Hier volgden we de zwammengemeenschap en het afbraakproces van riet in detail op.

1. Mycodiversiteit

Het eerste luik van deze studie, de taxonomische beschrijving van de fungi, vormde de basis van alle verdere onderzoek. Een goede taxonomische kennis van de bestudeerde organismen is noodzakelijk om de ecologie en de impact op ecosysteemprocessen van de specifieke organismen te onderzoeken.
Gedetailleerde taxonomische informatie wordt besproken in de **bijlage**. Alle 214 soorten (134 genera) die gevonden werden tijdens deze studie zijn gedocumenteerd met een beschrijving en illustraties voor de meeste soorten. De taxonomische databank is toegankelijk via het internet: http://biology.ugent.be/reedfungi. 59% van de taxa konden op naam gebracht worden en tenminste 20 soorten (9%) werden herkend als nieuw voor de wetenschap. 4 van deze nieuwe soorten werden reeds beschreven in korte publicaties. Andere soorten, die niet gedetermineerd konden worden, behoren ofwel tot soortscomplexen die dringend aan taxonomische revisie toe zijn; ofwel was er niet voldoende materiaal voorhanden om tot conclusies te komen. Ascomyceten vormen de dominante groep van soorten met ongeveer een gelijk aandeel in sexuele en asexuele stadia. Er werden relatief weinig basidiomyceten gevonden gedurende onze studie. Deze taxonomische verdeling is vergelijkbaar met de resultaten van andere studies die de zwamdiversiteit onderzochten op kruidachtige planten en grassen (inclusief riet) (b.v. Webster, 1956; Yadav & Madelin, 1968; Apinis et al., 1972; Gessner, 1977; Wong & Hyde, 2001). Wij vonden echter minder hyfomyceten in vergelijking met andere studies (b.v. Apinis et al., 1972). Dit verschil is vermoedelijk te wijten aan ons specifieke studiegebied en de gebruikte methodes. De milieucondities gecreëerd door de getijdenwerking op de schorren lijken ongeschikt voor veel hyfomyceten (hoofdstuk 8). Onze methodiek bestond enkel uit het oberveren van sporulerende zwammen in het veld waarbij de rietstalen maximaal twee weken werden bewaard in een koelkast bij 4 °C. In studies die veel hyfomyceten vonden op grassen werd sporulatie bevorderd door vochtige incubatie bij kamertemperatuur of werden culturen van oppervlakkig gesteriliseerd plantenmateriaal gemaakt.

In hoofdstuk 10 en 11 wordt alle informatie over mycodiversiteit samengevat en extrapoleren we de gegevens om te komen tot een wereldwijde schatting van de zwammen op riet. Onze gegevens tonen een ***'hyperdiversiteit'*** van fungi op riet. Een uitgebreide literatuurstudie, aangevuld met persoonlijke inventarisaties, leverde uiteindelijk een lijst op met 822 taxa (geschat op 682 soorten) ooit waargenomen als geassocieerd met riet. De globale soortendiversiteit wordt conservatief geschat op 863-1345 waarvan 78 tot 269 soorten beschouwd worden als gastheerspecifiek. Een dergelijke schatting is veel hoger dan de gemiddelde schatting van 6 gastheerspecifieke zwammen per plantensoort (Hawksworth, 2001). Samengevat kan de hoge diversiteit toegeschreven worden aan de specifieke structuur

van de plant, de klonale plantengroei en de grote ecologische variabiliteit waarbij de plant kan groeien gecombineerd met een kosmopolitische verspreiding. Deze factoren creëren een enorme waaier aan habitats voor fungi. In één enkele site vonden we eveneens een hoog aantal taxa (95 sporulerende taxa). Dergelijke hoge lokale diversiteit is waarschijnlijk te wijten aan de vele niches die beschikbaar zijn in een rietplant (met verscheidene interne en externe condities aanwezig) (zie hoofdstuk 1). Uiteindelijk is het de verscheidenheid van deze condities die een selectie veroorzaken in de mogelijke zwammen voorkomend op de rietplant. Deze zwammen zijn elk uniek in hun levensloop en het hoeft ons niet te verwonderen dat het uiteindelijk een wijd spectrum van soorten is dat de rietplant koloniseert.

We becommentariëren en benadrukken het belang van gedetailleerde habitatstudies (waarbij zowel temporele als ruimtelijke factoren worden onderzocht) om de diversiteit van zwammen te schatten op grotere schaal. Onze studie toont dat gedetailleerde inventarisaties de nauwkeurigheid van de schatting van wereldwijde diversiteit van zwammen aanzienlijk kunnen verbeteren (zie Hawksworth, 1991, 2001). Bovendien zullen gedetailleerde studies, zoals deze, aantonen dat de momenteel geschatte mycodiversiteit (Hawksworth, l.c.) te laag is.

Maar zijn al deze zwammen nu noodzakelijk voor een functioneel riet ecosysteem? Ons onderzoek leent zich alleen maar tot speculatie over dergelijke vraag. We veronderstellen (zoals ook andere auteurs, b.v. Swift & Anderson, 1993) dat het afbraaksysteem zeer gebufferd is. Dit betekent dat niet elk van de aanwezige soorten onvervangbaar is om een vergelijkbaar afbraaksysteem te hebben. Het belang van elk taxon zal verschillend zijn en afhankelijk van zowel de plaats en tijd in het afbraakproces. Indicaties hiervoor zijn (1) het feit dat rietplanten gekoloniseerd worden door verschillende ruimtelijk gescheiden deelgemeenschappen van zwammen die een soortensuccessie ondergaan (zie deel 1 proefschrift) en (2) de grote verschillen die we merken in de soortensamenstelling tussen gebieden (zie hoofdstuk 8).

Slechts weinig soorten zijn dominant op riet tijdens het afbraakproces (b.v. 11 taxa staan voor 60% van alle observaties in de studie site 'Saeftinghe'). Dit is een algemeen patroon (b.v. Sieber, 1985; Kowalski & Kehr, 1996). Nog minder soorten komen dominant voor in alle onderzochte studiegebieden (b.v. *Phoma* sp. III, *Massarina arundinacea*, en verschillende soorten die thuishoren in *Lophiostoma* en *Phaeosphaeria* (met bijhorende anamorfen)). Deze taxa zou men kunnen beschouwen als algemene gastheerspecifieke-endofyten. Er zijn echter meer gegevens nodig om dit te bevestigen. Gastheerspecificiteit wordt besproken in hoofdstuk 11. Volgens Petrini (1996) is een dominante endofyt vaak gastheerspecifiek. Het is echter wel belangrijk om rekening te houden met de schaal waarop het onderzoek gebeurt omdat het toekennen van een dominatiegraad afhankelijk is van de grootte en delen onderzocht aan een plant. Zo kunnen niet-systemische zwammen dominant zijn op een gastheer enkel in kleine niches. Zijn de overgrote meerderheid van de taxa dan zeldzaam? Doordat we in deze studie enkel gebruik maakten van observatie van sporulerende fungi kunnen we de vraag niet beantwoorden. Een

soort die zelden sporuleert kan toch algemeen aanwezig zijn in een plant als mycelium (Petrini, 1996). Moleculaire gegevens of indirecte observatie door het groeien van culturen uit oppervlakkig gesteriliseerd materiaal zijn nodig om dit te achterhalen. De niet dominante soorten kunnen in staat zijn om hun veroverde patch te verdedigingen tegen indringers en/of ze zijn beter aangepast aan specifieke niches in de rietplant die niet of minder geschikt zijn voor dominante rietkolonisatoren.

2. Ecologie en gemeenschapsstructuur van zwammen

Variabiliteit in de soortensamenstelling van zwammen die voorkomen op riet werd onderzocht op twee vlakken. Eerst werd de klemtoon gelegd op de soortensamenstelling in een brakwater getijdensysteem (**hoofdstuk 2-5)**. Vervolgens onderzochten we de soortensamenstelling tussen verschillende locaties in het Schelde estuarium in **hoofdstuk 8.**

In **hoofdstuk 2** analyseren we alle soortgegevens samen (3979 records) en onderzoeken we de gemeenschapsstructuur van zwammen in een rietkraag, in ruimte en tijd. ***Sporulerende zwammen komen zeer geordend voor op de rietplant zowel in de ruimte als in de tijd***. De soortensamenstelling tussen de verschillende plantendelen (bladschijf, bladschede en stengel) bleek significant verschillend en specifieke soorten of soorten met een hoge getrouwheid aan bepaalde delen van een rietplant werden gevonden. Multivariate analyse toont een verticaal distributiepatroon, met verschillende subgemeenschappen langsheen de staande rietplant en een subgemeenschap in de strooisellaag. Elk van deze gemeenschappen verandert in de tijd door successie van soorten gedurende de groei en ontwikkeling van de plant en tijdens de afbraak. Tenminste elf soortengroepen werden erkend en voor elk van deze groepen werden indicatorsoorten aangeduid. De observaties bleken herhaalbaar tussen verschillende jaren, dus de geobserveerde patronen zijn specifiek in ons studiegebied en niet toe te schrijven aan willekeurige kolonisatie.

In een volgend luik van het proefschrift, in **hoofdstuk 3**, **4** en **5**, bestuderen we de successie van sporulerende zwammen verder in detail, respectievelijk voor bladscheden, stengels en bladschijven.

Op elk van deze plantendelen werd een hoge diversiteit van zwammen gevonden (77 taxa op bladscheden, 49 taxa op stengels en 35 taxa op bladschijven). Alle plantendelen werden opgevolgd op drie verschillende hoogtes (indien mogelijk) van de rietplant (op levende en dode planten). Daarnaast werden de dode plantendelen opgevolgd in de strooisellaag tot wanneer ze

bijna volledig waren afgebroken. Multivariate analyse toont duidelijk ruimtelijke patronen in de distributie van de soorten op een rietplant. Bovendien kunnen we in elk van de onderzochte microhabitats een duidelijk temporeel patroon (successie) herkennen. De successie van zwammen op riet wordt beschreven volgens een *drie-stappen-sequentie*. Deze driestappen-successie van de zwammengemeenschap is vergelijkbaar met de observaties van Apinis et al. (1972), Gessner et al. (1993) en de algemene patronen beschreven door Dix and Webster (1995) en Cooke and Rayner (1984). We herkennen een initiële pioniersgemeenschap (fase I), die relatief open is voor zwammen om zich te vestigen. Een aantal stress-tolerante of ruderale taxa zijn abundant en samen met pathogene fungi vormen ze het relatief kleine aantal soorten dat sporuleert op riet gedurende deze fase. Het tweede stadium (fase II) is gekarakteriseerd als een meer gesloten gemeenschap met hoge diversiteit van meer combatieve soorten. Endofyten (als primaire saprofyten) sporuleren voornamelijk in deze fase. Deze metamorfose van endofyt tot saprofyt werd voordien al opgemerkt (b.v. Kowalski & Kehr, 1996; Parbery, 1996, Zhou & Hyde, 2001) en is waarschijnlijk een algemeen fenomeen. Vervolgens beginnen secundaire saprofyten de rietplant te koloniseren en kondigt de volgende fase in de successie zich aan. Deze derde fase (fase III) is gekarakteriseerd door een verarmde gemeenschap gedomineerd door een handvol taxa met doorgaans een lage sporulatiefrequentie. Deze soorten worden beschouwd als zijnde stress-tolerante en/of zeer combatieve taxa. Een mogelijke vierde fase (fase IV) is een gemeenschap die voornamelijk bestaat uit algemene bodemschimmels. Deze fungi werden echter niet gevonden met sporulatiestructuren op de plant in het veld maar een indicatie voor hun aanwezigheid zou de relatief hoge ergosterol concentratie kunnen zijn in het substraat op het einde van onze experimentele studieperiode. Na deze laatste fase van fungale afbraak domineren bacteriën geleidelijk aan het afbraakproces (b.v. Benner et al., 1986; Sinsabaugh & Findlay, 1995).

Specifiek bediscussiëren we het effect van seizoenaliteit en de gastheerfysiologie op successie van zwammen in **hoofdstuk 4**. Een ***opvallende observatie is het verband tussen sporulatie en de levenscyclus van de gastheer***. Blijkbaar kunnen veel (endofytische) soorten hun sporulatie uitstellen op de bladscheden totdat nieuwe bladschijven gevormd worden in het volgende groeiseizoen. Dergelijke opvallend goede timing tussen sporulatie van zwammen en de levenscyclus van een gastheer is waarschijnlijk algemeen (Parbery, 1996; Saikkonen et al., 1998) en werd recent ook geobserveerd bij eik-endofyten (Verkley, G.J.M., pers. comm.). Deze observatie kan een bijkomend belangrijk punt vormen in het ontstaan van gastheerspecificiteit bij bepaalde zwamsoorten.
In **hoofdstuk 8** onderzoeken we de respons van soorten op veranderende ecologische factoren in het Schelde-estuarium. We bemonsterden verschillende schorren langsheen de saliniteitsgradient (mesohalien tot zoet). Dit onderzoek werd uitgevoerd om een idee te krijgen

van de gevoeligheid van de soorten naar een breder spectrum van veranderende ecologische variabelen tussen gebieden. Multivariate analysetechnieken toonden dat de soortensamenstelling sterk gecorreleerd is met de overstromingsfrequentie en de saliniteit van het overstromingswater. De invloed van saliniteit op de soortengemeenschap wordt bediscussieerd en getest door een cultuurexperiment. Op basis van onze inventarisaties kunnen we de gevonden taxa opsplitsen in drie verschillende types: taxa die enkel voorkomen in brakwater omgeving, soorten die enkel voorkomen in een zoetwater habitat en taxa die tolerant zijn, en over de volledige saliniteitsgradiënt voorkomen. Voor elk van de types werden indicator soorten aangeduid. In twee locaties, een brakwater- en een zoetwaterschor, werd de verticale zonering van zwammen op riet onderzocht. We vonden in elk van de twee locaties een specifieke verticale zonering van soorten, met specifieke soorten voor een bepaalde saliniteit. De terrestrische soorten (niet beïnvloed door overstromingswater) tonen onderling een hogere similariteit in vergelijking met de (semi)aquatische soorten. Een aantal soorten kon in elk van beide locaties als indicator worden aangeduid voor een welbepaalde niche in de rietkraag. De invloed van overstromingshoogte op de verticale distributie van soorten op rietstengels wordt besproken. De diversiteit van zwammen in schorren is lager in vergelijking met biotopen die geen getij ondervinden. Dit is voornamelijk merkbaar in het lage aantal Agaricales, discomyceten en hyfomyceten.

3. Fungi en afbraakprocessen

Zwammen zijn algemeen gekende kolonisatoren van dood plantenmateriaal in verschillende milieus, maar specifieke informatie over de zwammengemeenschap op wetland planten, hun relatie tot het micromilieu en de link met het afbraakproces is weinig onderzocht (Gessner et al., 1997; Kuehn et al., 2004). Daarom onderzoeken we in **hoofdstuk 5** en **6** de diversiteit en successie van zwammen in de strooisellaag in combinatie met de zwambiomassa (ergosterol concentraties) en verschillende afbraakvariabelen. We analyseerden het stikstofverloop (en ook fosfor- en koolstof-patronen voor bladscheden en stengels in hoofdstuk 6) en de gewichtsafname tijdens het mineralisatieproces.
Onze resultaten tonen een ***grote fungale biomassa ontwikkeling in het dode riet zowel op het nog staande riet als in de strooisellaag***. Deze resultaten zijn in overeenstemming met andere studies voor zowel staande afbraak van riet (Gessner, 2001; Kuehn et al., 2004), afbraak in de strooisellaag (Findlay et al., 2002) en afbraak liggend in het water (Komínková et al., 2000). Deze grote biomassa is afhankelijk van de specifieke zwammengemeenschap in bepaalde microhabitats. Dit volgt uit de observatie dat een verandering in de positie van de rietstalen van een staande naar een liggende positie resulteerde in een massale extinctie waarin soorten elkaar vervangen en in een duidelijke afname van biomassa (zowel voor bladschijf als

voor bladschede). Deze transitie toont dat ***zwammengemeenschappen geadapteerd zijn aan bepaalde microhabitats*** en niet in staat zijn te sporuleren en te overleven in andere situaties (cf. Newell et al., 1989; Tanaka, 1991, 1993).

Elk plantendeel vertoonde een afbraaksnelheid vergelijkbaar met de snelste afbraakconstanten voor riet in andere studiegebieden (Gessner, 2000). Estuaria waar het plantenmateriaal regelmatig bevochtigd wordt, kunnen gunstige milieus zijn voor afbraak indien de overstromingsfrequentie niet té hoog is (Lee, 1990; Halupa & Howes, 1995) (zie hoofdstuk 1). Bladeren (schijven iets trager dan scheden) verloren 50% van hun initiële massa na 6 maanden afbraak. Stengels breken veel trager af. Hun afbraak is verwaarloosbaar gedurende de eerste 6 maand na afsterven en vervolgens deponeren in de strooisellaag. Na deze periode startte het afbraakproces. In vergelijking met bladeren worden stengels veel trager gekoloniseerd door zwammen. Deze vertraging in het afbraakproces is een belangrijk gegeven dat in experimentele studies in rekening moet gebracht worden.

In **hoofdstuk 6** hebben we getracht om de meest belangrijke ecologische variabelen aan te duiden die verantwoordelijk zijn voor het structureren van de zwammengemeenschap op riet tijdens de afbraak van stengels en bladscheden. Met behulp van indirecte en directe ordinatietechnieken onderzochten we onze set van gemeten milieuvariabelen. Uiteindelijk toonde het verloop van de celluloseconcentraties zich de meest gecorreleerde variabele met de soorten opeenvolging.

Samen met de verticale distributie van soorten op dode, rechtopstaande stengels onderzochten we ook het biomassapatroon van zwammen na één jaar rechtopstaande afbraak van stengels. Onze resultaten tonen dat ook stengels een hoge fungale biomassa bevatten in een rechtopstaande positie na een jaar afbraak in een rietkraag. Bovendien toonden de beide onderzochte stengels vergelijkbare verticale biomassapatronen. Mogelijke verklaringen voor deze verticale patronen kunnen zijn 1) plant specifieke karakteristieken (fysiologisch of anatomisch) en/of 2) milieu-omstandigheden (b.v. vochtigheid). Het belang van fungi gedurende de rechtopstaande afbraak van riet (Kuehn et al., 2004) wordt bevestigd door ons onderzoek. We vonden een hoge diversiteit (hoofdstuk 4) en een hoge biomassa (hoofdstuk 6) van fungi op staand dood riet. Onze resultaten tonen dat het nodig is om een verticaal patroon in de CO_2 uitstoot vanuit een rietkraag in rekening te brengen (cf. Kuehn et al., 2004).

Fungale biomassa kan een aanzienlijk aandeel van de nutriënten bevatten die aanwezig zijn in het dode riet. We schatten dat de aanwezige zwammen (biomassa) maximaal 34 ± 7% van de totale stikstof bevatten van de bladschijven wanneer ze nog vasthangen aan de rietplant. Eens de bladschijven afvallen, vermindert dit percentage tot ongeveer 12%. Onze gegevens

suggereren dat zwammen belangrijk zijn in het vasthouden van stikstof in de bladeren tijdens het afsterven van de bladeren aan de plant, maar hun belang is minder groot in stikstofretentie in de strooisellaag (hoofstuk 5).

In december, net na het afsterven van de bovengrondse delen van de rietplant, vonden we in de bladscheden dat 104 ± 14% van de totale stikstofpool en 94 ± 14% van de totale fosforpool geïntegreerd zijn in de fungale biomassa. Op hetzelfde moment waren de stengels nauwelijks gekoloniseerd door zwammen en bevatten de fungi minder dan 10% van de beschikbare nutriënten stikstof en fosfor. Gedurende het volledige afbraakproces werd het aandeel stikstof in het rietmateriaal aanwezig in zwambiomassa geschat op 64 ± 13% en 102 ± 15% respectievelijk voor bladscheden en stengels. Over dezelfde periode werd het aandeel van fosfor in het rietmateriaal aanwezig in zwambiomassa geschat op 37 ± 9% en 52 ± 15% respectievelijk voor bladscheden en stengels. Deze resultaten suggereren dat zwammen belangrijk zijn in het vasthouden van nutriënten in de bladscheden, maar niet in de stengels gedurende het afsterven. Verder zijn ze belangrijk in het weerhouden van nutriënten in de strooisellaag. Bovendien suggereren we de mogelijke actieve opname van nutriënten door stengelfungi uit het omringende milieu (Schelde water) (hoofdstuk 6).

In **hoofdstuk 7** zoemden we verder in op de mogelijke interacties van zwammen in vochtige ecosystemen en onderzochten we de mogelijke betrokkenheid van zwammen in het weerhouden van metalen in dood riet materiaal. Dit onderzoek is een relevant onderwerp omdat veel rietvegetaties (ook in het Schelde estuarium) gecontamineerd zijn met zware metalen en een beter inzicht in de impact van vegetaties en dood plantenmateriaal op metaal retentie in wetlands is nodig (Catallo, 1993). Om dit te onderzoeken bepaalden we de metaal-concentraties in afbrekende bladschijven, bladscheden en stengels. We stelden vast dat de metaalconcentraties in alle plantendelen toenamen in functie van de tijd. Deze accumulatie van metalen in plantenafval is mogelijks een zeer belangrijke parameter in het begrijpen van de metaaltransfer naar de basis van de voedselketen in schor-ecosystemen. Het onderzoek suggereert de ***mogelijks belangrijke impact van fungi op het weerhouden van metalen in de rietstengels en bladscheden***. Misschien is dit ook het geval voor bladschijven maar voor dit weefseltype was er een lage correlatie tussen metaalconcentraties en fungale biomassa. Vermoedelijk maskeerde een hoge passieve metaalopname en een contaminatie door sediment met geassocieerde metalen de mogelijke impact van fungi in de metaalretentie.

Hoofdstuk 9 is een samenvattend hoofdstuk over de ***vastgestelde en mogelijke impact die zwammen hebben op het Schelde ecosysteem***. Het belang van fungi in het estuarium laat zich voelen op verschillende vlakken. Ten eerste is er de ***hoge diversiteit*** van zwammen, 166 verschillende taxa waargenomen op riet in het Schelde estuarium. Ten tweede toonden we dat

deze zwammen een ***grote biomassa*** kunnen opbouwen in het substraat en we vonden dat de fungi tot 10% van de totale koolstof konden bevatten (b.v. in bladeren net voor deze afvallen). In dode rechtopstaande stengels en bladscheden vonden we dat zwammen de overgrote hoeveelheid aan ***nutriënten incorporeerden*** en over het algemeen meer dan de helft van de aanwezige nutriënten (N en P) in het plantenmateriaal van de strooisellaag. In de bladschijven is er ook een aanzienlijke retentie van stikstof zolang ze aan de plant hangen, maar in mindere mate eens deze terecht komen in de strooisellaag. Door extrapolatie kunnen we schatten dat in de rietkraag (exclusief strooisel) per m² ongeveer 45 g zwambiomassa aanwezig is in de bladeren (bladschede + schijf). Na één jaar afbraak schatten we dat er in de stengels ongeveer 10.8 en 11.2 g zwambiomassa aanwezig is per m² respectievelijk in de strooisellaag en in de nog rechtopstaande stengel. Het mogelijke belang van zwammen als voedselbron voor invertebraten en het mogelijke belang van zwammen voor de gastheerplant is kort besproken.

Referenties

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1972). Colonisation of *Phragmites communis* leaves by fungi. Nova Hedwigia 23: 113-124.

BENNER, R., MORAN, M.A. & HUDSON, R.E. (1986). Biogeochemical cycling of lignocellulosic carbon in marine and freshwater ecosystems: relative contributions of prokaryotes and eukaryotes. Limnol. Oceanogr. 31: 89-100.

CATALLO, W.J. (1993). Ecotoxicology and wetland ecosystems: current understanding and future needs. Environ. Toxicol. Chem. 12: 2209-2224.

CHRISTENSEN, M. (1989). A view of fungal ecology. Mycologia 81: 1-19.

COOKE, R.C. & RAYNER, A.D.M. (1984). Ecology of saprotrophic fungi. Longman Group Limited, London.

DIX, N.J. & WEBSTER, J. (1995). Fungal Ecology. Chapman & Hall, London.

FINDLAY, S.E.G., DYE, S. & KUEHN, K.A. (2002). Microbial growth and nitrogen retention in litter of *Phragmites australis* compared to *Typha angustifolia*. Wetlands 22: 616-625.

GESSNER, M.O. (2000). Breakdown and nutrient dynamics of submerged *Phragmites* shoots in the littoral zone of a temperate hardwater lake. Aquatic Bot. 66: 9-20.

GESSNER, M.O. (2001). Mass loss, fungal colonisation and nutrient dynamics of *Phragmites australis* leaves during senescence and early decay. Aquatic Bot. 69: 325-339.

GESSNER, M.O., THOMAS, M., JEAN-LOUIS, A.M. & CHAUVET, E. (1993). Stable successional patterns of aquatic hyphomycetes on leaves decaying in a summer cool stream. Mycol. Res. 97: 163-172.

GESSNER, R.V. (1977). Seasonal occurrence and distribution of fungi associated with *Spartina alterniflora* from a Rhode Island estuary. Mycologia 69: 477-491.

HALUPA, P.J. & HOWES, B.L. (1995). Effects of tidally mediated litter moisture content on decomposition of *Spartina alterniflora* and *S. patens*. Mar. Biol. 123: 379-391.

HAWKSWORTH, D.L. (1991). The fungal dimension of biodiversity: magnitude, significance and conservation. Mycol. Res. 95: 641-655.

HAWKSWORTH, D.L. (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. 105: 1422-1432.

KOMÍNKOVÁ, D., KUEHN, K.A., BÜSING, N., STEINER, D. & GESSNER, M.O. (2000). Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of a large lake. Aquat. Microb. Ecol. 22: 271-282.

KOWALSKI, T. & KEHR, R.D. (1996). Fungal endophytes of living branch bases in several European tree species. *In* REDLIN, S.C. & CARRIS, L.M. (EDS.) Endophytic Fungi in Grasses and Woody Plants. American Phytopathological Press, St.-Paul, MN. 67-86.

KUEHN, K.A., STEINER, D. & GESSNER, M.O. (2004). Diel mineralization patterns of standing-dead plant litter: implications for CO_2 flux from wetlands. Ecology 85: 2504-2518.

LEE,S.Y. (1990). Net aerial primary productivity, litter production and decomposition of the reed *Phragmites communis* in a nature reserve in Hong Kong: management implications. Mar. Ecol. Prog. Ser. 66: 161-173.

MITSCH, W.J. & GOSSELINK, J.G. (2000). Wetlands (3rd edition). Van Nostrand Reinhold, New York.

NEWELL, S.Y., FALLON, R.D. & MILLER, J.D. (1989). Decomposition and microbial dynamics for standing, naturally positioned leaves of the salt-marsh grass *Spartina alterniflora*. Mar. Biol. 101: 471-481.

PARBERY, D.G. (1996). Trophism and ecology of fungi associated with plants. Biological Reviews of the Cambridge Phylosophical Society 71: 473-527.

PETRINI, O. (1996). Ecological and physiological aspect of host specificity in endophytic fungi. *In* REDLIN, S.C. & CARRIS, L.M. (EDS.) Endophytic Fungi in Grasses and Woody Plants. American Phytopathological Press, St.-Paul, MN. 87-100.

SAIKKONEN, K., FAETH, S.H., HELANDER, M. & SULLIVAN, T.J. (1998). Fungal endophytes: a continuum of interactions with host plants. Rev. Ecol. Syst. 29: 319-343.

SIEBER, T.N. (1985). Endophytische Pilze von Winterweizen (*Triticum aestivum* L.). Dissertation ETH Nr. 7725. Swiss Federal Institute of Technology, Zürich, Switzerland.

SINSABAUGH, R.L. & FINDLAY, S. (1995). Microbial production, enzyme activity, and carbon turnover in surface sediments of the Hudson River estuary. Microb. Ecol. 30: 127-141.

SWIFT, M.J. & ANDERSON, J.M. (1993). Biodiversity and ecosytem function in agricultural systems. *In* SCHULTZE, E.-D. & MOONEY, H.A. (EDS.) Biodiversity and Ecosystem Function. Ecological Studies 99. Springer-Verlag, Berlin. 15-41.

TANAKA, Y. (1991). Microbial decomposition of reed (*Phragmites communis*) leaves in a saline lake. Hydrobiologia 220: 119-129.

TANAKA, Y. (1993). Aerobic cellulolytic bacterial-flora associated with decomposing *Phragmites* leaf-litter in a seawater lake. Hydrobiologia 263: 145-154

WEBSTER, J. (1956). Succession of fungi on decaying cocksfoot culms. I. J. Ecol. 44: 517-544.

WONG, M.K.M. & HYDE, K.D. (2001). Diversity of fungi on six species of Gramineae and one species of Cyperaceae in Hong Kong. Mycol. Res. 105: 1485-1491.

YADAV, A.S. & MADELIN, M.F. (1968). The ecology of microfungi on decaying stems of *Urtica dioica*. Trans. Brit. Mycol. Soc. 51: 249-259.

ZHOU, D. & HYDE, K.D. (2001). Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi. Mycol. Res. 105: 1449-1457.

Appendix

Taxonomy of species

Introduction

- Visual screening of the reed fragments for fungi was always done at magnification 180×
- Reed fragments were wetted in water to make subepidermal sporulation structures more visible
- Nearly all observations and measurements were performed on freshly collected, vital fungi. Few species are figured in a dead state from herbarium specimen (this is indicated).
- Studied specimens refer to the key collections used to make the description and illustrations. For some taxa dried cultures are also deposited in the herbarium. All herbaria collections can be searched in the database
- All exsiccata are deposited in GENT and are indicated by a VR-number: See WWW (Http://biology.ugent.be/reedfungi). GVR-numbers are record numbers referring to my personal database and are not necessarily associated with a herbarium collection. Several taxa were mounted as permanent slides according to the method proposed by Volkmann-Kohlmeyer & Kohlmeyer (1996)
- Ecological data are presented (substrate, spatial occurrence and temporal occurrence). Phenological data are only given if taxa were recovered several times, providing more reliable information about their occurrence (see also Chapter 4, 5, 6 and 9)
- For the descriptions, if possible, 10 spores were measured for the range of spore size. In some cases the spore size range was investigated for twenty spores and an average value is included in the size range; other measurements are generally based on 5 measurements (basidia, asci, cystidia, paraphyses etc.). Some species (those which got a *nom. prov.* and are considered new to science) were studied in more detail and all structures were measured at least 10 times
- Nearly all taxa have illustrations (drawings and/or digital pictures). See Http://biology.ugent.be/reedfungi

Index

Table 1. The species list (in the same order the descriptions are presented) with the groups being (B: basidiomycetes; Bh: heterobasidiomycetes; BTe: teliomycetes (rusts and smut fungi); Ad: discomycetes (Ascomycota); Ap: pyrenomycetes (Ascomycota); C: coelomycetes; H: hyphomycetes and O: oomycetes.

Descriptions	
Group	**Taxa**
B	Basidiomycete, steriel mycelium (Not discussed)
B	*Bolbitius* sp.
B	*Coprinus kubickae* Pilát & Svrček
B	*Coprinus urticicola* (Berk. & Broome) Buller
B	*Maireina monacha* (Speg.) W.B. Cooke
B	*Marasmius limosus* Boud. & Quél.
B	*Melanotus phillipsii* (Berk. & Broome) Singer
B	*Panaeolus fimicola* (Pers.) Gillet
B	*Pellidiscus pallidus* (Berk. & Broome) Donk
B	*Pholiota pityrodes* (F. Brig.) Holec
B	*Psathyrella almerensis* Kits van Wav.
B	*Psathyrella typhae* (Kalchbr.) A. Pearson & Dennis
B	*Resinomycena saccharifera* (Berk. & Broome) Redhead
B	*Tubaria furfuracea* f. *romagnesiana* (Arnolds) Volders
Bh	*Tetragoniomyces uliginosus* (P. Karst.) Oberw. & Bandoni
Bh	*Tremella spicifera* Van Ryck., Van de Put & P. Roberts
Bh	*Trimorphomyces papillionaceus* Bandoni & Oberw.
BTe	*Puccinia magnusiana* Körn.
BTe	*Puccinia phragmitis* (Schumach.) Körn.
BTe	*Sporobolomyces* sp.
BTe	*Ustilago grandis* Fr.
Ad	*Albotricha acutipilosa* (P. Karst.) Raitv.
Ad	*Ascobolus stercorarius* (Bull.) J. Schröt.
Ad	*Bisporella scolochloae* (De Not.) Spooner

Descriptions	
Group	**Taxa**
Ad	*Cistella fugiens* (Phill. ex Bucknall) Matheis
Ad	*Cryptodiscus* sp.
Ad	*Lachnum controversum* (Cooke) Rehm
Ad	*Lachnum tenuissimum* (Quél.) Korf & Zhuang
Ad	*Laetinaevia* sp.
Ad	*Lophodermium arundinaceum* (Schrad.) Chevall.
Ad	*Mollisia* cf. *palustris* (Roberge ex Desm.) P. Karst.
Ad	*Mollisia hydrophila* (P. Karst.) Sacc.
Ad	*Mollisia retincola* (Rabenh.) P. Karst.
Ad	*Orbilia septispora* Baral
Ad	*Phragmiticola rhopalospermum* (Kirschst.) Sherwood
Ad	*Psilachnum eburneum* (Roberge ex Desm.) Baral
Ad	*Rutstroemia lindaviana* (Kirschst.) Dennis
Ad	*Scutellinia* cf. *umbrarum* (Fr.: Fr.) Lambotte
Ad	*Stictis* sp.
Ad	*Stictis* sp. II
Ad	*Stictis stellata* Wallr.
Ad	*Trichopeziza albotestacea* (Desm.) Sacc.
Ad	*Unguiculella eurotioides* (P. Karst.) Nannf.
Ap	*Acrocordiopsis* sp.
Ap	*Anthostomella punctulata* (Roberge) Sacc.
Ap	*Anthostomella tomicoides* Sacc.
Ap	*Apiospora montagnei* Sacc.
Ap	Asco sp. incertae sedis II
Ap	Asco sp. incertae sedis III
Ap	*Botryosphaeria festucae* (Lib.) Arx & E. Müll.
Ap	*Buergenerula typhae* (Fabre) Arx
Ap	*Ceratosphaeria* sp.
Ap	*Cercophora* sp.
Ap	*Claviceps microcephala* (Wallr.) Tul.
Ap	*Didymella glacialis* Rehm
Ap	*Didymella proximella* (P. Karst.) Sacc.
Ap	*Didymella* sp.
Ap	*Discostroma* sp.
Ap	*Gibberella zeae* (Schwein.) Petch
Ap	*Halosphaeria hamata* (Höhnk) Kohlm.
Ap	*Hydropisphaera arenula* (Berk. & Broome) Rossman & Samuels
Ap	*Keissleriella culmifida* (P. Karst.) Bose
Ap	*Keissleriella linearis* E. Müll.
Ap	*Lasiosphaeria* sp.
Ap	*Lewia infectoria* (Fuckel) M.E. Barr & E.G. Simmons
Ap	*Lophiostoma appendiculatum* Fuckel
Ap	*Lophiostoma arundinis* (Pers.) Ces. & De Not. s.l.
Ap	*Lophiostoma caudatum* Fabre
Ap	*Lophiostoma semiliberum* (Desm.) Ces. & De Not.

Descriptions	
Group	**Taxa**
Ap	*Magnisphaera spartinae* (E.B.G. Jones) J. Campb., J.L. Anderson & Shearer
Ap	*Massarina aquatica* J. Webster
Ap	*Massarina arundinacea* (Sowerby: Fr.) Leuchtm.
Ap	*Massarina fluviatilis* Aptroot & Van Ryck.
Ap	*Massarina* sp. III
Ap	*Massariosphaeria grandispora* (Sacc.) Leuchtm.
Ap	*Massariosphaeria mosana* (Mouton) Leuchtm.
Ap	*Massariosphaeria palustris* (E. Müll.) Leuchtm.
Ap	*Massariosphaeria* sp.
Ap	*Massariosphaeria typhicola* (P. Karst.) Leuchtm.
Ap	*Metasphaeria coccodes* (P. Karst.) Sacc. sensu Munk
Ap	*Microthyrium nigroannulatum* J. Webster
Ap	*Morenoina phragmitidis* J.P. Ellis
Ap	*Mycosphaerella lineolata* (Roberge ex Desm.) J. Schröt.
Ap	*Ophioceras dolichostomum* (Berk. & M.A. Curtis) Sacc.
Ap	*Paraphaeosphaeria michotii* (Westend.) O.E. Erikss.
Ap	*Passeriniella obiones* (P. Crouan & H. Crouan) K.D. Hyde & Mouzouras
Ap	*Phaeosphaeria culmorum* (Auersw.) Leuchtm.
Ap	*Phaeosphaeria eustoma* (Fuckel) L. Holm s.l.
Ap	*Phaeosphaeria fuckelii* (Niessl) L. Holm
Ap	*Phaeosphaeria graminis* (Fuckel) L. Holm
Ap	*Phaeosphaeria luctuosa* (Niessl) Otani & Mikawa
Ap	*Phaeosphaeria nigrans* (Roberge in Desm.) L. Holm
Ap	*Phaeosphaeria phragmiticola* Leuchtm.
Ap	*Phaeosphaeria phragmitis* (Hollós) Leuchtm.
Ap	*Phaeosphaeria pontiformis* (Fuckel) Leuchtm.
Ap	*Phaeosphaeria* sp. I
Ap	*Phaeosphaeria* sp. II
Ap	*Phaeosphaeria* sp. III
Ap	*Phaeosphaeria* sp. IV
Ap	*Phaeosphaeria vagans* (Niessl) O.E. Erikss.
Ap	*Phomatospora berkeleyi* Sacc.
Ap	*Phomatospora dinemasporium* J. Webster
Ap	*Phomatospora phragmiticola* O.K. Poon & K.D. Hyde
Ap	*Phomatospora* sp. I
Ap	*Phomatospora* sp. II
Ap	*Phomatospora* sp. III
Ap	*Phomatospora* sp. IV
Ap	*Pleospora abscondita* Sacc. & Roum.
Ap	*Pleospora herbarum* (Pers.) Rabenh. ex Ces. & De Not.
Ap	*Podospora* sp. I
Ap	*Podospora* sp. II
Ap	*Pseudohalonectria adversaria* Shearer
Ap	*Pseudohalonectria* aff. *falcata* Shearer
Ap	*Rosellinia musispora* Van Ryck. & Verbeken
Ap	*Savoryella lignicola* E.B.G. Jones & R.A. Eaton
Ap	*Schizothecium hispidulum* (Speg.) N. Lundq.

Descriptions	
Group	**Taxa**
Ap	*Schizothecium* sp.
Ap	*Sphaerodes* sp.
Ap	*Wettsteinina* cf. *niesslii*
Ap	*Wettsteinina moniliformis* Van Ryck. & Aptroot
C	*Aposphaeria* sp.
C	*Ascochyta* cf. *arundinariae* Tassi
C	*Ascochyta* cf. *leptospora* (Trail) Hara
C	*Camarosporium feurichii* Henn.
C	*Camarosporium* sp.
C	Coelomycete sp. I
C	Coelomycete sp. II
C	Coelomycete sp. III
C	Coelomycete sp. IV
C	Coelomycete sp. V
C	Coelomycete sp. VI
C	*Coniothyrium* sp.
C	*Cytoplacosphaeria rimosa* (Oudem.) Petrak s.l.
C	*Dinemasporium graminum* (Lib.) Lév.
C	*Diplodina* sp.
C	*Hendersonia* aff. *culmiseda* Sacc.
C	*Hendersonia culmiseda* Sacc.
C	*Hendersonia* sp. I
C	*Hendersonia* sp. II
C	*Hendersonia* sp. III
C	*Microdiplodia* sp.
C	*Microsphaeropsis arundinis* (J. Ahmad) B. Sutton
C	*Microsphaeropsis* sp. I
C	*Microsphaeropsis* sp. II
C	*Neottiosporina australiensis* B. Sutton & Alcorn
C	*Phaeoseptoria* sp.
C	*Phialophorophoma* sp.
C	*Phoma* sp. I
C	*Phoma* sp. II
C	*Phoma* sp. IIa
C	*Phoma* sp. III
C	*Phoma* sp. IV
C	*Pseudorobillarda phragmitis* (Cunnell) M. Morelet
C	*Pseudorobillarda* sp.
C	*Pseudoseptoria donacis* (Pass.) B. Sutton
C	*Septoria* sp.
C	*Septoriella phragmitis* Oudem.
C	*Septoriella* sp(p).
C	*Sphaerellopsis filum* (Biv. ex Fr.) B. Sutton
C	*Stagonospora cylindrica* Cunnell
C	*Stagonospora elegans* (Berk.) Sacc. & Traverso

Descriptions	
Group	**Taxa**
C	*Stagonospora* incertae sedis I
C	*Stagonospora* incertae sedis II
C	*Stagonospora macropycnidia* Cunnell
C	*Stagonospora* sp. I
C	*Stagonospora* sp. II
C	*Stagonospora* sp. III
C	*Stagonospora vexata* Sacc. sensu Diedicke
H	*Alternaria alternata* (Fr.) Keissl.
H	*Arthrinium phaeospermum* (Corda) M.B. Ellis
H	*Arthrinium* state of *Apiospora montagnei* Sacc.
H	*Arthrobotrys* sp.
H	*Bactrodesmium atrum* M.B. Ellis
H	*Cerebella andropogonis* Ces.
H	*Chrysosporium* sp.
H	*Cladosporium* sp(p).
H	*Cochliobolus* cf. *australiensis* (Tsuda & Ueyama) Alcorn
H	*Corynespora* sp.
H	*Cylindrocarpon* sp.
H	*Dactylaria* sp.
H	*Deightoniella roumeguerei* (Cavara) Constant.
H	*Dictyochaeta simplex* (S. Hughes & W.B. Kendr.) Hol.- Jech.
H	*Dictyosporium oblongum* (Fuckel) S. Hughes
H	*Drechslera biseptata* (Sacc. & Roum.) M.J. Richardson & E.M. Fraser
H	*Epicoccum purpurascens* Ehrenb. & Schltdl.
H	*Fusarium* sp. I
H	*Fusarium* sp. II
H	*Fusarium* sp III
H	*Helicomyces tenuis* Speg.
H	*Helicosporium phragmitis* Höhn.
H	*Hymenella arundinis* Fr.
H	Hyphomycete sp. I
H	Hyphomycete sp. II
H	Hyphomycete sp. III
H	Hyphomycete sp. IV
H	Hyphomycete sp. V
H	Hyphomycete sp. VI
H	*Monacrosporium* sp.
H	*Monodictys* cf. *putredinis* (Wallr.) S. Hughes
H	*Myrothecium cinctum* (Corda) Sacc.
H	*Myrothecium masonii* M.C. Tulloch
H	*Penicillium* sp.
H	*Periconia cookei* E.W. Mason & M.B. Ellis
H	*Periconia digitata* (Cooke) Sacc.
H	*Periconia glyceriicola* E.W. Mason & M.B. Ellis
H	*Periconia hispidula* (Pers.) E.W. Mason & M.B. Ellis
H	*Periconia igniaria* E.W. Mason & M.B. Ellis
H	*Periconia minutissima* Corda

Descriptions	
Group	**Taxa**
H	*Rhinocladiella* sp.
H	*Stachybotrys atra* Corda
H	*Stachybotrys bisbyi* (Sriniv.) G.L. Barron
H	*Torula herbarum* (Pers.) Link ex S.F. Gray
H	*Trichoderma koningii* Oudem.
H	*Verticillium* sp.
O	*Saprolegnia* sp. (not discussed)

Taxonomic descriptions

Basidiomycetes (incl. heterobasidiomycetes)

Cf. ***Bolbitius*** sp.

Pileus 2-2.5 cm diam., first conical later spreading, with umbo, light ochraceous-brown, strongly striate, darker at the centre, hygrophanous; margin slightly fibrillated. Stipe 45-60 x 4 mm, tough, cylindrical, thickened at the base. Lamellae free, light brown, ventricose; edge smooth or slightly serrulate. Spore print ochre-brown. Spores 8.8-10.4 x 5.6-6.4 μm, ellipsoid, light-brown, thick-walled, smooth, with one or two guttule(s); germ pore inconspicuous. Basidia 20-25 x 10 μm, clavate, 4-spored, sometimes with granular content; sterigmata pointed. Pleurocystidia absent but numerous sterile (basidiole-like) cells present in between the basidia. Cheilocystidia 38-47 x 14-18 μm, abundant, utriform, sometimes with small projections on the top. Pileipellis cellular composed of subglobose cells, ± 20 μm diam.

Ecology: on stem and leaf litter.

Studied specimens: VR17

Remarks

1. Macroscopically this fungus was assigned to *Bolbitius* because of the fragile, striate, hygrophanous appearance of the cap, the free lamellae, spore colour and the ephemeral basidiocarp. Microscopically the genus is characterized by the cellular pileipellis, the thick walled, smooth spores, with (mostly) a germ pore, abundant cheilocystidia, and the absence of clear pleurocystidia while multiple sterile cells present between the basidia (brachycystidia) (Watling, 1982).
2. The species was collected only once and basidiocarp colours in fresh state were not well described, spores are rather small compared to other representatives of the genus.

This makes the determination uncertain even at genus level. The herbarium collection holds only one well preserved fruit body.

Coprinus kubickae Pilát & Svrček, Česká Mykol. 21 : 142 (1967).

Pileus 3-7 mm diam. when expanded, at first whitish later brownish-grey; veil not abundant, flocculose. Stipe up to 20 mm long, white; base swollen, tomentose; basal hyphae with clamp connection. Lamellae free, about 30, first white then blackish. Spore print unknown. Spores 7.5-9.5 x 7.5-8 μm, subglobose to broadly ellipsoid, smooth, chestnut brown, with pale germ pore and apiculus; germ pore excentric. Basidia 22-30 μm, 4-spored. Pleurocystidia 55-65 x 18-24 μm, utriform, subcylindrical. Cheilocystidia not observed. Veil hyphae, 4-8 μm wide, diverticulate, thin-walled, hyaline up to brownish.

Ecology: on reed litter.
Phenology: 1 – 2 – 3 – **4 – 5 – 6 – 7 – 8** – 9 – 10 – 11 – 12

Studied specimens: VR716

Remarks
A common species in humid conditions during early summer. One of the few Agaricales occurring on brackish tidal marshes. Species forms basidiocarps on reed litter on the sediment or in aquatic reed stands on floating reeds or on standing dead reed parts (mostly leaf sheaths) just above water surface. This species was also isolated from wood lying in the water and is probably a widespread and common species in wetlands (Anastasiou, 1967; Uljé & Noordeloos, 1997). This species is well characterized by the small basidiocarps, the habitat, the subglobose spores and the presence of clamp connections (Uljé & Noordeloos, 1997). Another common *Coprinus* species from wetlands is *C. friesii* which was not found during this study but is characterized by ovoid-rhomboid spores and strongly thick-walled, not or slightly coloured veil (Bas, 1971; Uljé & Noordeloos, 1997).

Coprinus urticicola (Berk. & Broome) Buller, Trans. Brit. Mycol. Soc. 5: 485 (1917).

Pileus up to 11 mm diam. when expanded, at first conical, white; veil white. Stipe 20-30 x 0.5 mm, white; base of stipe enlarged and tomentose. Lamellae free, first white then blackish. Spore print dark brownish. Spores 5.5-9 x 4.5-6 μm, ellipsoid, pale brown, smooth, with germ pore. Basidia 13-16 x 6 μm, 4-spored. Pleurocystidia not observed. Cheilocystidia 28-30 x 12-14 μm, few observed, thin-walled. Veil hyphae 5-9 μm wide, thin-walled, without clamps, hyaline, diverticulate.

Ecology: on stem litter.

Studied specimens: VR315

Remarks
Rather common species in *Phragmites* stands. The species can be recognized by the small, pure white basidiocarps with woolly scales of veil. Microscopically the often conical, ellipsoid or ovoid, pale coloured spores and thin-walled elements of veil are good characters to identify *C. urticicola* (Uljé & Noordeloos, 1997).

Maireina monacha (Speg.) W.B. Cooke, Beih. Sydowia 4: 90 (1961).
Syn. Merismodes bresadolae (Grélet) Singer

See Van Ryckegem & Dam (1999).

Basidiomes cyphelloid, plane till cupulate discs up to 1.5 mm diam., cupulate when young stretched when mature, centrally attached, without stipe or with very short stipe (up to 0.2 mm), solitary or gregarious (3-4 basidiocarps), if gregarious with tendency to fuse. Hymenium white to whitish-buff, felty, drying buff in the centre. Outside light brown, hairy. Margin with lose woolly hairs, curved inwards. Hairs up to 400 µm long, 4-6 µm wide, brownish, sometimes hyaline, near the base of the basidiocarps tobacco-brown, cylindrical, rounded tip, not conspicuously narrowed to the base, entirely thick-walled, with crystals; crystals variable in size, sometimes absent near the top (in 5% KOH the hairs remain brown, do not swell and the crystals remain visible). Spores 11.5-13-15 x 4.5-6.5-9.1 µm, ellipsoid, sometimes curved, thin-walled, often with 1-(2) large guttules, smooth, non-amyloid, no colour reaction in KOH; apiculus conspicuous up to 1.6 µm long; germination often after 24 hours in water; germ tube at the adaxial side just above the apiculus. Basidia 50-74 x 8-10 µm, clavate; sterigma 6.5-11 µm long. Cystidia absent. Trama and subhymenium tightly interwoven hyphae; hyphae torulose, with abundant oil drops, with clamp connections (also many false clamp connections), not dextrinoid (or at most some very light yellow coloration), not metachromatic, with small, hyaline crystals, mainly in the subhymenium.

Ecology: on stems.
Phenology: **1 – 2 – 3 – 4 – 5 – 6 – 7** – 8 – **9 – 10 – 11 – 12**

Studied specimens: VR36

Remarks
The ecology and phenology of this species is mysterious: the species occurs on a wide range of hosts (Cooke, 1961) and was found during our inventarisation on *Phragmites* almost in all months of the year without clear preference for a specific period. This species was always found on reeds growing in brackish conditions specifically on dead stems with more than one

year standing decay or in the litter layer on old dead stems (however the reader should keep in mind that the brackish habitat was the most visited, so strong predictions concerning habitat preference are inappropriate). See also Heller & Keizer (2004).
There exist two forms of this species: one with obvious clamp connections on the basidia and one without clamp connections (all our collections) (pers. comm. 4/01/03 Carlos Enrique Hermosilla). It is thus not excluded that more than one species is involved.

Marasmius limosus Boud. & Quél., Bull. Soc. Bot. Fr. (323): 27 (1877).
No personal illustrations

Pileus 0.75-2.5 mm diam., greyish brown, striate, hemispherical, then convex, central a small umbo. Lamellae pale greyish, white, adnexed to a wide collarium. Stipe 10-65 x 0.2 mm, paler near the top, black at the base, filiform, glabrous, tough. Spore print unknown. Spores 7.5-10 µm x 5-6 µm, broadly ellipsoid, often curved, hyaline. Basidia (not observed) but 17-22 x 6-8 µm, 2(4) spored, clavate, clamped according to Antonín & Noordeloos (1993). Pleurocystidia clavate, apically diverticulate. Cheilocystidia up to 20 x 9 µm, clavate, utriform, diverticulate at the top with hyaline projections.

Ecology: on stem, leaf and leaf sheath.
Phenology: 1 – **2 – 3 – 4 – 5** – 6 – 7 – **8 – 9 – 10 – 11 – 12**

Studied specimens: VR20

Remarks
Very common species in non-tidal wetlands, in tightly packed litter, the basidiocarps collapse very quickly in open air conditions. Most records are from August till October but occasionally found during early spring. The species occurs on all kinds of monocotyledons in wetland habitats. Additional description in Antonín & Noordeloos (1993).

Melanotus phillipsii (Berk. & Broome) Singer, Beih. Sydowia 7: 84 (1973).

This species was collected once on *Phragmites* and illustrated but not described in detail. For a description see Breitenbach & Kränzlin part 4: 328 (1995) and in Flora Agaricina Neerlandica part 4: 52 (as *Psilocybe phillipsii* (Berk. & Broome) Vellinga & Noordel.).

Studied specimens: VR25

Panaeolus fimicola (Pers.) Gillet, Hyménomycètes: 621 (1874).

Pileus 8-15 mm diam., parabolic, striate, light-brown till rust coloured, surface not flocculose, smooth, hygrophanous, cream-coloured at the margin till darker brown to the centre; margin

smooth. Stipe 70-90 x 3-4 mm, cylindrical, buff-brown to cream coloured; base tomentose, darker than top, striate, no volva remnants, no rhizomorphs. Lamellae adnate to almost free, brown with white edge. Spore print brownish-black. Spores 11.4-*12*-13 x 5.6-*6.4*-7.2 µm, dark brown, maturing irregularly, ellipsoid-fusiform, sometimes slightly curved, with conspicuous apical germ pore. Basidia ± 20 x 7 µm, 4-spored, clavate. Pleurocystidia absent. Cheilocystidia 25-40 x 7-12 µm, utriform, irregularly undulating at the top, top enlarged. Pileocystidia comparable to cheilocystidia but larger.

Ecology: on almost completely decomposed remnant of reeds (leaves and stems) in a wet habitat.

Studied specimens: VR28

Remarks
A species normally occurring in grasslands, roadsides and occasionally on dung. The shape of the spores and the cheilocystidia are typically *P. fimicola* although the weakly adnate lamellae are atypical and argue for *P. acuminatus* (Kits van Waveren, 1979).

Pellidiscus pallidus (Berk. & Broome) Donk, Persoonia 1: 90 (1959).

Basidiome cyphelloid, 0.5-1 mm diam., flattened, loosely adnate, with upright margin, white, very thin; margin hairy, floculose. Hymenium whitish, 25-30 µm thick. Subiculum absent but basidiocarps are interwoven by lose, hyaline hyphae superficially on the substrate. Hairs up to 100 x 3 µm, hyaline, smooth, septated, thin-walled, flexible, narrowed on the top. Hyphae without clamp connections, 3 µm wide, hyaline. Spores 7-9.5 x 3-4.5 µm, hyaline, ellipsoid, smooth, with a small apiculus. Basidia 17-22 x 6-7 µm, clavate, 4-spored; sterigma up to 3 µm long, spiny. Basidioles abundant. Cystidia absent.

Ecology: we found it on leaf blades of *P. australis*; however it is also found on other substrates in several conditions (Dam, N., pers. comm.).

Studied specimens: VR265

Remarks
The (overmature?) spores should become light brownish and verrucose (Reid, 1963).

Pholiota pityrodes (F. Brig.) Holec, Libri Botanici 20: 84 (2001).

Bas. *Agaricus pityrodes* F. Brig. (1844).

Syn. *Phaeomarasmius pityrodes* (F. Brig.) M.M. Moser, Blätterpilze- und Bauchpilze, ed. 1: 146 (1953); invalid combination. (For complete list of synonyms see Holec, 2001)

Macroscopical description: fresh material not observed. Basidiocarps tiny, pileus 0.5-3 cm diam., dry, straw-yellow, ochre-brown at centre, with appressed, rusty scales.
Spores 5.6-*6.7*-7.4 x 2.8-*3.2*-3.6 µm, broadly cylindrical, subphaseoliform in lateral view, with inconspicuous germ pore, almost hyaline. Basidia not observed. Pleurocystidia abundant 30-45 x 10-15 µm, with refractive inclusions (chrysocystidia), utriform, tapered at the top, abundant. Cheilocystidia 30-33 x 4-7 µm, utriform, sometimes capitate, without refractive inclusions. Pileipellis with scales: consisting of strongly incrusted, thick-walled cells.

Ecology: on basal parts of standing dead stems and stems buried in the soil.

Studied specimens: Melsen (Merelbeke), De Putten, IFBL D3.42.14, 4/09/98 Walleyn R. 1390 (GENT).

Remarks
This species is different from *P. conissans* (Fr.) M.M. Moser [= *P. graminis* (Quél.) Singer] by the hyaline spores and the conspicuous incrusted pileus scales (Holec, 2001; Jacobsson, 1997).

From the literature study it is suspected that *Naucoria typhicola* Henn., Verhandl. des Bot. Ver. Prov. Brandenburg, 33: 11 (1891) at least sensu Schweers (1941) and Jansen (1969) [= *Conocybe typhicola* (Henn.) Schweers (1941)] is a further synonym of *P. pityrodes*. (For descriptions of *N. typhicola* see Schweers 1941, Jansen, 1969). *N. typhicola* was synonymized by Kits van Waveren (1985) with *Psathyrella typhae*. None of the above authors saw the type specimen. To elucidate this problem the Hennings type specimen should be checked.

Psathyrella almerensis Kits van Wav., Persoonia Suppl. Vol. 2: 280 (1985).

Pileus 10-20 mm diam., conical-convex, striate, ochre to red brown, smooth, hygrophanous, with darker centre. Stipe 30-45 x 1.5-2.5 mm, cylindrical, white, slightly swollen at the base, hollow, pruinose, whitish apex. Lamellae buff, broadly adnate, slightly ventricose; edge white. Spore print dark brown to blackish. Spores (9) 9.6-10.8 (11.5) x 5-7 µm, light brown, ellipsoid-ovoid, adaxial slightly flattened, smooth, with germ pore, with 1-4 guttules, with small apiculus. Basidia 23-30 x 9-12 µm, clavate, 4-spored; sterigma 3-5 µm. Pleurocystidia not abundant, 35-50 x 9-15, utriform to lageniform. Cheilocystidia 28-50 x 10-15 µm, abundant, subutriform to lageniform, rather variable in shape.

Ecology: On dead leaves and stems.
Phenology: 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – **9 – 10** – 11 – 12

Studied specimens: VR16, VR29, VR33 VR49, VR724.

Remarks

Species occurring in wetland habitats on remnants of *Phragmites*, *Typha*, *Cirsium*. The species resembles very much *P. typhae* and *P. basii*. The latter species seems indistinguishable from *P. almerensis* with the characteristics given by Kits van Waveren (1985) (smaller basidiocarps, smaller spores (8-10 x 5.5-6 μm) and pleurocystidia being more lageniform). Studying different collections shows that pleurocystidia shape can vary within one collection from utriform to lageniform. A re-examination of the type-collections could clear out the differences between those two species. See also De Meulder (1999a).
From our data *P. almerensis* seems more common on *Phragmites* than *P. typhae* as the latter species was only once collected on *Phragmites australis*.

Psathyrella typhae (Kalchbr.) A. Pearson & Dennis, Trans. Brit. Mycol. Soc. 31: 185 (1948).

This species was only once collected on *Phragmites* and is distinct from *P. almerensis* by the absence of a germ pore and absence of pleurocystidia (Kits van Waveren, 1985).

Studied specimens: Belgium, Molenkreek, IFBL B2.57.22, 31/08/98, No herbarium collection could be made (nematodes destroyed material).

Resinomycena saccharifera (Berk. & Broome) Redhead, *Canad. J. Bot.* **62**(9): 1850 (1984).

This species was collected once (VR806) on *Phragmites* but not described in detail.
See Antonín & Noordeloos, (2004) for a description and illustrations of the species.

Tubaria furfuracea f. ***romagnesiana*** (Arnolds) Volders, Sterbeeckia 21/22: 19 (2002).

Pileus 1-4 cm diam., convex to flattened, smooth, ochre-brown; margin of young basidiocarps slightly lighter and clearly striate; no veil. Stipe up to 40 x 4 mm, solid, ochre, with whitish stripes and small white scales, at the base tomentose white. Lamellae broadly adnate, slightly paler than pileus; trama with hyphae 7-9 μm wide. Spore print unknown. Spores 7-8.5 x 4-5 μm, ellipsoid, broadly rounded, very light brown, without germ pore, with clear apiculus. Basidia 20-22 x 7-8 μm, broadly cylindrical to clavate, 4-spored. Cheilocystidia 30-40 x 4-6 μm, often slightly subcapitate, enlarged to 8-7 μm.

Ecology: on rather decomposed stems at the margin of reed stand.

Studied specimens: VR172

Remarks

The species complex of *Tubaria furfuracea*, *T. hiemalis* and *T. romagnesiana* was critically revised by Volders (2002) bringing the latter two species to rank of respectively variety and formae. *T. furfuracea* f. *romagnesiana* has typically small spores and a hymenophoral trama not wider than 15 µm.

Heterobasidiomycetes

Tetragoniomyces uliginosus (P. Karst.) Oberw. & Bandoni, Canad. J. Bot. 59: 1035 (1981).

Basidiomes mycoparasitical on a sclerotium. Sclerotium 1-5 mm broad, up to 4 mm high, cerebriform, orange to organe-brown, soft, like gelatine, shining, sometimes hollow. Hyphae 3-5.5 µm wide, with clamp connections, hyaline, often filled with guttules, with haustoria. Sclerotium(host)-cells variable in shape and size (distorted, swollen), branched, without visible clamp connections, constricted at septa. Probasidia 10-12 µm diam. subglobose to ellipsoid, with basal clamp connection, hyaline, at first two-celled, later a four-celled chiastobasidia. Basidia 10-13 (15) µm diam., chiastobasidia, spherical to ovoid, verrucose (see remark), thick-walled, soon dehiscent, full with small guttules.

Ecology: on stems, leaves and leaf sheaths, also found on *Carex* leaves.
Phenology: 1 – 2 – 3 – 4 – 5 – 6 – 7 – **8 – 9 – 10** – 11 – 12

Studied specimens: VR26

Remarks
This peculiar species seems typical for humid habitats (but see Van de Put et al., 2000), with basidia which are thought to be especially well adapted for water dispersal (Oberwinkler & Bandoni, 1981). The basidia are in fact not verrucose as seen by SEM but porous, the spore wall is a radial canalized structure. The sclerotium was found to have dolipores (Oberwinkler & Bandoni, 1981)and the host was cultured, studied and described as a new species, *Waitea nuda* by Clémençon (1990).
The species easily develops in moist chamber (even secondarily on paper tissue?!), perhaps because of fragmentation of the original sclerotium.

Tremella spicifera Van Ryck., Van de Put & P. Roberts, Mycotaxon 81: 185 (2002).

Basidiomes 200-600 µm diam., hyaline, watery-gelatinous, in patches, growing parasitically on perithecia of *Massarina arundinacea*. Hyphae 2-3 µm wide, in places inflated up to 4-6 µm wide, hyaline, clamped, frequently anastomosing, with thin-, refractive, or gelatinously thickened walls. Spores 4-5 µm, globose, with distinct apiculus, hyaline, aguttulate, sometimes producing secondary spores. Basidia 10-15 x 6-9 µm, tremelloid, globose to ovoid, clavate to capitate, with longitudinal or sometimes transverse septa. Sterigmata 2-3 (-4), mostly up to 20 x 2 µm, exceptionally up to 65 µm long. Conidiogenous cells arising from same hyphae as basidia,

sometimes in fascicles, lageniform, 4-6 µm wide at base, neck up to 30 x 1.5 µm, retaining when old the collapsed basal clamp-connexions of the conidia. Conidia dikaryotic, 5-8 (-12) x 2.5-4 µm, elliptical to cylindrical, hyaline, with many small guttules, formed laterally and terminally on the conidiophores.
Ecology: all the collections of the host (and parasite) were collected from brackish tidal marshes on stems.
Phenology: **1 – 2 – 3 – 4 – 5** – 6 – 7 – 8 – 9 – 10 – 11 – **12**

Studied specimens: VR271, VR291, VR575 (Holotype); GVR3237 (pictures) Belgium, Scheldt estuary, tidal marsh Burcht, 28/11/2002, IFBL C4.26.31, developing on *Stagonospora vexata*, no herbarium collection

Remarks
For a full discussion on this species see Van Ryckegem et al. (2002). This mycoparasite was also found on *Stagonospora vexata*.

Trimorphomyces papilionaceus Bandoni & Oberw., System. Appl. Microbiol. 4: 105 (1983).

(Basidiomes) Sporodochia 500 µm diam., gelatinous, hyaline, compact, parasitic on *Arthrinium* sp. Conidiophore 12-20 µm long, cylindrical, sometimes septated, with several conidiogene cells apically. Conidiogene cells 12-20 x 3-4 µm long, cylindrical- lageniform, successive production of conidia. Conidia 3-4 x 2-3 µm, zygo-conidia, butterfly or 'H'-shape, in chains, direction not always the same.

Ecology: on stem

Studied specimens: Belgium, tidal marsh along Scheldt estuary, Burcht, IFBL C4.26.31, 15/11/98. No herbarium collection.

Remarks
Tremella-like parasite on *Arthrinium* sp. often associated with grasses but also found on *Heracleum spondylium* (Spooner, 1994). The etymology of the name *Trimorphomyces* refers to the fact that this species shows three different stadia: a sexual stage with tremalloid basidia, a yeast-like stage and a conidial stadium with zygo-conidia (Oberwinkler & Bandoni, 1983).
The sexual stage has not been observed on our collections as was the case in another Belgium collection (see Van de Put, 2001).

Urediniomycetes (Rust-fungi)

Puccinia magnusiana Körn., Hedwigia 15: 179 (1876).

Telia 0.5-2 mm long, striate, ellipsoid up to broadly cylindrical, on both sides of the leaf, gold-brown to dark brown, granular, often abundant, orientated in the length direction of the substrate. Teliospores 33-51 x 14-22 μm (excl. stipe), clavate, 1-septate; stipe short, as long or shorter than spore, yellowish. Paraphyses present, 80-90 x 15-22 μm, capitate, hyaline to light yellow, smooth.

Ecology: on leaves and leaf sheaths
Phenology: **1 – 2** – 3 – 4 – 5 – 6 – 7 – **8 – 9 – 10 – 11 – 12**

Studied specimens: VR10, VR50, GVR1136: The Netherlands, The Scheldt estuary, Saeftinghe, IFBL B4.33.43, on leaf sheath (figure)

Remarks
Uredinia and urediniospores are formed on different *Ranunculus* species (Cummings, 1971). Compare with *P. phragmitis*.

Puccinia phragmitis (Schumach.) Körn., Hedwigia 15: 179 (1876).

Telia 2-6 mm long, thick, ellipsoid to broadly cylindrical, on both sides of the leaf, granular, dark brown, orientated in the length of the substrate. Teliospores 40-60 x 18-25, clavate, 1-septate, gold-yellow to brownish; stipe long, up to 240 μm, longer than spore, yellow. Paraphyses absent.

Ecology: on leaves and leaf sheaths.
Phenology: **1 – 2 – 3** – 4 – 5 – 6 – 7 – **8 – 9 – 10 – 11 – 12**

Studied specimens: VR2

Remarks
Uredinia and urediniospores are formed on *Rumex* spp. (Cummings, 1971) (e.g. *Rumex hydrolapathum* pers. obs.). Both rusts are frequently recorded on *Phragmites*, with *P. phragmitis* proportionally more found on the leaves, while *P. magnusiana* seems to occur proportionally more often on the leaf sheaths. Both species are easily separated from each other based on the overall larger telia and longer teliospore stipe and the absence of paraphyses for *P. phragmitis*.

***Sporobolomyces* sp.**

Colonies on the host surface up to 1 mm diam., salmon-coloured, almost hyaline, compact resembling a sporodochium. Mainly out of inflated cells (up to 10 μm wide) in short chains,

hyaline, with sometimes sterigmata on these cells. Spores 4-5 x 2.5-3 µm, kidney-shaped, with small apiculus, agutulate, asymmetrical.

Ecology: on green and moribound leaves and leaf sheaths, often on and between the hairs of the ligula.

Studied specimens: VR558

Remarks
Although this species is often recorded from green plant leaves after indirect observation (plating or genetic screening) (inclusive *Phragmites* leaves see e.g. Taligoola 1969; Apinis et al., 1972a) it was rarely encountered in the field with observable colonies under the dissecting stereomicroscope (screening magnification:180x).

Ustilaginomycetes (Smut-fungi)

Ustilago grandis Fr., Syst. Mycol., III: 518 (1832).

Sori in culms, dark brown till black, confluent, often surrounding the entire stem, mostly on the upper half of standing stems, underneath a membrane from host origin which has a swollen appearance and remains after rupture supported by small columns of plant tissue (containing wood vessels?), after sporulation stems remain with a streaked appearance. Spores subglobose, globose sometimes slightly irregular, 7-10 x 8-11 µm, olivaceaous, brown, apparently smooth but densely and very finely echinulate; with several small guttules when fresh.

Ecology: on stems.

Studied specimens: VR3, VR809

Remarks
This species is not common but may be locally abundant. According to Weeda et al. (1994) this fungus is responsible for weakening the standing shoots and hence, enhances the tumbling of the shoots into the water or on the sediment. However this may be true in some cases, it is thought that other species play a more important role in weakening of standing dead stems before breaking (e.g. *Massarina arundinacea*).

Ascomycetes – teleomorphic state

Ascomycota comprise the group with the highest species richness. Together with their asexual (anamorphic) stages they are the main contributors in the decay process of *Phragmites australis*. Descriptions of ascomycetes are split up in two artificial sections: discomycetes and pyrenomycetes. Discomycetes are recognizable by the free exposure of a hymenium (apothecia). Typically these taxa comprise disc-shaped fungi superficially on the host. Pyrenomycetes are characterized by their flask-shaped fruit bodies (perithecia – pseudothecia) exposing the spores (or asci) through a small channel (ostiole). However some taxa could incorrectly be taken for pyrenomycetes because they superficially look like it. For example ostropalian fungi are considered to be discomycetes but form immersed apothecia exposing their hymenium only in a mature state.

Discomycetes

Albotricha acutipilosa (P. Karst.) Raitv. (1970). Scripta Mycol. 1: 40.
Syn.: *Dasyscyphella acutipilosa* Baral & E. Weber (1992). Bibl. Mycol. 140: 103.
Ill. Svampe 30: 36 (1994).

Apothecia 0.6-1.5 mm diam., whitish, becoming ochre coloured when dry, with short stipe, often in small groups aggregated. Stipe up to 0.5 mm long, hairy. Receptaculum white, covered with long hairs; ectal excipulum textura prismatica. Hymenium white, to cream-brownish when dry. Hairs at margin and on receptaculum 130-180 x 3-4 μm, narrowed to the top, blunt, hyaline to light yellow, 7- 11 septate, smooth. Paraphyses lanceolate, 70-80 x 3.5-4.5 μm, up to 30 μm longer than the asci, 2-3 septate. Asci 45-56 x 5- 5.5 μm, cylindrical, rounded apex, 8-spored, spores biseriate, IKI^+, with crosiers. Ascospores 8-10 x 1.5-2.5 μm, guttulate, fusiform, ends rounded up to pointed, some irregular.

Ecology: on stems.

Studied specimens: VR130

Remarks
The proposed recombination of the species in *Dasyscyphella* by Baral & Weber (1992, Bibl. Mycol. 140: 103) is not accepted and the species is retained in *Albotricha* according Raitviir (2002).

Ascobolus stercorarius (Bull.) J. Schröt., Krypt.-Fl. Schlesien 3(2): 56 (1893).
Syn.: *Ascobolus furfuraceus* Pers. per Hook

Apothecia 3-5 mm diam., yellow-greenish, with short inconspicuous stipe, cupulate till disc-shaped. Receptaculum yellow-greenish, granular; ectal excipulum textura globosa, subglobular cells with yellow cell walls. Hymenium yellow to greenish, with protruding asci with dark spores, creating a black-punctuated hymenial surface. Paraphyses septated, not or with a slightly enlarged tip up to 6 µm. Asci 150-200 x 16 µm, clavate, 8-spored, biseriate, IKI$^-$, but ascus wall amyloid. Spores 17-20 x 10-12 µm, brown, elliptical to fusiform, rounded, with anastomosing striae.

Ecology: on very rotten reed material in litter layer.

Studied specimens: VR34

Remarks
For a detailed description of this species see Van Brummelen (1967: 106) under *Ascobolus furfuraceus* Pers. per Hook.

Bisporella scolochloae (De Not.) Spooner, Kew Bull. 38: 557 (1984).

Apothecia up to 1 mm diam., cupulate to flat, brightly yellow, in small groups aggregated or solitary, sessile. Receptaculum and hymenium yellow, smooth; ectal excipulum with anticline, thick-walled hyphae. Paraphyses with one large to several smaller guttules, sometimes branched near the top. Asci 62-84 x 7 µm, cylindrical-clavate, narrowed at the top, biseriate, IKI$^-$. Ascospores 13-15 x 3 µm, fusiform, hyaline, up to 3-septate, slightly constricted at the septa.

Ecology: on stems and leaf sheaths.

Studied specimens: VR30; VR179; VR819

Remarks
According to Kirk & Spooner (1984) this species is a parasite of *Apiospora* sp. [with anamorphic state *Arthrinium* sp. fide Ellis (1971)]. However this relationship could not be confirmed by us. The species was several times observed growing on (old?) perithecia, of an unidentifiable species.

Cistella fugiens (Phill. ex Bucknall) Matheis, Friesia 11(2):92 (1977).

Apothecia 0.2-0.7 mm diam., hyaline, saucer-shaped, slightly incurved margin, sessile to subsessile. Receptaculum granular; ectal excipulum textura prismatica with more globular cells near the margin; with visible tomentose aspect of hairs on the margin (loupe). Hairs 15-21 x 5-9 μm, clavate, finely incrusted, hyaline. Paraphyses 30-40 x 1-1.5 μm, cylindrical, septated, with multiple guttules, tip not or slightly enlarged. Asci 27-36 x 5-7 μm, cylindrical, weakly clavate, 8-spored, bi-uniseriate, IKI^+ (weakly), with crosiers. Ascospores 7-11 x 1-2.5 μm, cylindrical, clavate, hyaline, smooth, with few small guttules at the ends, aseptate or sometimes one median septum.

Ecology: on stems and on old perithecia of probably *Massarina arundinacea*.

Studied specimens: VR43, VR192, VR244.

Remarks
According to Dennis (1949) this species is 4 spored. However all our collections showed asci with eight spores. Baral (1985 in Baral & Krieglsteiner) reports on a remarkable variability of the spore size (5-11 x 1-2.5 μm) and found 8 spored asci.

Cryptodiscus **sp.** [*Cryptodiscus minutus* nom. prov. Declercq & Van Ryck.]

Apothecia 100-300 μm diam., first totally covered by the host epidermis, later visible as an opening in the epidermis which curls up and forms the margin of the apothecium, immersed. Ectal excipulum cells subglobose. Hymenium ochraceous to pale yellow, thin. No periphyses observed. Paraphyses 47-52 x 2 μm, septated, apex slightly enlarged. Asci 30-40 x 6-7 μm, J^-, wall redbrown in IKI (hemi-amyloid), clavate to cylindrical, broadly rounded at apex, 8 spored, biseriate. Ascospores 11-14 x 2-3 μm, fusiform, 1-septate becoming 3-septate, not constricted at septa, hyaline, with lots of small guttules.

Ecology: immersed in leaf sheaths.

Studied specimens: VR51; VR381

Remarks
A small *Cryptodiscus* was also described by Baral (HB7267) (pers. comm.) on *Phragmites*. Based on the description and figures this seems to be a different species (?). A comparably small spored, incompletely described and unnamed *Cryptodiscus* sp. was found on *Lycopodium alpinum* (Holm & Holm, 1981).

Lachnum controversum (Cooke) Rehm, Hedwigia 27: 165 (1888).

Apothecia 0.5-1.6 mm diam. at first cupulate later spreading open, white to cream-colored; stipe 0.2-0.8 mm long, white, with hairs. Receptaculum textura prismatica, with hairs. Hymenium white becoming yellow to brown-red when drying, smooth. Paraphyses 61-80 x 3-4 µm, lanceolate, up to 20 µm longer than the asci, with multiple refractive bodies, often with 1-2 septa near the base. Asci 43-50 x 4-5 µm, cylindrical, with short stipe, IKI^+, without croziers, in bundles. Ascospores 6.5- 9 x 1.5-2 µm (*sensu stricto*); 8.5-12.5 x 2-3 µm (GVR 1856; microscopical pictures available; *sensu subcontroversum* = *L. controversum sensu* Le Gal (1939)), cylindrical-fusiform, with rounded ends, with few small guttules near the ends. Hairs 65-80 x 3-4 µm, cylindrical, completely incrustrated, sepated, apex not or only very slightly enlarged.

Ecology: on leaf sheaths, stems and leaf blades in the litter layer. Not in tidal marshes.

Studied specimens: VR817; VR826; GVR1856, Switzerland, Southeastern shore of Lake Neuchâtel, oligotrophic hardwater lake (46°54'N, 6°54'E), 4/13/2003, Collector M.O. Gessner (no herbarium specimen; pictures available)

Remarks
Lachnum subcontroversum Baral nom. prov. (1985 in Baral & Krieglsteiner) would only differ from *L. controversum* by the larger spores. We did not measure with enough detail each collection to verify if there are indeed two species involved in this group; however, our large spore range for *L. controversum* suspects we interpreted the species in a large sense in most cases. At least one documented record showed generally larger spores and could fit for *L. subcontroversum* (see above). More detailed study is needed on this group.

Lachum tenuissimum (Quél.) Korf & W.Y. Zhuang, Mycotaxon 22: 501 (1985).

Syn. *Dasyscyphus pudicellus* (Quél.) Sacc.; *Dasyscyphys tenuissimus* (Quél.) Dennis; *Lachnum pudicellum* (Quél.) Schröter

! Dennis (1963) points out that, 'tenuissimum' as an epithet has priority on 'pudicellum', however Baral (1985 in Baral & Krieglsteiner) doubts that the type description given by Dennis (1963) is conspecific with what is generally understood under *L. pudicellum* and therefore rejects the use of 'tenuissimum'.

Apothecia 0.5-1 mm diam. at first cupulate later spreading open, white; stipe 0.2-1.1 mm long, white, with hairs. Receptaculum textura prismatica, with hairs. Hymenium white, becoming yellow to brown-red when drying, smooth. Asci 43-50 x 4-5 µm, cylindrical, short stipe, biseriate, IKI^+, no crosiers but in bundles. Paraphyses 61-80 x 3-5 µm, lanceolate, up to 25 µm longer than the asci, with refractive bodies, often with 1-2 septa near the base. Ascospores 6.5-11 x 1.5-2.2 µm, hyaline, cylindrical-fusiform, with rounded ends, with few small guttules near

the ends. Hairs 65-75 x 3-4 μm, cylindrical, completely incrustrated, septated, apex mostly enlarged up to 6 μm diameter, however some hairs are not or only very slightly enlarged.

Ecology: on stems, leaf sheath and leaf blades in the litter layer. Both species (*L. controversum* and *L. tenuissimum*) are found abundant in non-tidal reeds from April to October.

Studied specimens: VR 821, GVR2739, Belgium, Boerekreek (Sint-Laureins, Sint-Jan-in-Eremo), IFBL C2.18.11, 3/7/2002 (no herbarium collection only drawing)

Remarks
Although in literature *L. controversum* and *L. tenuissimum* are considered to be well separated, we had some difficulties in recognizing them on *P. australis*. It is important to observe marginal hairs of *L. tenuissimum*, those are most inflated. *L. tenuissimum* seems to have paraphyses with less oil content compared to *L. controversum*. Apothecia of *L. tenuissimum* generally look elegant while *L. controversum* looks more robust when mature.
At this stage I recognize two species on *P. australis*, however more species are reported on reed and critical examination of all *Lachnum* taxa is necessary. I feel some cryptic, well resembling species are present in humid marsh habitats. Some might be host specific but other could occur on several hosts. Other *Lachnum* or *Lachnum*-like species which could occur on *Phragmites*, although less common, are *Lachnum elongatisporum* Baral (see Raitviir & Sacconi, 1991: small species with elegant apothecia, with small asci up to 45 x 4.5 μm and slender spores 7.5-13 x 1-1.5 μm); *Brunnipila palearum* (Desm.) Baral (with brown hairs, see Baral (1985 l.c.)); *Lachnum nudipes* (Fuckel) Nannf., *Lachnum carneolum* (Sacc.) Rehm and possibly other taxa.

Laetinaevia sp

Apothecia 150- 300 μm diam., downy, cupulate at first but spreading open and becoming convex, with white hyphae at the base, cream to white receptaculum, sessile, receptaculum 80 μm thick, hymenium 70 μm. Peridium textura prismatica but difficult to have a good view, some part are more like textura intricata, probably hyphoid hairs are present but a clear observation of these structures could not be done, they look probably similar to the paraphyses. Paraphyses 2 μm wide, cylindrical, septate, top slightly enlarged, hyaline, guttulate but guttulation seemed already disturbed in collected material, seldomly branched (at base), some top cells showed a hemiamyloid reaction (or where it some marginal hairs lost in the squash slide?). Asci 55-65 x 10-11 μm, clear hemiamyloid apex (red reaction), after KOH treatment deep blue coloration, conical tip, with clear stipe, crosiers present, 8-spored, biseriate. Spores 17-18 x 3,5 μm, fusiform, mostly curved, 0-1 septate, guttulate (see remark above), with mucous sheath but quickly disappearing, spores often adhering together.

Ecology: on leaf sheath.

Studied specimens: VR749

Remarks
Determination with Rehm (1896), Feltgen (1899, 1901) and Hein (1976) does not lead to a species name. Closest resembling species is *Laetinaevia carneoflavida* but this species has wider and shorter spores. Only one collection of this species was made with a few ascomata on the reed host.

Lophodermium arundinaceum (Schrad.) Chevall., Fl. Gén. Env. Paris (Paris) 1: 435 (1826).

Apothecia 1-1.5 x 0.5 mm diam., ellipsoid to bean-shaped, black, immersed splitting up the epidermis in the length direction of the stem or leaf sheath; margins of the apothecium lighter coloured, eventually hymenium completely free, greyish-hyaline. Paraphyses 110-140 µm x 1-1,5 µm wide, with curled tip. Asci 100-130 x 7-8 µm,, cylindrical, clavate, thin-walled, 8-spored, IKI^-. Ascospores 56-76 x 1.5 µm, scolecospores, aseptate, straight or slightly bended, with several small guttules, very narrow mucous sheath, multiseriate in ascus or spirally when dead.

Ecology: this species is relatively frequently collected typically in early spring (beginning of March till May) but can exceptionally be found up to October. It is indicative for leaf sheaths in the middle canopy.

Studied specimens: VR150

Mollisia hydrophila (P. Karst.) Sacc., Syll. fung. 8: 345 (1889).

Ascomata 0,5- 1 mm diameter, 200-300 µm high, light greyish, transparent, soft, first as bud like, dark primordia and later spreading open to form regular discs, sessile with very short stipe (microscope), with slightly concave hymenium pressing the margin to the substrate or undulated, flat discs with a clear, lighter margin, gregarious to densely aggregated (becoming angular by mutual pressure). Subiculum rudimentary to well developed, with dark brown to black hyphae 3,5-4,5 µm wide. Ectal excipulum textura angularis, basal cells near stipe dark brown, other basal cells light brown; margin cells with clavate (up to 10 µm wide) end cells with refractive vacuole body. Medular excipulum hyaline, textura intricata, non-gelatinized, with rhomboid, calciumoxalate crystals, often abundant near the stipe base, but in some apothecia scattered or almost absent. Paraphyses as long as asci or slightly longer, 3-4 µm wide, apically not or only very slightly widened, rounded, straight, with one large vacuole body filling the top cell (sometimes divided in 2-3), 1-2 septa near the base. Asci 75-84 x 5-6 µm, ascospores biseriate, spores in the upper 20-30 µm, apex slightly conical, euamyloid, IKI^{++}, apical apparatus flat and small in living state; base with crosiers. Ascospores 10-13 x 2-3 µm,

slightly scutuloid, base tapering to fusiform, aseptate, hyaline, with some small guttules at both ends.

Ecology: less common than *M. retincola*. In the litter layer of non-tidal reed beds. Most often during spring and summer.

Studied specimens: VR812; VR820

Remarks
A weak KOH reaction (light yellow coloration) was noticed in (Melsen material VR812) the ectal excipulum and in the hymenium (more subtle) for a short period. Probably this reaction is only noticed on fresh material (and on not too thin sections).

Mollisia cf. palustris (Roberge ex Desm.) P. Karst., Acta Soc. Fauna Fl. Fenn. II (6): 135 (1885).

Apothecia 0,3 - 1 mm diam., discoid, sessile, brownish-grey, margins slightly darker, a brown rudimentary subiculum is sometimes present, no calciumoxalate crystals present, marginal cells hyaline to brown, sometimes somewhat tapered, up to 15 µm long. Peridium textura globulosa, basal cells brown, no KOH reaction. Paraphyses 3 µm wide, cylindrical, with large guttule in upper part, septate (2-3 septa), not enlarged at tip. Asci 45-60 x 4,5-6 µm, IKI^+, with croziers cylindrical-clavate, biseriate. Ascospores 7-11 x 1-2.5 µm, fusiform, tapered to one end, without or with some small guttules towards the ends.

Ecology: because of the taxonomic confusion about this species, ecological notes seem not consistent. We find this species up in the canopy on standing dead leaf sheaths and we retrieved the taxon from the litter layer in non-tidal marshes often on leaf sheaths.

Studied specimens: VR46 (this collection stands close to *M. evilescens* with brown margin; marginal cells conspicuous brown, slightly tapering up to 15 µm long; spores 7-8 x 1,5 µm; no crystals observed; collection sparse); VR125 starts to form a subiculum, crystals not absent?; VR159 no crystals, rudimentary subiculum in this collection; VR777 in canopy of reed stand: habitus picture

Remarks
The delineation of this taxon is far from clear to us at present. Additional fresh collections should be made with new drawings and pictures of the habitus should be taken.
The difference with *Mollisia hydrophila* should be checked out properly as it seems that in some cases the only difference between the two spp. seems to be the absence/presence of crystals in the medulla. A rudimentary subiculum seems to be present in some collections of *M. palustris*. The difference between *M. palustris* and *M. evilenscens* is far from clear, some collections seem to

state the middle of the two species. *M. palustris* would be distinct in culture characteristics from *M. retincola* (*M. hydrophila* in Le Gal & Mangenot, 1961: 330) as smooth colonies are produced while the latter species would have abundant aerial mycelium.
Mollisia species can change a lot in appearance when moisted or not!

Mollisia retincola (Rabenh.) P. Karst., Myc. fenn. 1: 209 (1871).
We follow Nannfeldt (1985) and do not recognize *Peziza kneiffii* Wallr. (1833) conspecific with *Peziza retincola* Rabenh. (1860). Hence the latter is recognized as basionym.

Ascomata 0.5-2 mm diam., at first bud-like, later spreading open, sessile, solitary or gregarious, greyish yellow, ochre yellow (more ochre coloured when dry), margin more greyish; outer excipulum textura angularis, inner excipulum filled with conspicuous calciumoxalate crystals; clear yellow outflow from material when threatened with KOH (microscope). Subiculum often abundant, with dark brown thick-walled hyphae, 3-4 µm diam.; red colouration of substrate was often observed underneath the subiculum. Paraphyses about 100µm long, 3-4 µm wide, with one (2) large guttule(s) filling the upper cell, 1-2 septate near the base. Asci 94-115 x 8 µm, cylindrical, 8-spored, J^+, IKI^+, with crosiers, biseriate. Ascospores 15-25 x 2.5-3 µm, cylindrical, straight to slightly curved, mostly aseptate, some 1-septate, with many small guttules near the ends (lipid content 3 see Baral, 1992).

Ecology: *Mollisia retincola* is a very common colonizer of basal parts of standing dead reed stems during spring. It seems to be restricted to *Phragmites* and confined to fresh water reeds. Apothecia are abundantly formed just above water level (or humid soil).

Studied specimens: VR212; VR 811

Orbilia septispora Baral, Z. Mykol. 55(1): 126 (1989).

Ascomata 100-250 µm diam., superficial, irregular, yellow-orange, sessile. Hymenium slightly more yellowish. Paraphyses 50-55 x 1.5 µm, abruptly enlarged at the tip (3-4.5 µm wide), without septa. Asci 52-57 x 7-8 µm, relatively thick-walled, cylindrical-clavate, with foot often forked, IKI^-, biseriate. Ascospores 10-12 x 2-2.5 µm, cylindrical-fusiform, sometimes slightly bended in the lower part of the spore, 0-1 septate, sometimes 2 septa, hyaline, with some small guttules and an inconspicuous refractive body apically.

Ecology: rarely found during this study and hence ecology not well documented. On leaf sheaths and stems.

Studied specimens: VR4

Phragmiticola rhopalospermum (Kirschst.) Sherwood, Mycotaxon 28: 170 (1987).
Basionym = *Odontotrema rhophalospermum* Kirscht., Ann. Mycol. 36: 377 (1938).
Proposed synonyms

= *Sphaeropezia arundinariae* Cash, J. Wash. Acad. Sci 30: 300 (1940).

= *Eupropolella arundinariae* (Cash) Dennis, Kew Bull. 30: 361 (1975).

Observations on herbarium material:
Ascomata up to 0,8 mm long, elliptical, solitary or aggregated, splitting epidermis open, erumpent, dark brown to black-greyish excipulum exposing greyish (dry) to pinkish (wet) hymenium. Peridium textura globulosa to prismatica. Hamathecium filaments forming epithecium +/- 10 µm high, with slime matrix without colour reaction in KOH/ IKI, slender, slightly enlarged at apex, few septa, sometimes small crystals on top of the tips. Asci 70-85 x 8,5-10 µm, IKI^+, cylindrical to utriform (broadest part under the middle), narrowed towards apex. Ascospores 19-21 x 2,5-3,5 µm, cylindrical to clavate, slightly narrowed towards base, 3-septate, smooth, hyaline, slime coat was not observed.

Studied specimens: VR725 (Determination Van Ryckegem, duplicate from Danish collection sent by Thomas Læssøe, TL1106)

Remarks
From literature it became clear that *Sphaeropeziza arundinariae* Cash (1940) is a later name for *Odontotrema rhophalospermum* Kirscht. (1938) and should be placed in synonymy.

Psilachnum eburneum (Roberge ex Desm.) Baral, Beih. Z. Mykol. 6: 87 (1985).

Ascomata 0.1-0.25 (0.8) mm diameter, small, cupulate, with short stipe or almost sessile, cream-coloured; receptaculum granular with minute hairs; ectal excipulum textura prismatica, cells 15-17 x 4-5 µm. Hymenium hyaline to cream-coloured. Paraphyses sparse, 40-45 x 1.5-3 µm, septate in lower half, with large guttule in top cell. Asci 35-45 x 5-7 µm, cylindrical, with rounded to slightly fusiform top, IKI^+, only slightly tapering towards the base, biseriate, often with one large guttule in the ascus under the spore part, disappears when mature (overmature asci), with crosiers, difficult to observe, asci easily dehiscent. Ascospores 7-11 x 1.5-3 µm, rather variable in size (even within one collection!), cylindrical, fusiform or slightly scutuloid, straight or slightly curved, hyaline, with 1-3 small guttules near the ends, aseptate. Hairs 10-25 µm long, slightly enlarged top, sometimes with refractive guttule, hyaline.

Ecology: on leaf blades, sheaths and stems in the litter layer. During spring and summer.

Studied specimens:VR813 (pictures and drawing).

Rutstroemia lindaviana (Kirschst.) Dennis, British Cup Fungi: 65 (1960).

Ascomata 1-1.5 mm diam., light brownish, becoming darker when aging, growing on black slerotized leaf or leaf sheath tissue, several apothecia arising from one sclerotium. Stipe 0,5-4 mm long, variable short to long (see drawing), always clearly stipitate, brown. Receptaculum smooth, brown, external excipulum textura prismatica. Paraphyses 40-55 (60) x 4-4.5 µm, some deeply rooted, with several guttules, hyaline. Asci 40-55 x 4-4.5 µm, cylindrical, rounded top, short stipe, 8-spored, without crosiers, IKI^+, spores biseriate. Ascospores 4.5-6.5 x 1.5-2.5 µm, hyaline, ellipsoid, smooth, with several small guttules (index 2).

Ecology: on leaf blades and sheaths in the litter layer. During spring and summer.

Studied specimens: VR180; VR759

Scutellinia cf. umbrarum (Fr.: Fr.) Lambotte, Fl. Mycol. Belge, suppl. 1: 300 (1887).

Ascomata 2-4.5 mm, sessile, discoid, flat, pale orange-red; receptaculum orange-brown, with dark brown hairs. Paraphyses 2-3 µm, with enlarged, clavate top (up to 10 µm wide), multi-septate. Asci 228-250 x 20 µm, cylindrical, broadly rounded, 8-spored, IKI^-. Ascospores 18-21 x 11-13 µm, broad ellipsoid, hyaline, multiguttulate, with warts which are sometimes connected, uniseriate. Hairs 250-400 µm, bifurcate, multiseptate, brown.

Studied specimens: VR18

Remarks
Only one collection which agrees well with the description for this species in Breitenbach & Kränzlin (1981). However Schumacher (1990) described *S. umbrarum* with spores characterized by wrats which are not interconnected and with only one large guttule.

***Stictis* sp** [*Stictis dissociativus* Van Ryck. nom. prov.]
Etymology: referring to the species characteristics which are typical for two genera

Ascomata 200-300 µm diam., 150-160 µm high, black, initialy immersed later breaking epidermis open, solitary to gregarious, apothecoid; ostiole central, spreading open during development, powdery whitish-grey margin; periphyses 11-25 x 2-2.5 µm, abundant, 1-2-septate, hyaline, seldom branched at base. Peridium brown, with sometimes blue-green tint, thinner at base of ascoma, upper part with small rhomboid crystals in a zone of about 20 µm wide (clearly visible in KOH), marginal hyphae hyaline to brown and hyaline hyphae with small crystals on surface which do not dissolve in KOH. Subhymenium 15-20 µm wide, compact polygonal cells, light ochraceous yellow. Paraphyses abundant, 80-120 x 1-2 µm, filiform, top

reraly enlarged, septate. Asci 110-120 x 13-20 µm, IKI $^{-}$, (KOH+IKI)$^{-}$, initially cylindrical, later central part broader and tapering towards apex, slender stipe; apical apparatus inconspicuous, in young state ascus appears like bitunicate; 8 ascospores first lineair, sometimes spiraliform in middle part of ascus (dead state), eventualy disintegrating, zone above subhymenium (KOH + IKI)$^{+}$, it seems that the slime mucus around the paraphyses colours blue; slime mucus around the paraphyses in IKI^{+-}, orange-red. Ascospores about 100 x 1.5-2.5 µm within ascus, articulating in 2.5-4 x 2-2.5 µm fragments (when dead 2.5-4 x 1.5-2 µm), hyaline, sometimes 1-2 small guttules per fragment.

Ecology: hitherto we found this species to be restricted to several brackish reed marshes (both tidal and non-tidal). It is restricted to the middle height canopy on leaf sheaths and the species can occasionally be found in the litter layer after the culms fell down. This species develops slowly. The fruit bodies are visible from December but are mature the end of March.

Studied specimens: VR433

Remarks
Difficult determination as disintegration of spores and the presence of periphyses are two features normaly not united in one genus *sensu* Sherwood (1977a,b). *Schizoxylon* is characterized by disarticulated spores and absence of periphyses while *Stictis* normaly shows no disarticulating spores and has periphyses. However *Stictis stigma* Cooke & Ellis and *Stictis monilifera* (Sherwood, 1977b) have periphyses and articulating spores, but not conform to our species.
Acarosporina has also disarticulating spores but amyloid asci (Sherwood, 1977a; Johnston, 1985).
A collection of this species was studied by H.O. Baral and he confirmed the inability to name this taxon with the Sherwood monography. Probably this species is new to science.

Culture remained sterile. An anamorph recorded for *Stictis* spp. is *Stictospora* according Johnston (1983).

***Stictis* sp II** [*Stictis megagraminicola* Van Ryck. nom. prov.]
Etymology: referring to the larger sized asci and ascospores compared to *Stictis graminicola* Lasch

Ascomata 300-350 µm diam., apothecoid, opening by pore, urceolate (250 µm high), immersed, not erumpent, black; margin white, inconspicuous. Peridium out slender, hyaline interwoven hyphae, in cross section with periphyses up to 40 x 1,5 µm, filliform, unbranched or branched at base; crystalline layer ± 40 µm wide, hyaline crystals, sparse; subhymenium ± 10 µm thick, IKI^{-}. Hamathecium filaments filiform, 1 µm wide, as long as asci, seldomly branched, often slightly tapering towards the top. Asci 180-195 x 5-7 µm, IKI^{-}, cylindrical,

apical apparatus distinct, 8 spores, helicoidally in dead ascus. Ascospores 150- 170 x 1-1.5 μm, filiform, septate, cells 7-9 μm long, hyaline.

Ecology: on standing dead leaf sheaths.

Studied specimens: VR761

Remarks
This species differs from *S. graminicola* by the filiform periphyses and larger asci and spores. The size of the asci and spores are much larger than those of *Stictis pusilla*. A collection of this species was studied by H.O. Baral and he confirmed the inability to name this taxon with the Sherwood (1977a) monography. Probably this species is new to science.

Stictis stellata Wallr., Fl. crypt. Germ. (Nürnberg) 2: 144 (1833).

Ascomata 250-500 μm diam., round, breaking through the epidermis, first with a wide white collar with many large crystals; mature (overmature) apothecia without the conspicuous margin and a black, smooth margin remains. Hymenium pale yellowish-orange, flesh coloured, 100-110 μm thick, in dry conditions contracting and not adhering to the excipulum, subhymenium 40-70 μm thick; periphyses abundant, up to 45 μm x 2 μm, septated at the base, rounded at the top. Paraphyses slender 1-1.5 μm wide, tips enlarged up to 3 μm; forming an epithecium of about 15 μm thick, IKI^{+} (red brown), $(IKI+KOH)^{+}$ (blue). Asci 250-300 x 6-7 μm, no colour reactions seen, conspicuous apical apparatus. Ascospores 240-270 x 1-1.5 μm, filiform, septate, cells 6-7 μm long.

Ecology: on standing dead leaf sheaths.

Studied specimens: VR694

Trichopeziza albotestacea (Desm.) Sacc., Miscell. Mycol. I: 8 (1884).
= *Albotricha albotestacea* (Desm.) Raitv., Scripta Mycol. 1: 40 (1970).

Ascomata up to 3 mm diam., about 100-150 μm thick, with a short stalk superficial on resource; hymenium flesh coloured; receptaculum orange-reddish with long straight hairs; ectal excipulum textura globulosa. Paraphyses up to 90 μm long, 4- 7 μm wide, exceeding the asci by 20-30 μm,, 2-3 septate, top cell about 35 μm long and tapered. Asci 55-65 x 4.5-5 μm, IKI^{++} (deep blue), cylindrical, narrowed towards the base, 8-spored, ascospores biseriate, grouped in top 30 μm; crosiers (?) not tight. Ascospores 9-15 x 1.5-2 μm, relatively variable in length, narrowly fusoid, hyaline, smooth, with several small guttules near the ends (lipid content 2).

Hairs ± 200 μm long tapering into a point, rounded or branched near the top, smooth in the upper part, granular in lower part, with brownish droplets on the outside.

Ecology: on stems during spring-early summer in the litter layer.

Studied specimens: VR818

Unguiculella eurotioides (P. Karst.) Nannf., Trans. Brit. Mycol. Soc. 20: 194 (1936).

Ascomata 0.1-0.3 mm diam., light yellow, sessile, often aggregated, cupulate-discoid to concave; margin downy, receptaculum finely granular, pale yellow; ectal excipulum textura prismatica. Paraphyses 35-50 x 1-1.5 μm, curled on the top, septate. Asci 30-35 x 4 μm, cylindrical-clavate, 8-spored, IKI$^-$, biseriate. Ascospores 6-8 x 2 μm, ellipsoid, appear relatively thick-walled, smooth, hyaline, generally aseptate but becoming 3-septate. Hairs 20-30 μm long, typical recurved hooks, base wider, weakly refractive.

Ecology on stems in the litter layer.

Studied specimens: VR32

Pyrenomycetes

Acrocordiopsis **sp.** [*Acrocordiopsis aestuari* Van Ryck., nom. prov.]
Etym. referring to the estuarine environment the species occurs.

Ascomata 0.4- 1 mm diam. up to 2 mm long elongated along the long axis of the substrate, 300-500 μm high, black, carbonaceous, semi-globose or conical, superficial to slightly immersed, solitary to aggregate with few ascomata; ostiole slitlike, poorly developed, not papillate. Peridium thick-walled, 2-layered, carbonized, up to 200 μm thick, hyaline parenchyma layer of prismatic cells about 50 μm thick (to be confirmed again). Hamathecium cellular pseudoparaphyses abundant, 2.5 μm diam., septated every 11-17 μm, clearly anastomosing. Asci 115- 160 x 20-25 μm, cylindrical, very short stipe, ocular chambre, pedunculate, eight spored, uniseriate, bitunicate. Ascospores 24-31 x 13-16 μm, broadly ellipsoid with rounded ends, 1-septate, septum median or slightly submedian, constricted at the septa, apical cell slightly enlarged, smooth, hyaline (later becoming brown), filled with small guttules; mucous sheath sharply delimited, entire, swelling in water up to 5 μm wide.

Ecology: on intertidal, old stems in the litter layer.

Studied specimens: VR690, VR641, VR767

Remarks
This species seems to fit pretty good the genus *Acrocordiopsis* (see Borse & Hyde, 1989) (Hyde, pers. comm.) and differs from the two other species described in the genus by their spore characteristics. However, genetic confirmation is needed before placing the species definitively in *Acrocordiopsis* because a clear refractive apical thickening in the asci was not observed and cellular pseudoparaphyses (in contrast to trabeculae) seems to be abnormal within *Acrocordiopsis*.

Anthostomella punctulata (Roberge ex Desm.) Sacc., Michelia 1: 374 (1878).

Ascomata 200-400 µm diam., spherical to ovoid, brown-black, immersed, solitary or gregarious, when together causing black discoloration of substrate; clypeus moderately developed, black; ostiole, central, papillate, central, protruding. Peridium textura angularis; outer cells brown, thick-walled, inner cells thin-walled and light yellow. Pseudoparaphyses 1.5-2.5 µm wide, hyaline, septated, slender, guttules. Asci 50-65 x 5-7.5 µm, IKI^+ (clearly), cylindrical, uniseriate ascospores, unitunicate. Ascospores 5- 8 x 2-3.5 µm, dark chestnut brown, smooth, ovoid-allantoid, relative variable in shape, biguttulate, very unclear furrow.

Ecology: on stem, leaf sheath and leaf blade.

Studied specimens: VR280, VR704

Remarks
Anthostomella minima Sacc. (see Francis, 1975) is possibly a species close to *A. punctulata* and reported from *P. australis*. It would differ from *A. punctulata* by smaller spores. The type material of *A. minima* is not traceable and is not kept in PAD, were most of Saccardo's type specimens are located (Francis, 1975). Francis (1975) doubts the status of this species and suspects that *A. punctulata* and *A. minima* are the same species, however without putting them officially in synonymy.

Anthostomella tomicoides Sacc., Atti Soc. Venet.-Trent. Sc. Nat., Padova 4: 101 (1875).

This plurivorious species (often on sedges in wetland habitat) was found once on *P. australis* and differs clearly from the more specific *A. punctulata* by the much larger spores (13-15 x 4-6.5 µm) being two-celled, one cell brown, one small hyaline appendage.

Ecology: on stem material.

Studied specimens: VR807

Apiospora montagnei Sacc., Nuovo Giorn. Bot. Ital. 7: 306 (1875).

Ascomata 140-160 µm diam., immersed, black, spherical, aggregated. Stroma up to 5.5 x 0.5 mm, linear, black, orientated in the length of the reed stem, at first subepidermal, splitting the epidermis slit-like. Ostiole papillate, protruding the stroma. Asci 92-100 x 12-13 µm, cylindrical-clavate, bitunicate(?), 8-spored; ascustip thickened, IKI^-. Pseudoparaphyses 2.5-5 µm wide, septated, some cells inflated, vanishing, hyaline. Ascospores (25) 28-32 (35) x 5.5-7 µm, geniculate, tapered, smooth, hyaline, many guttules, with one septum near sub-median near the end of the spore, end-cell 5-7 µm.

Ecology: on stems.

Studied specimens: VR303, VR241, VR282, VR764

Remarks
The anamorphic state *Arthrinium,* (see hyphomycetes) would differ from *A. phaeospermum* by the smaller conidia (up to 7 µm diam.). Collection VR764 showed both the sexual and asexual stage. However, the *Arthrinium* state had a size range of 6.5-8.5 (10) µm, having a range large enough to be possibly catalogued in one of the two anamorphs. Furthermore the size range of the ascospores mentioned in literature is very large (Hudson, 1963; Hudson & McKenzie, 1984). We found a relatively small range, consistent in all of the collections. Above observations make us wonder if *A. montagnei* is effectively a widespread, plurivorious species occurring on different monocotyledons or is in need of a narrower concept. On *Phragmites* stems, *A. montagnei* could be mistaken, both macro- and microscopically, for *Botryosphaeria festucae* if asci and ascospores are not readily available.
Dennis (1981) includes *Apiospora* in the Amphisphaeriaceae and keys the species out as if it would have a J^+ apical apparatus. However this is a mistake. Currently *Apiospora* is in the Sordariomycetes characterized by (among other things) unitunicate asci. However we doubt current position because we assume asci to be bitunicate.

Asco sp. incertae sedis 2

Ascomata 80-150 µm diam., spherical, solitary in substrate or hypersaprotrophical in old fruit bodies, yellow- light brown, immersed; ostiole up to 230 µm long x 30-50 µm wide, hyaline, flushy on the top. Peridium textura epidermoidea (difficult to observe!), thin-walled. Hamathecium filaments 3 µm wide, septate, constricted at septa, hyaline with small guttules. Asci 60-85 x 8.5-17 µm, lageniform, broadest below the middle, bitunicate (?), and slightly thickened at top (± refractive) or unitunicate (?) with inconspicuous apical apparatus (see young asci), IKI^-, cylindrical, stretching when mature, short pedicel and rounded foot, in bundles, 8-spores, bi-triseriate at first, uniseriate later. Ascospores 22-30 (35) x 5.5-6.5 µm,

fusiform, 3-septate, very slightly or not constricted, hyaline, one large guttule and often some some ones in each cell.

Ecology: On *P. australis* stems, leaf blades and leaf sheaths in a further state of decay in the litter layer. Not rare, observed the whole year round.

Studied specimens: VR236, VR763: good collection; GVR2241 on leaf blade, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', 31/10/2001, IFBL B4.33.43.

Remarks
The ascospores of this species show resemblance to *Keissleriella culmifida*. However fruitbody structure is different: textura epidermoidea, no bristles on the ostiole, but generally a well developed ostiole. I am unsure about the ascus-type. The species should be compared with *Micronectriella agropyri* Apinis & Chesters (TYPUS: IMI 102471) [Trans. Br. mycol. Soc. 47: 432 (1964)]. Our collections show good resemblance to the original description of this species, however spore size mentioned was smaller (16-22 x 4-6 μm) and a peridium type was not mentioned.

Asco sp. incertae sedis 3
(no figures)
This species was collected only once and can not be confused with any other taxon we encountered before on reed. However the bad, immature collection makes it impossible to give a good description and drawings. Further material is needed. A provisional description is given below.

Ascomata +/- 300-350 μm diam., spherical, brown, immersed; ostiole central protruding, wide. Peridium textura angularis, dark brown, rather thin walled. Hamathecium filaments not observed. Asci ± 110x 21 μm, only few, cylindrical, broad base and rounded on the top, bitunicate, thick-walled, 8-spored, with overlapping biseriate spores. Ascospores 30-40 x 10-15 μm, only measured inside ascus, broadly fusiform to ellipsoid, first septum, supramedian (to be confirmed), slightly constricted, additionally one or two septa seem to develop, hyaline, slime sheath (characteristics to be confirmed), with guttules.

Studied specimens: VR526

Botryosphaeria festucae (Lib.) Arx & E. Müll., Beitr. Krytopgamenfl. Schweiz 11(1): 38 (1954).

Ascomata 400-500 μm diam., pyriform, relatively thick-walled; peridium brownish sometimes with a faint of purple-red; generally in rows (up to 1 cm) in stroma, upper half of perithecium outside the stroma. Stroma black, linear, with multiple perithecia on rows, close to each other. Ostiole papillate, short. Asci 100-145 x 19-25 μm, clavate, bitunicate, thick-walled at the top, 8-spored, fragile, soon vanishing, seemingly loozing spores through ostiole channel. Pseudoparaphyses 6.5-10 μm wide, abundant, with guttules, constricted at the septa. Ascospores 18-40 x 8-12 μm, rather variable, inequilateral ellipsoid to mostly reniform, sometimes smaller towards the end, filled with small guttules, hyaline, biseriate in ascus.

Ecology: found on stems in the litter layer and especially on the middle part of standing dead stems, being dead for about a year or longer. Species seemed to prefer brackish conditions. During autumn.

Culture characteristics:
Culture (VR570) initially whitish, slow growing on corn meal agar, densely branched, featherlike appearance. After a while forming yellow pigments, multiple hyphal coils in culture slides. Remains sterile with the formation of chlamydospore like structure (thickened hyphal cells) (see figures).

Studied specimens: VR82, VR570, VR729

Remarks
Fusicoccum state of *Botryosphaeria festucae* was found inside the same stroma (VR729) together with the teleomorphs with separate locules filled with subhyaline conidia 12-19 x 3-4 μm, fusiform-cylindrical, bended, aseptate, thin-walled (see figures); conidiogenous cells not observed. See Sutton (1980), Denman et al. (2000). The anamorph was found only once, while the teleomorph was found frequently.

Rebentischia typhae Fabre, Annls Sci. Nat., Bot., sér. 6 (9): 88 (1878).
non *Buergenerula typhae* (Fabre) Arx, Revue Mycol. 41: 267 (1977).

Ascomata up to 1 mm diam., relatively large, semi-immersed to superficial, blackish-brown, conical. Ostiole conspicuous, central, round, with a small neck or more often as a hole in the peridium (lysigenous), often several ostioles per perithecium. Paraphyses 2 μm wide, hyaline, septate, abundant. Asci 120-170 x 14-15 μm, clavate, bitunicate, ascus top slightly thickened, long stipe, IKI⁻. Ascospores 34-45 x 8-9 μm (without appendage), yellow-brown, 5-7 septa, clavate, constricted at septa; appendage 5-7 μm long, basal, hyaline, it seems more cellular than a mucous appendage.

Ecology: on stems in the litter layer, only in reed vegetations in freshwater circumstances. During spring.

Studied specimens: VR61 VR705; GVR1664 (culture), on stem, 25/04/2001, Belgium, Scheldt estuary, Het Kijkverdriet, Steendorp (Temse), IFBL C4.54.11 (no exsiccatum)

Culture

The species forms an *Aposphaeria* or *Pleurophomopsis* like anamorph in culture (permanent slides) (see figures). Both anamorphs mainly differ in the conidiomata structure with *Aposphaeria* stated to have thin-walled and *Pleurophomopsis* to have thick-walled conidiomata (Sutton, 1980). Conidiomata superficial, (some thick-walled, other thinner) formed on CMA; peridium textura angularis-intricata, cells dark greenish-black. Conidia 3-4 x 1.4-2 μm, hyaline, cylindrical-ellipsoid. Conidiogene cells integrated on short conidiophores 1-2 septate, up to 15 μm long, sometimes branched basally. Conidiogene cells 8-11 x 3-4.5 μm, enteroblastic, phialidic, not annelate.

We did not observe this anamorph on field collections.

Remarks

There is confusion in literature about this species and type studies are required to clear the systematic position of this species; probably there are some look-alikes involved in the discussion. Based on ana- and teleomorph characteristics and the observed bitunicate asci we suspect that our species is not well placed in the genus '*Buergenerula*' (Magnaporthaceae, unitunicate asci). The type species of the genus *Buergenerula* is characterized by a *Passalora*-anamorph, has (sub)hyaline ascospores with characteristic septation and wide hamathecium filaments. *B. typhae* was transferred from *Rebentischia* (Tubeufiaceae, bitunicate asci) by von Arx (1977) (no type study). He transferred the species because he regarded it different from the type specimen of *Rebentischia* (*R. pomiformis* P. Karst.) which has "no periphyses and darker ascospores, each with an appendage-like, hyaline cell at their base". Spore size and shape in von Arx (l.c.) were similar to our observations. The ascomata were smaller and immersed in reed leaves. Furthermore von Arx (l.c.) observed a two-layered ascuswall with a refractive, non amyloid ring visible at the apex. We never observed this species on leaves, nor did we observe an apical ring. However our collections had bitunicate asci and a clear cellular appendage (see pictures) and no ostiolar periphyses were observed, although the latter character should be studied in more detail. Based on our description (large pseudothecia with obvious ostiole, bitunicate asci) and the anamorph isolated we could place our specimens in *Lophiostoma*. Barr (1980) concluded that all specimens labelled *Buergenerula typhae* she examined were a large variant of *Lophiostoma caudatum* Fabre (as *L. daryosporum*). This species has similar spore morphology compared to our collections but ascospores generally have no or only a very small slime appendages at the end and clearly has ascomata with flattened ostioles (see below). For the time being we consider the appendages peculiar (a basal hyaline cell more than a slime

appendage like in *Lophiostoma*) and keep the species in *Rebentischia* which was mentioned to have an *Asteromella* anamorph (Barr, 1980; Sutton, 1980) which also closely resembles the genus *Aposphaeria* or *Pleurophomopsis*. However future molecular work could show that this species fits within Lophiostomataceae.

Ceratosphaeria sp. [*Ceratosphaeria gessnerii* Van Ryck., nom. prov.]
Etymol. in honour of the collector and wetland scientist M.O. Gessner

Ascomata up to 1000 µm long, 600 µm high, 600 µm wide, brown, upper part of ascomata thicker walled, weakly clypeate; ostiole protruding, weakly papillate, ostiole channel +/- 25 µm diam. Peridium surface view textura epidermoidea upper part ascomata, bottom textura prismatica, cross section showing polygonal cells in several strata. Innermost stratum comprising several cells of large rhomboid cells, second stratum of thicker walled, small, brown-yellow cells. Hamathecium filaments scanty, 4 µm diam., septated, as long as asci, tapering, growing from the base of ascogene hyphae. Asci 440-500 x 13-15 µm, very long, cylindrical, tapering at the base, with thimble shaped apical apparatus, uni-biseriate only the ends of the spores crossing. Ascospores 75-100 x 7-11 µm, fusiform- navicular, smooth, at both ends small appendages 2-3 µm long, 6-9 septate, slightly constricted at septa, multi-guttulate.

Ecology: on stems submerged in freshwater (only know from Switzerland).

Studied specimens: VR620, 628, 629, 639

Remarks
K.D. Hyde kindly investigated the material and referred it to *Ceratosphaeria* and not to *Annulatascus* as was initially suggested by us. However the species is in several aspects curious and perhaps not congeneric with *Ceratosphaeria* (see Hyde et al., 1997) (Hyde pers. comm..). Molecular work is really needed. However, several attempts to culture the species on CMA and on sterile reed material remained unsuccessful. Our species is also not included in the work of Tsuda & Ueyama (1977).

Cercophora sp.

Ascomata 700-850 µm high, ovoid, superficial, light brown, granular appearance under the stereomicroscope, superficial brown mycelium at the substrate; peridium with long agglutinated, flexibel hairs (2µm wide, septate) (hyphae) and short hairs consisting out of a few cells. Ostiole black, conical, with periphyses. Asci 250-300 x 13-17, slender, long stipe, unitunicate, with refractive apical ring (± 5 µm wide), IKI^-. Paraphyses abundant, septate, difficult to observe. Ascospores at first geniculate, some straight cylindrical, 60-75 x 6-10 µm, hyaline, with some guttules, staying aseptate for a long time forming an apical cell and the

remaining basal cell often becomes one septated; apical cell, ellipsoid, dark brown, 26-30 x 13-15 μm, with one large guttule; secondary appendages apical and basal, striate, hyaline; apical appendage, long, 40 μm; basal appendage often a short appendage (5 μm), however some spores had also a long basal appendage up to 35 μm.

Ecology: on stems.

Studied specimens: VR310, permanent slide and one perithecium remaining.

Remarks
This species seems new to science, not included in Lundqvist (1972) nor in Hilber & Hilber (1979). Seems near to *C. caudata* (Curr.) Lundq. , however the latter species is characterized by immersed perithecia, smaller ascospores and smaller appendages.

Claviceps microcephala (Wallr.) Tul., Annls Sci. Nat., Bot., sér. 3 20: 49 (1853).

Stromata with stipe and spherical head; multiple perithecia immersed and protruding with ostiole. Ergots (sclerotia) smaller than 1 cm and formed instead of the seed. No detailed microscopically study was performed on the perithecia.

Ergots can be found on the inflorescences during autumn and winter. In an immature state the ergots are often accompanied by their *Sphacelia* state (Figure). Conidia 5-8.5 x 3-4 μm, hyaline, oblong, smooth, aseptate, with small guttules (see Loveless, 1964).
During winter ergots fall on the sediment and produce the stromata next spring (May-June).

Studied specimens: GVR910, on ergot, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', 20/09/2000, IFBL B4.33.43.

Remarks
This species has some strange gap in his life cycle because it is considered that *Claviceps* spp. infect their host by the ascospores during spring flowering and subsequently spread in the plant population by their conidial *Sphacelia* state which is growing on the young sclerotia. This *Sphacelia* state is attractive to insects (honeydew) which help with the spread of the parasite. However the ascomata are mature at a stage the reed is not flowering, this suggest that the species probably has a type of heteroecism before the reed plant is infected similar to the one reported by Stäger (1905) for the form of *Claviceps purpurea* occurring on *Brachypodium sylvaticum*. The ergots formed on this host are dormant during winter and form their stromata in spring. The ascospores produced infect *Millium effusum*, and on this host the conidia of the *Sphacelia* stage are formed but no ergots. Later in the summer, the flowers of *B. sylvaticum* emerge, and

these become infected by the *Sphacelia*-conidia produced on *M. effusum* (Wheeler, 1968: 202). If *Claviceps* is not host specific, several grass species in the vicinity could serve as a primary host.

***Didymella glacialis* Rehm, Hedwigia 21: 121 (1882).**

Ascomata 100-160 μm diam., spherical, brown; ostiole central, 20-30 μm diam., protruding through the epidermis, initially filled with hyaline cells, at the base of ostiole darker, ostiole sometimes very conspicuous, almost as wide as ascomata (125 μm wide x 100 μm high) formed out of hyaline cells making it easy to recover species on substrate (See GVR1687). Peridium greyish-brown, textura unclear +/- textura prismatica or intricata. Hamathecium filaments abundant. Asci 65-70 x 7-8 μm, cylindrical, tapering towards base, with clear foot, bitunicate, 8-spored. Ascospores 13-17(-19) x 3-4 μm, fusiform to ellipsoid, often bended, ends acutely rounded, 1-septate in middle, constricted, 1 cell sometimes wider, widest at the septum, cells biguttulate, with tapering polar slime caps up to 4 μm long (not often seen).

Culture – A7 (GVR834) dried culture; no anamorph formed; perithecia abundant in CMA; mycelium felty, white, with moderate aerial mycelium, slow growth (1 cm week^{-1}). Figures.

Ecology: leaf and leaf sheath *Phragmites australis*; also recorded on *Carex*. Common species which seems to have a higher recurrence in brackish water conditions (tidal or not). This species was also described as *Didymella* sp. by Taligoola (1969) on *P. australis* from a brackish tidal marsh.

Studied specimens: GVR256, 17/02/1999, leaf sheath, Belgium, Oostpolderkreek (Sint-Laureins, Bentille), C2.18.14; GVR834 on leaf blade, 30/08/2000, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43; GVR1687, leaf sheath, 23/05/2001, ibid.

Remarks
Our collections closely resemble the description of the original type (see Kirk & Spooner, 1984) and are in agreement of Corlett's (1981) interpretation of the taxon.

***Didymella* sp.** [*Didymella moribunda* Van Ryck., nom. prov.]
Etymol. referring to the appearance of ascomata during senescence and soon after leaf dead.

Ascomata 200 - 250 μm diam., spherical, dark brown, weakly developed clypeus, immersed; ostiole not papillate nor protruding the epidermis, epidermis seems to be lysed by fungus. Peridium textura angularis, out thin walled cells. Hamathecium filaments up to 8 μm wide, scanty, septated. Asci 65-75 x 12-13 μm, cylindrical clavate, bitunicate, 8-spores, biseriate. Ascospores 15-19 x 6-8 μm, fusiform, ellipsoid with rounded ends, 1 septum slightly submedian or almost median, constricted at septum, some small guttules.

Ecology: on leaves (blades and sheaths) in the basal and middle canopy soon after senescence, during summer and early autumn.

Studied specimens: GVR890, on leaf blade, 20/09/2000, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43

Remarks
We could not find an appropriate name for this species which is most probably an undescribed taxon. However more study and culture experiments to check for an anamorph are needed.
Other resembling *Didymella* species occurring on grasses are, *D. hebridensis* Dennis (spores 17-20 x 4-6 μm), *D. subalpina* Rehm (spores 12-14 x 5 μm), *D. exitialis* (spores 12-17 x 4-5.5 μm, median to slightly submedian septum) , *D. phleina* (spores 16-18 x 4-5 μm, median to slightly submedian septum), *D. eupyrena* (spores 18-21 x 6-8 μm, septate below the middle, pseudothecia thick-walled and larger, considered to be restricted to Urticaceae (Corlett, 1981)), *D. asphodeli* (spores 16-19 x 5-6, septate below the middle).

Didymella proximella (P. Karst.) Sacc. Syll. fung. I: 558 (1882).

Ascomata up to 300 μm long, 250 μm wide and high, compressed in length direction of stem between large vascular bundles, solitary or sometimes aggregated and than fruit bodies interconnected with clypeoid, blackened tissue; ostiole lysigenous, at first with hyaline periphyses, seen as yellow spots on fruit bodies, not papillate, 35-45 μm diam. Peridium textura prismatica to textura angularis, pale brown, 10-15 μm thick, cells about 7 μm diam., smaller and more thick-walled cells near ostiole, clypeus dark brown. Hamathecium filaments abundant, filiform, 1-1,5 μm wide. Asci 85-90 x 13-15 μm, clavate, with short stipe, bitunicate, biseriate. Ascospores 20-25,5 x 6-8 μm, ellipsoid to fusiform, often slightly curved, with one septum mediane to slightly under the middle, constricted, upper cell broader and mostly also longer, hyaline, cells biguttulate, smooth, no slime sheath.

Studied specimens: VR415 (drawing and pictures available)

Remarks
This is the largest of the three observed *Didymella* species on *P. australis* during our research. Our observations fit well with description of the species (Corlett, 1981); fruitbodies were somewhat larger than mentioned in literature and hamathecium seem more filiform than presented in Corlett & Smith (1978). *D. proximella* has a *Stagonospora*-like anamorph (Corlett & Smith, 1978). Conidia hyaline, fusiform to broadly fusiform, 22.5-34 x 6.5-9.5 μm, straight or slightly bent, rounded at apex, 2-3 septa, slightly constricted. This anamorph was not observed on *P. australis* during our research.

***Discostroma* sp.** [*Discostroma intitia* Van Ryck., nom. prov.]
Etym. referring to the fact this species is one of the first to sporulate on reeds after emergence.

Ascomata 200- 250 µm diam., spherical flattened, black, immersed; clypeus present (not always clear), black; ostiole central, protruding, at first filled with hyaline cells. Peridium unclear textura angularis (epidermoidea?) becoming textura prismatica near ostiole, thickwalled carbonaceous, exterior with lots of hyphae. Hamathecium filaments 3µm wide, abundant, septate, hyaline together with asci in common subhymenium, with free apex, with small guttules. Asci 45-50-70-75 x 7-8 µm, IKI^+, cylindrical, conical apex, no stipe, apical plug 1.5 µm wide, ascospores biseriate. Ascospores 12-17 x 2.5-3.5 µm, fusiform to clavate, in ascus long aseptate but becoming 1-septate, not constricted at the septa, hyaline, with very small guttules, no slime coat.

Ecology: On the tough bud scales surrounding the conical stem points and on the most lower (still green or moribund) leaf sheaths of *Phragmites* during first three months of the growing season (parasite).

Studied specimens: VR577, VR582, VR719, VR728

Remarks
Although not rare, most of the found ascomata are in poor condition. It seems this species is often able to grow on the reed but has difficulties to become mature and sporulate. The ascomata are often found with lots of oil(?) drops inside. Spores resemble those of *Scirrhia rimosa,* another species occurring on *P. australis.* However this species is totally different in many aspects (bitunicate, larger asci, pseudothecia in large stromata). *S. rimosa* was never found be me; notwithstanding it should be common according to Dennis (1981).
Our specimens match well with the description of *Sydowiella juncina* Spooner [in Kirk & Spooner, 1984] except for the fact our collections had IKI^+ reaction of the apical apparatus and paraphyses with a slightly enlarged tip instead of a slightly narrowed tip. We should compare our collections with the type-collection of *S. juncina.*
For the time being, taking the lugol reaction as diagnostic, this species fits best in the generic concept of *Discostroma* (Barr, 1990). However no species within this genus seems to match with our observations.

Gibberella zeae (Schwein.) Petch, Ann. Mycol. 34: 260 (1883).

Ascomata 200-220 µm diam., black-shinning, superficial, in small group; excipulum violet-purple coloured under the microscope, textura angularis. Ostiole 15µm diam., weakly papillate. Asci 60-65 x 10-12 µm, clavate, unitunicate, 8-spored, short stipe, IKI^-. Hamathecium filaments with inflated cells up to 15 µm wide, constricted at septa, hyaline. Ascospores 20-25

x 4-5 µm, fusiform, hyaline, becoming 3-septated, slightly constricted, aguttulate, bi-triseriate in ascus.

Ecology: on leaf sheaths and stems in the litter layer; sporadic on *Phragmites*.

Studied specimens: VR279

Halosphaeria hamata (Höhnk) Kohlm., Can. J. Bot. 50: 1956 (1972).

Synonym: *Remispora hamata* (Höhnk) Kohlm. (1961).

Ascomata 130-200 µm diam., immersed, hypersaprotrophical, often in old perithecia of *Massarina arundinacea*, solitary, pyriform; peridium excipulum textura angularis, dark coloured when solitary in substrate but peridium light coloured when growing protected in old perithecia of other species. Ostiole variable, up to 500 µm long and 50 µm wide; mostly 100-200 long, 20-27 µm wide, sometimes absent and easily lost, subhyaline till brownish, central; periphyses present. Asci 40-55 x 25-30 µm, clavate, short stipe, deliquescing vary rapidly at maturity leaving mass of lose ascospores in the ventral cavity of the perithecium. Ascospores 19-30 x 7-12 µm, hyaline, 1-septate, with one large guttule and some small in each cell; appendages 5-10 µm, at both sides, formed by loosening of epispore, at first cap-like and later like thread-like hooks.

Culture deposited in MUCL (MUCL 45117).

Ecology: common on reed material in a later phase of decay. The taxon was found along the complete salinity gradient in the Scheldt estuary and in all other wetlands visited which had a historical connection to the estuary.

Studied specimens: VR101; GVR2826, 2/05/2002, on leaf blade, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43

Remarks
Halosphaeria spp. would have a *Periconia* anamorph (Kohlmeyer, 1972). Several *Periconia* species were found on *P. australis*, however no anamorph was formed in our culture collections nor could we suspect a *Periconia* anamorph for *H. hamata* from our field observations.

Hydropisphaera arenula (Berk. & Broome) Rossman & Samuels, Stud. Mycol. 42: 30 (1999).
Basionym: *Sphaeria arenula* Berk. & Broome, Ann. Mag. Nat. Hist. Ser. 2, 9: 320 (1852)
= *Nectria arenula* (Berk. & Broome) Berk., Outl. Brit. Fung. : 394 (1860)
= ?*Nectria graminicola* Berk. & Broome, Ann. Mag. Nat. Hist. Ser. 3, 3: 376 (1859)

Ascomata 300-400 μm diam., perithecia, orange-yellow, supperficial, few together, perithecia dry discoid with papillate central ostiole. Peridium two layered, outer out of larger hexagonal to globose cells, inner wall out of smaller hexagonal cells. Hamathecium filaments absent. Asci 57-75 x 9-10 μm, conical, tapering to top, widest part in the middle, with small refractive apical apparatus (lugol reaction not recorded), biseriate. Ascospores 17-23 x 4-5 μm, fusiform, straight to slightly bended, one median septum, slightly constricted, apical cell slightly or not enlarged, smooth, hyaline, mostly two guttules in each cell.

Studied specimens: VR739, VR765

Remarks
We named this species initially *Nectria graminicola*. However *N. graminicola* is propably a later name of *Nectria arenula* based on the suspicion of Booth (1959) and his very similar description and illustration.

Keissleriella culmifida (P. Karst.) Bose, Phytopathol. Z. 4: 188 (1961).

Ascomata 200-220 μm diam., immersed, spherical, dark brown; peridium, excipulum textura angularis out of thick-walled cells; many hyphae at the outside; some greenish-dark grey appearance under microscope. Ostiole papillate, dark mouth, sometimes lighter (ochre), with hyphae surrounding the ostiole having tendency to form a clypeus; with periphyses. Bristles surrounding the ostiole mouth, 25- 40(-80) x 4-6 μm wide at base, dark brown, thick-walled, aseptate, pointed. Hamathecium filaments few. Asci 55-80 x 9-13 μm, bitunicate, ellipsoid, short stipe, widest under the middle, 8-spored, biseriate. Ascospores (13-)16-26 x (4)-5-6 μm, fusiform, cylindrical, hyaline to light yellow, 3-septate, constricted, widest at the second cell, often in each cell one large guttule and several small, occasionally a mucous sheath (2-3 μm wide), entire.

Ecology: on leaf sheaths and stems in the litter layer

Studied specimens: VR131, VR505

Keissleriella linearis E. Müll., Kew Bull. 19: 120 (1964).

Ascomata 300-400 μm diam., at first immersed and visible as white spots on the stems later erumpent, somewhat elongated along the long axis of the substrate; ostiole weakly papillate, surrounded by bristles, up to 70 μm long x 5 μm wide at base, dark brown, rounded at apex, abundant; clypeus weakly developed. Peridium textura prismatica. Hamathecium filaments 2 μm wide, abundant, branching, anastomosing, hyaline, slender, with guttules. Asci 120-125 x 14- 17 μm, clavate, pedicel (5-10 μm), bitunicate, biseriate. Ascospores 34-40 x 6.5-9 μm,

fusiform, straight to slightly curved, 1-3 septate (2:1:2), deeply constricted at median septa, less at secondary septa; mucilaginous coat tight, entire, at first constricted in the middle and swelling in water (like *Massarina aquatica* see below); central cells 1 large guttule, outer cells 2 large guttules and often some small ones; senescent spores yellow-brown becoming echinulate, often gathered on top of ostiole on bristles.

Anamorph: there are good indications that *Stagonospora vexata* (see coelomycetes) is the anamorphic state of *K. linearis*. Both taxa were observed several times in close association and culture characteristics of both taxa were similar. However, the connection could not be confirmed because cultures remained sterile. In literature the anamorphic state of *Keissleriella* proves to be placed in several Phoma-like genera (*Dendrophoma*, *Phoma*, *Ascochyta* (hyaline 1-septate conidia)). This could fit with our observation because *Stagonospora vexata* has also a microconidial state which is Phoma-like. However other taxa mentioned to be anamorphic states of *Keissleriella* species are *Dinemasporium* sp. and *Diplodia* sp. Those latter connections could be considered suspicious.

Ecology: abundant on standing dead stems *P. australis* being in a standing dead position more than 1.5 years. In moist chamber fruit bodies were found to develop on leaf sheaths. Occasionally found on (fallen) stems in the litter layer.

Studied specimens: GVR670, 27/06/2000, stem, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43 (no exsiccatum); VR802

Remarks
Notice the resemblance of this species with *Massarina aquatica* in spore morphology. In general the spores of *M. aquatica* appear to be smaller (up to 7 µm). Furthermore, the ascomata seem larger and more superficial, hymenium has a yellowish appearance when the fruit body is cut under the stereomicroscope and bristles are absent from the latter species. However one should not focus too much on the bristles, they could be immature or absent on older fruit bodies of *K. linearis*. *M. aquatica* seems to be restricted to stems in the litter layer.

'Lasiosphaeria' sp.– *Ruzenia taligoolia* nom. prov**.**

Etymol. in honour of H.K. Taligoola who was a pioneer in the research on phragmiticolous fungi and the first to make a provisional description of this unknown species

Ascomata 300-500 µm diam., superficial, black, spherical; peridium relatively thick-walled, with dark brown thick-walled hyphae, some with crystals on the outside, mainly fringed around the base of the perithecium. Ostiole ± 25-30 µm inner diam., papillate, black. Hamathecium

filaments 2-3 µm wide, abundant, septated. Asci 100-120 x 10-15 µm, cylindrical, short pedicel, unitunicate, 8-spored, with a typical mushroom shaped apical apparatus, IKI$^-$. Ascospores 27-32 x 5-7 µm, allantoid, hyaline, entirely filled with small guttules, aseptate, biseriate, no appendages.

Ecology: on leaf sheath and stem *Phragmites australis*.

Studied specimens: VR126.
Exsiccatum in bad condition, only young perithecia present?
Additional collections without herbarium material: all on the same date, 1 second collection Roeslarekreek and 1 collection Molenkreek.

Remarks
We should consult (however not in our library) Hilber R. & Hilber O. 2002 and Miller A.N. 2003. [Phylogenetic studies in the Lasiosphaeriaceae and the key genus, *Lasiosphaeria*. - University of Illinois at Chicago and Field Museum, PhD, Chicago, 222 pp.]

Recent molecular work confirmed the paraphyletic constitution of the genus *Lasiosphaeria* s.l. (Miller & Huhndorf, 2004). Based on spore morphology our specimen looks to belong to the newly establish genus *Ruzenia* (with *Lasiosphaeria spermoides* as type species). However we should study the ascomatal wall morphology in more detail. To our knowledge only one '*Lasiosphaeria*' is mentioned from grasses, *Lasiosphaeria dactylina* Webster, but this species looks very different from our collections (Hilber et al., 1987).
The same species was found on *Phragmites* by Taligoola (1969) and named as *Lasiosphaeria* sp.
Description Taligoola (1969):
Perithecia globosa or subglobosa, 460-530 x 420-500 µm, black with thick hairs; they are partly immersed or superficial and may be aggregated. The neck is short and conical. Asci cylindrical-clavate, 100-120 x 8-10µm, uniseritate to biseriate, the apex does not stain in Meltzer's iodine. They are expelled in a cluster when a perithecium is squashed. Ascospores allantospores, 1-celled, 25-33 x 5-8 µm, hyaline, smooth
Occurrence on highly decomposed submerged parts or litter at Attenborough; quite often occurring together with Ophiobolus sp.

Lewia infectoria (Fuckel) Barr & Simmons, in Simmons**,** Mycotaxon 25: 294 (1986).

Ascomata 200-240 µm diam., spherical, black, immersed, aggregated. Ostiole indistinct, small, black, bordered with hyaline cells (5 µm diam.). Peridium textura angularis, relatively thick-walled. Pseudoparaphyses +/- 3µm wide, hyaline, with septa, slightly or not constricted at septa. Asci 85-90 x 12-13 µm, clavate, short-stiped, 8 spores (sometimes 4-spored), bitunicate, biseriate. Ascospores 18-22 x 7-8 µm, broadly elliptic, muriform, becoming 5-septate (3

primary septa), only end cells not longitudinally septated, constricted, yellow-brown, with entire mucous sheath.

Studied specimens: VR121

Lophiostoma cf. appendiculatum Fuckel, Jb. nassau. Ver. Naturk. 27-28: 29 (1874) [1873]
=? Lophiostoma appendiculatum Niessl

Ascomata up to 500 µm long, 100 µm wide, largest part of fruit body immersed; peridium black, relatively thick-walled. Ostiole protruding the epidermis, small, moderately compressed. Hamathecium filaments 2.5-3 µm wide, abundant, hyaline, anastomosing, septated every 20-30 µm, not constricted. Asci 135-140 x 13.5-21 µm, cylindrical, with pedicel, bitunicate. Ascospores 33-42 x 9-11 µm, at first pale brown, becoming darker when older, end cells slightly paler, fusiform, slightly curved, strongly guttulated, 5-septate, constricted at septa, smooth, primary septum supramedian, third cell swollen, smooth; appendages at both ends, 1-2 µm long, rounded, hyaline, not coloured in kongo red.

Studied specimens: VR711

Description of this species is similar to the interpretation of *Lophiostoma appendiculatum* by Holm & Holm (1988); however *L. appendiculatum* is considered to be mostly 7-septate. Our collection differs from *Lophiostoma caulium,* a variable taxon, by the longer and wider ascospores, the small appendages and the relatively light coloured ascospores. It differs from L. *arundinis* and from *Lophiostoma* sp. (see under *L. arundinis*) by the larger and wider ascospores.

We could isolate the species (VR711) and in culture a *Pleurophomopsis* anamorph (see Leuchtmann, 1985; figure) was formed (permanent slide available). Conidiomata mostly superficial on agar (CMA). Conidiophores short, 1-2 septated up to 40 x 2 µm long, with sympodial conidiogene cells, sometimes conidiogene cells sessile on the peridium cells. Conidiogene cell 8-15 x 3-3.5 µm, cylindrical, tapered, phialidic, sometimes a clear collaret. Conidia 2-3 x 1-1.5 µm, hyaline, ellipsoid-cylindrical, smooth, aguttulate.

Lophiostoma arundinis (Pers.) Ces. & De Not. Comment. Soc. Critt. Ital. 1: 220 (1863).

Ascomata 500-1000 µm long, ± 500 µm wide and ± 750 µm high, half-immersed, at first subepidermal, later clearly visible, bean-shaped, variable, black, sometimes in short rows; peridium thick-walled, slightly carbonaceous. Ostiole with a strong flattened papilla in the length direction of or in a slight angle with the host stem, sometimes several ostioles for one perithecium. Hamathecium filaments abundant, thin, filiform, hyaline, septate, not constricted. Asci 120-143 x 11-13 µm, cylindrical, clavate, bitunicate, 8-spored, biseriate. Ascospores 29-43

x 6-8.5 µm, fusiform, 5-septate, constricted at septa, at first hyaline but soon brown within the ascus, end cells often conspicuously paler, slightly curved, with one large and several small guttules in each cell, sometimes with little mucous caps (? see remarks).

Ecology: on stems, often on basal parts of standing dead stems.

Studied specimens: VR56, VR586; VR782, VR801

Remarks
This taxon enholds records probably belonging to two species. However, we recognized the distinct taxa in a later phase of the research. Therefore, the data referring to this taxon in our Ph.D. should be interepreted with care and refers to *L. arundinis sensu lato* (i.e. two distinct species). During writing this down it was found that Leuchtmann (1985) also noticed the excistence of two separate taxa. We recognize a species with smaller ascospores (26-35 x 6.5-7.5 µm), with more rounded endcells, which are not paler and with sometimes mucous appendages (*L. arundinis sensu stricto*) and a species with larger ascospores (32-43 x 7-8 µm) with tapered and lighter coloured endcells (*Lophiostoma* sp.). Both species occur together on *Phragmites*.
Culture: VR586. Growth characteristics in culture differ between the two species (Leuchtmann, 1985). Leuchtmann found that *L. arundinis sensu stricto* grows slowly in culture and does not form any anamorph. *Lophiostoma* sp. would grow faster and produce a *Pleurophomopsis* anamorph but no teleomorph in culture. However contrary to Leuchtmann, we found no anamorph and only a teleomorph after culturing the *Lophiostoma* sp. (sensu Leuchtmann, 1985). We did not culture *Lophiostoma arundinis sensu stricto*.

L. arundinis sensu stricto (VR782 this collection showed small ascospores in the range up to 34 µm and with slime caps on the spores!).
Lophiostoma sp. (sensu Leuchtmann, 1985) seems more common (VR586, VR801).

Lophiostoma caudatum Fabre, Annls Sci. Nat., Bot., sér. 6 **9**: 66 ? or 103? (1878).

Ascomata 500-600 µm diam. longer than wide, semi-immersed, black, often in rows. Ostiole flattened in the length of the fruit body. Hamathecium filaments, 2µm wide, abundant, branched, interwoven. Asci 70-115 x 12-14 µm, clavate, bitunicate, 8-spored with spores biseriate at the top and becoming uniseriate in the lower part. Ascospores 25-30(-34) x 6-7 (8) µm, widest in the middle, with broadly rounded apex and tapering base, yellow-gold brown, 5-6-septate, slightly constricted, sometimes a small slime appendage (1.5-2 µm long) is visible at the basal end.

Ecology: on stems in litter layer.

Studied specimens: VR102: good collection

Remarks
This species is less common than *Lophiostoma arundinis* and *L. semiliberum* and restricted to oligohaline and fresh water conditions. The spores resemble those of *"Buergenerula" typhae* however the latter has generally larger spores with a small cellular appendage.

Lophiostoma semiliberum (Desm.) Ces. & De Not., Comment. Soc. Critt. Ital. 1: 220 (1863).

Ascomata 500-1000 µm long, ± 500 µm wide and 400-750 µm high, ellipsoid, black, semi-immersed. Ostiole flattened laterally in length of the perithecium. Hamathecium filaments abundant, filiform, 1.5-2 µm wide, sometimes septate. Asci 100-130 x 12-15 µm, cylindrical-clavate, thick-walled, 8-spored. Ascospores 28-39 x 6-8 µm, fusiform, hyaline, 1-septate (rarely seen 3-sepate), strongly constricted in the middle, with 8-10 large guttules, sometimes small polar slime caps.

Ecology: on stems in the litter layer and on basal standing parts of dead stems. Often together with *L. arundinis* s.l.

Studied specimens: VR40, VR779

Remarks
On one field collection (Van Ryckegem 779 (GVR3202)) this common species was associated with a *Pleurophomopsis* anamorph (see Picture) formed within the perithecium near the ostiole while asci and mature spores where present within the perithecium. The anamorph agrees well with the description of the *Pleurophomopsis* state separately isolated on culture medium by Leuchtmann (1985).
Some authors (e.g. Eriksson, 1967) regarded *L. semiliberum* as immature states of *L. arundinis*. However the difference between the two species is clear (see also Chesters & Bell, 1970; Leuchtmann, 1985). Spores of *L. arundinis* become brown within the asci and are soon multiseptate. Spores of *L. semiliberum* stay hyaline (or if dead they turn brown) and are at most 3-septated. The confusion probably originates from the fact that all *Lophiostoma* species can grow in close association with each other on stems of *P. australis*.

Magnisphaera spartinae (E.B.G. Jones) J. Campb., J.L. Anderson & Shearer, Mycologia 95: 547 (2003).

Basionym: *Haligena spartinae* E.B.G. Jones, Trans. Brit. Mycol. Soc. 45: 245 (1962).
Synonym: *Halosarpheia spartinae* (E.B.G. Jones) Shearer & J.L. Crane, Bot. Mar. 23: 608 (1981).

Ascomata 250-350 μm diam., at first immersed later lifting up and splitting the epidermis, spherical, black, thick-walled, solitary. Ostiole papillate. Hamathecium filaments absent? Asci 140-150 x 37-45 μm, clavate, short stipe, unitunicate, deliquescing vary rapidly at maturity, IKI$^-$. Ascospores 47-66 x 15-16 μm (without appendages), ellipsoid, hyaline, 3-5 septate, at maturity 5 septate, constricted at septa; one median septum and two septa close to each other at both ends; central cells multiguttulate, the small apical and the basal cells with few guttules; appendages 7-11 x 0.5-1 μm, at both ends, first more cap like but becoming tapered and soon disappearing in mineral water, hyaline.

Studied specimens: VR306; Van Ryckegem (1999) nr. 32, master thesis: 27/03/1999 Belgium, Scheldt estuary, Burcht (Schor bij het Galgenweel), IFBL C4.26.31 (no exsiccatum, figure)

Remarks
Considered to be a marine ascomycete (Kohlmeyer & Kohlmey, 1979); originally described from *Spartina townsendii*. Occasionally on stems in the litter layer in brackish environment. However this species seems to be able to occur in rather freshwater conditions as it was recently recovered from *Typha* in a freshwater wetland (De Meulder, 2002). However this wetland was connected in earlier days with the Scheldt estuarine ecosystem.

Massarina aquatica J. Webster, Trans. Brit. Mycol. Soc. 48: 451 (1965).

Ascomata variable up to 1 mm long and 0.5 mm high, ellipsoid, linear on the substrate, at first immersed with black clypeus and later almost superficial; peridium relatively thick-walled, carbonaceous; if perithecia are cut open a typical yellowish hymenium is observed. Ostiole weakly papillate or more lysogenous, sometimes more than one ostiole per perithecium. Hamathecium filaments 1.5 μm up to 3 μm wide, septate, hyaline, abundant. Ascospores 25-37 x 4,5-6(-7) μm, fusiform, at first 1-septate, hyaline, becoming 5-septate, constricted at the median septum and becoming more and more constricted during maturation, sometimes spores break up in two parts, if overmature spores turn brown and echinulate, with two large guttules and several small; slime coat obvious, up to 5 μm around the spores (swelling in mineral water); constricted in the middle, spores bi- to triseriate in ascus.

Ecology: on stems in the litter layer or in the water.

Anamorph: *Tumularia aquatica* (Ingold) Descals & Marvanová. This anamorph was not seen on our collections.

Studied specimens: VR263

Remarks

See *Keissleriella linearis*.

Massarina arundinacea (Sowerby: Fr.) Leuchtm., Sydowia 37: 179. (1984).

Ascomata 150-300 µm, black, spherical-pyriform, often solitary on leaf blades and sheaths and densely grouped in potentially long rows aggregated in lose stromata tissue; on stems perithecia develop slowly, at first below the epidermis and erumpent to nearly superficial in a later phase; on leaves perithecia immersed; peridium thick-walled, 15-26 µm, dark greyish-black cells; ostiole short papillate. Hamathecium filaments hyaline, with guttules, abundant, septate (cellular), not constricted. Asci 125-145 x 13-15 µm, clavate, broadly rounded, with a short stipe and a clear foot, 8-spored, bi-triseriate. Ascospores 22-33(-38) x 4-5(-6) µm, fusiform, slightly curved, with median septum, constricted, above median septum spores slightly enlarged, becoming first 2-septate with additional septum in the upper cell and later sometimes a third septum in the lower, more tapered cell, hyaline to yellowish when (over)mature; slime coat entire if material fresh, eventually coat subsides and forms a slime appendage at the base of the spore. Spores germinate at both ends.
Culture (VR829): no anamorph formed

Ecology: one of the most common species on stems of P. australis in the litter layer and on basal parts of standing stems. Occasionally this species can be found on leaves (sheaths and blades) (see figure for details during succession).

Studied specimens: VR344; VR635, VR829

Remarks
Massarina phragmiticola Poon & K.D. Hyde (1998) looks similar and was not compared with *M. arundinacea* in the discussion of the type. It could prove to be a synonym of *M. arundinacea.*

Massarina fluviatilis Aptroot & Van Ryck., Nova Hedwigia 73: 162 (2001).

Ascomata 180-210 µm diam., 180-200 µm high, (sub)globose, immersed, black, clustered in small groups or short rows with protruding, central, black, inconspicuous, small papillate ostiole; clypeus black out dark brown hyphae 1-1.5 µm diam. Peridium 20-25 µm thick, composed of carbonaceous thick-walled, polygonal cells, with hyaline hyphae at the exterior. Pseudoparaphyses numerous, 3-4 µm diam., septate at 7-8 µm, not branched or anastomosing. Asci 69-82 x 18-23 µm, oblong to cylindric, broadly rounded at apex, tapering to short stipe, bitunicate, with irregularly bi-triseriate ascospores. Ascospores 24-31 x 7-10 µm, broadly fusiform with rounded ends, usually 2-septate, becoming 3-septate (2:1:3); first septum submedian, second cell most swollen; ascospore constricted at septa mainly at median septum,

mostly hyaline, browning with age; with many, small guttules, surrounded by a wide expanding mucilaginous sheath, which is up to 5 µm thick, not constricted median.
Host: *Phragmites australis* (Cav.) Steud.

Ecology: found in fresh and brackish tidal marshes, on dead leaf sheaths still attached on standing one year old reed stems. At a height of approximately 1,5 m to the surface.

Studied specimens: VR509 (TYPE); VR750

We should compare *M. fluviatilis* in detail with the type of *Leptosphaeria contecta* Kohlm.

Massarina sp III cf. *Phaeosphaeria albopunctata* (Westend.) Shoemaker & C.E. Babc., Can. J. Bot. 67: 1566 (1988).

Ascomata 300-350 µm diam., dark brown, slightly lifting the epidermis, solitary in rows; ostiole circular not papillate, +/- 50 µm diam. Peridium textura angularis, thin-walled, light brown. Hamathecium filaments 4 µm diam., septated, cellular, anastomosing, hyaline. Asci 155- 25 µm, bitunicate, short stipe, apicaly thickened. Ascospores 42-57 x 10-13 µm, hyaline becoming brown when senescent, fusiform, 3-5-septate, sometimes 7-septate, primary septum submedian (0.7-0.8), 2nd or 3th cell broadest, constricted at septa (fresh!); slime coat sharply delimited, entire, 5-6 µm wide.

Ecology: Together with *Stagonospora elegans* on upper part of culm on leaf sheath.

Studied specimens: VR766

Remarks
This species is perhaps conspecific with *Phaeosphaeria albopunctata*! If so *P. albopunctata* should be transferred to *Massarina*.

Massariosphaeria grandispora (Sacc.) Leuchtm., Sydowia 37: 172 (1984).

Ascomata 300-500 µm, immersed, black, bean-shaped, in rows but not in a stroma, growth colours the substrate red; peridium thin-walled at the base, thicker-walled near the ostiole, textura unclear; ostiole lenticular, not papillate. Hamathecium filaments cellular, 3 µm wide, septate, hyaline, anastomosing. Asci 102-119 x 15-20 µm, clavate, short stipe, clear foot, bitunicate, 8-spored, bi-triseriate. Ascospores 33-36 x 7-8 µm, fusiform, straight or bended, hyaline to pale yellow (when overmature also with echinulate appearance), 7-8-septate, constricted, broadest at the 4th cell, multiple small guttules; slime coat entire, up to 5 µm wide.

Studied specimens: VR54

Remarks
Shoemaker & Babcock (1989) recombines this species in *Lophiotrema*. However I would keep this species in *Massariosphaeria* for the time being because Shoemaker & Babcock missed the literature of Holm & Holm (1988). The latter authors amended the heterogenous genus *Lophiotrema* and pointed a specific peridium structure with a clear textura angularis and hyalofragmospores with maximally 3 septa for this genus. However the slit-like ostiole observed could be an argument to place *M. grandispora* within *Lophiotrema* while the reddish colouration of the host resource, is a common characteristic for *Massariosphaeria* colonization.

Massariosphaeria mosana (Mouton) Leuchtmann, Sydowia 37: 170 (1984).

Ascomata up to 700 µm long, 450-500 µm wide, black, semi-immersed, large, relatively thick-walled, no reddish colouration of the substrate was observed; ostiole central, circular, not papillate. Peridium textura prismatica, brownish black, thick-walled. Hamathecium filaments 2 µm wide, septate, hyaline, branched, abundant. Asci 100-120 x 13-20 µm, cylindrical-clavate, bitunicate, abundant, 8-spored, biseriate. Ascospores 35-45 x 7-10 µm, clavate, fusiform, basally more tapered, pale brown to dark brown, echinulate, 7-8 septa, fourth cell widest, with several guttules in each cell; slime coat conspicuous, entire, swelling and appearing sometimes with pointed ends (? artefact), 5-6 µm wide.

Ecology: on stems and leaf sheaths in the litter layer of freshwater habitats

Studied specimens: VR634, VR788

Remarks
This species comes close to *M. typhicola* but differs in having thick-walled and larger ascomata.

Massariosphaeria palustris (E. Müll.) Leuchtm., Sydowia 37: 171 (1984).

Ascomata about 200 µm diam., immersed, spherical to bean-shaped, black; substrate is purplish red coloured; ostiole short, papillate. Hamathecium filaments abundant, hyaline, septate. Asci 115-130 x 30 µm, few, clavate, bitunicate, 8-spored, bi-triseriate. Ascospores 35-45 x 11-13 µm, ellipsoid-fusiform, with rounded ends, light brown, minutely echinulate, 6-8 septa, constricted, with several guttules in each cell; slime coat entire, not very obvious.

Studied specimens: VR313

Only one collection conforms to Leuchtmann's (1984) description. Taxon differs from *M. typhicola* by the wider ascospores (perhaps this will prove to be an extreme form of the already very variable *M. typhicola*).

Massariosphaeria sp. [*Massariosphaeria* (*Chaetomastia*?) *aquadulcis* Van Ryck. nom. prov.]
Etymol. name refers to the fresh water environment we found this species to be very frequent

Ascomata 300-600 µm long, 250-400 µm wide, +/- 300 µm high, bean-shaped, elongated in the direction of the substrate axis, black, immersed, lifting up the epidermis; ostiole 30 µm inner diam., becoming papillate (sometimes more than one ostiole per ascoma), black, periphysate. Peridium textura prismatica, thin-walled, smooth, black to greyish cells on average 7 µm long. Hamathecium filaments abundant, 1,5 µm wide, septate, cells 15-20 µm long, not constricted, hyaline, anastomosing. Asci 100-110 x 10-12 µm, cylindrical-clavate, widest part subapical, short stipe with clear foot, bitunicate, biseriate. Ascospores 26-33 x 6-7,5 µm, fusiform, mostly slightly bended, becoming 5-septate (sequence 3:2:1:2:4), primary septum supramedian (lower part about 1-2 µm longer), constricted at median septum, cell above median septum enlarged, basal part more tapered, echinulate, rust-coloured, often with sightly paler end cells, one large guttule and sometimes some additional small ones per cell; slime coat entire, inconspicuous.

Ecology: on stems laying in the litter layer or submerged in fresh water.

Studied specimens: VR339, VR337, VR412, VR490, VR692, VR708, VR789

Remarks
There is no red colouration of the substrate.
Massariosphaeria typhicola (P. Karst.) Leuchtm., Sydowia 37: 168 (1984).
= *Chaetomastia typhicola* (P. Karst.) Barr, Mycotaxon 34:514 (1989)
= *Leptosphaeria baldingerae* Fautr. & Lamb., Rev. Mycol. 19: 3 (1897).

Ascomata 200-400 µm diam., immersed, spherical to bean-shaped, black, causing a red colouration of the substrate. Peridium not studied in detail. Ostiole short, papillate. Hamathecium filaments abundant, hyaline, septated, cellular. Asci 85-120 x 15-19 µm, abundant, clavate, cylindrical, bitunicate, 8-spored, bi-triseriate. Ascospores 28-50 x 5.5-10 µm, ellipsoid, fusiform, with rounded ends, light brown, densely echinulate, 6-8 septa; slime coat entire, several guttules in each cell.

Culture: (VR617) No anamorph observed; perithecia developed but remained sterile
Anamorph: *Aposphaeria*-like (Leuchtmann, 1984)

Studied specimens: VR334, VR617, VR622; GVR3156, 30/09/2002, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43; GVR3367, 26/02/2003, Belgium, Scheldt estuary, freshwater tidal marsh, Het Kijkverdriet, Steendorp (Temse), IFBL C4.54.11

Remarks
Barr (1989) included *Massariosphaeria* in *Chaetomastia*. Not so much on account of objections against the fusion but more for a reason of convenience I keep *Massariosphaeria* in the sence of Crivelli (1983) and Leuchtmann (1984). The only drawback to follow the concept of Barr (1989) is the fact the genus concept has a (too?) wide interpretation.
The red coloration of the host resource by *M. typhicola* is very obvious! The species is plurivorious, occurring on a whole range of monocots (we have personal records from *Typha* and *Juncus*) and in close association with fresh water. On top of the ostiole it is possible to observe the expulsion of whole asci, indicating the spores are possibly initially dispersed within the ascus.

The species referred to by Taligoola et al. (1972) as *Leptosphaeria culmifraga* shows very similar ecology to what we determined as *M. typhicola* and is perhaps the same species.

Metasphaeria coccodes (P. Karst.) Sacc. *sensu* Munk (1956: 304).

Ascomata 200-350 µm diam., solitary or aggregated in small groups, no stromata or clypeus was seen; dark brown, spherical; ostiole central, round, not papillate, porus in epidermis. Excipulum 10-15 µm thick, cells relatively thick-walled (up to 1 µm); textura unclear, near ostiole textura prismatica, up to textura angularis; basally more a textura intricata and even textura epidermoidea. Hamathecium hyaline, septate (probably cellular), not very clear. Asci 75-80 x 16-18 µm, bitunicate, slightly thickened at apex, broad-oblong, rounded basally, no stipe, spores tri-tetraseriate. Spores 21-28 x 4-5 µm, hyaline, fusiform straight or slightly bented, swollen above the median septum, first 1-septate, becoming 3-septate; first septum supramedian to median; no appendages or guttulation could be observed in the herbarium collection.

Studied specimens: Ascomycete sent to me by T. Læssøe, collected by B. Spooner (Herbarium? KEW?)

Remarks
This species resembles very much the taxon that was described by Munk (1957) as *Metasphaeria coccodes* (P. Karst.) Sacc. (Basionym *Leptosphaeria coccodes* P. Karst.) and pointed as type of *Metasphaeria* Sacc. However *Metasphaeria* was already typified by Clement & Shear (1931) by *M. sepincola*, now called *Saccothecium sepincola* (Fr.) Fr. making *Metasphaeria* a "nomen nudum" (it was originally used as a form genus with hyaline phragmospores). *M. coccodes* was recombined by Eriksson and Yue (1986) in the genus *Massarina*. *M. coccodes* (P. Karsten) O.E. Eriksson & J.Z. Yue was not recognized in the latest review of the genus *Massarina* by Aptroot (1998). Aptroot states that this species is a *Lophiostoma*, related to or identical with *L. semiliberum* (Desm.) Cesati

& De Not. and noticed that the ostioles vary between round and slit-like to even somewhat branched.

The species described by Munk (1956, 1957) is not conspecific with the original type collection of P. Karsten (and seen by Eriksson & Yue, 1986 and Aptroot, 1998). He did not see the authentic material (Eriksson, 1967).
Remains the question about our species, which resembles very closely the description of Munk (1957) and is certainly not a *Lophiostoma*. This species could be a *Massarina* species or if additional septa were not formed we could think of a species close to *Didymella*. For the moment being it's not clear to me in what genus this species belongs and it is termed here as "*Metasphaeria coccodes* (P. Karsten) Sacc." sensu Munk (1956, 1957).

Microthyrium nigroannulatum J. Webster, Trans. Brit. Mycol. Soc. 35: 208 (1952).

Ascomata catathecioid 70-80 μm diam. superficial, circular, flattened, not more than 50 μm high, brown, with dark black ring built out of cells with thick cell walls surrounding the ostiole. Ostiole circular, about 7-10 μm diam. Asci 20-24 x 6-7 μm, apex thick walled, thickest in the middle, ovoid, 8-spored. Ascospores 7-9 x 2μm, aseptate or with one median septum, fusiform, hyaline, with several small guttules.

Studied specimens: VR73

Remarks
We use here the basionym probably the name under which this species is best known. It has been positioned in several genera, in chronological order: *Trichothyrina*, *Micropeltopsis* and there is a planned paper to transfer it to *Lichenopeltella* (http://www.indexfungorum.org/).

Morenoina phragmitis [as '*phragmitidis*'] J.P. Ellis, Trans. Brit. Mycol. Soc. 74 : 304 (1980).

Ascomata thyriothecia ± 200-500 μm long, ± 300 μm wide, simple or irregular, solitary but often confluent, black, superficial; upper peridium breaks open irregularly; visible as lighter lines exposing inner hymenium. Hamathecium filaments short but soon vanishing as mucus between asci. Asci 20-25 x 12-15 μm, ovoid, thick-walled near apex, 8-spored, ascospores clustered. Ascospores 9-12 x 4-4.5 μm, hyaline, 1-septate, strongly constricted, basal cell tapered, upper cell rounded, no guttules, no slime coat, not verrucose, ascospores show a clear shrinking in Cotton blue.
Within the confluent of ascomata we often can find an anamorph. Conidia 1.5-3 x 0.5 μm, hyaline, aguttulate, aseptate. Conidiogene cells not observed.

Ecology: on stems and leaf sheaths. Often in a standing position (but also in litter layer) from late spring till late autumn, common but easily overlooked or interpreted as sterile mycelia. On both plant parts often near the nodes.

Studied specimens: VR757

Remarks
Morenoina paludosa J.P. Ellis stands *M. phragmitis* very near and is found in marshes on *Carex*, *Juncus* and *Cladium* but would be characterized by an anamorph with larger conidia (5-7 x 1.5-2 µm) and longer ascospores (up to 11.5 µm) (Ellis, 1980). However the argument of larger ascospores seems to fail as we recorded slightly larger ascospores for our collections than those mentioned by Ellis [Ellis (1980): up to 10 µm long] and hence our measurement could fit for *M. paludosa*. However, our anamorph showed very small conidia typical for *M. phragmitis*.

Mycosphaerella lineolata (Roberge ex Desm.) J. Schröt., Krypt.-Fl. Schlesien 3(2): 339 (1894).

Ascomata 40-100 µm diam., spherical, in rows near ribs of leaf sheath, solitary with distance between ascomata; ostiole short, protruding epidermis. Peridium textura angularis, thin-walled (10 µm), cells small (5-7 µm diam.), brown, (one seen with in each cell a guttule). Hamathecium filaments absent. Asci 32-40 x 12-16 µm, few, oblong-pyriform, apically thick-walled, no stipe, sitting in well developed subhymenium, 8-spored, 3-4-seriate. Ascospores 12-21 x 3-5 µm, clavate, 1-septate, septa supramedian, with upper part slightly enlarged, basal cell elongates during spore maturation, not or slightly constricted, hyaline, with 2 (3) guttules in each cell.

Septoriella state of *Mycosphaerella lineolata*
Found by two separate culture experiments (VR611, VR715):

Conidiomata 100-300 µm diam., dark brown, immersed or superficial, with papillate ostiole which becomes in culture up to 100 µm long. Peridium textura angularis, up to 20 µm thick, cells with guttules, greyish brown under microscope, with hyphae at the exterior. Condiophores absent. Conidiogene cells small, 3-5 µm diam., ampulliform, enteroblastic, with inconspicuous periclinale thickening. Conidia 17-30 x 3-4 µm, cylindrical, light brown, dark brown in mass, mostly 3-septate, but up to 7-septate observed, slightly or not constricted, with small guttules near septa or aguttulate, smooth, with slime-appendage staining red in Kongored.

Ecology: common on dead leaf sheaths in the middle canopy during the summer (culms in a standing dead position for about half a year or slightly longer).

Studied specimens: VR611, VR715; GVR1016, on stem, 20/09/2000, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43

Remarks
Our collection is very close to the type description of *Mycosphaerella lineolata* (Roberge ex Desm.) J. Schröt. in Kryptogamen Flora von Schlesien 3(2): 339 (1894). [Type description: Ascomata 60-70 µm, asci 40-50 x 12-15, spores 16-18 x 3-5 µm]. Type collection on *Scirpus*. basionym *Sphaeria lineolata* Roberge in Desmazières, Ann. Sci. Nat. ser. 2, 19: 351. 1843. (asci 50 µm, spores 12.5 µm); Desmazières, Plantes Cryptog. France ed.1 no. 1263 (Corlett, 1991).
Mycosphaerella lineolata could well fit into the section *Longispora* (Barr, 1972; Kuijpers & Aptroot, 2002). Reported anamorphs belong to the form-genera *Phloeospora* or *Septoria sensu lato* (Verkley & Priest, 2000). Our anamorph could be placed within *Septoria sensu lato*. However not in *Septoria sensu stricto* as described by Sutton (1980). The best match for our appendaged coelomycete seems to be *Septoriella* (Nag Raj, 1993).
The *Septoriella* state found by culture experiments seems very close to a dominant, variable taxon named *Septoriella* sp(p). in the canopy of *P. australis* formed on the living, moribund and initially dead leaves (blades and sheaths). At this point we are unable to recognize a generic difference between *Mycosphaerella* anamorphs and *Phaeosphaeria* anamorphs which seem very similar [?]. *Phaeosphaeria* anamorphs are often placed in the form genus *Stagonospora* (e.g. Leuchtmann, 1984). If our recorded *Septoriella* sp(p). and *Mycosphaerella lineolata* form the holomorph, *M. lineolata* is probably endophytic on *Phragmites* with initially forming its anamorph soon after senescence of the host and forming its teleomorph the next summer on standing dead plant parts.

Probable synonym:

- *Mycosphaerella phragmitis* (J.B. Ellis & Everhart) Lavrov, Trudy Tomsk. Gosud. Univ. Kuibyševa Tomsk. Gosud. Pedagog. Inst. 110(4): 141. 1951. Ascomata 100-110 µm; asci 30-45 x 6-7; spores 11-13 x 2-3 µm. Original description (basionym) *Sphaerella phragmitis* J.B. Ellis & Everhart, Proc. Acad. Nat. Sci. Philadelphia, 1983, p. 446. Ascomata 100-110 µm; Asci 30-45 x 7-9 µm; Spores 9-12 x 3-4 µm. On *Phragmites*, USA. (Corlett, 1991).

Other *Mycosphaerella* species recorded from *Phragmites*:

- *Sphaerella perpusilla* (Desmazières) Cesati & de Not., Comment. Soc. Crittog. Ital. 1(4): 237. 1863. Basionym: *Sphaeria perpusilla* Desmazières, Ann. Sci. Nat. ser. 3, 6:80. 1846. (Asci 50 µm; spores 5 µm), France. Syn. *Laestadia perpusilla* (Desmazières) Sacc. Syll. Fung. 1: 423. 1882. (Corlett, 1991).
- *Sphaerella vaginae* (Lasch) Fuckel, Jahrb. Nassauishen Vereins Naturk. 23-24: 100. 1869-1870. Spores 14 x 3-4 and aseptate. Germany (Corlett, 1991). *Phoma* sp.?

Ophioceras dolichostomum (Berk. & Curt.) Sacc., Syll. Fung. 2: 358 (1883).
= *Ophioceras commune* Shearer, Crane & Chen, Mycologia 91: 146 (1999)

Ascomata 350- 450 wide, 300-350 μm high, spherical to somewhat compressed, immersed or partly superficial; ostiole 0.75- 1 mm long, black with hyaline tip. Peridium textura angularis, ostiole textura porrecta with most upper part of ostiole irregular textura intricata. Hamathecium filaments 3.5-4 μm wide, hyaline, septate, not constricted. Asci 110-120 x 9.5-10 μm, cylindrical, unitunicate, apical apparatus visible in cotton blue, evanescent, spores multiseriate not spiralised. Ascospores 82-108 x 2-2.5 μm, scolecospores, straight or flexuous, 3-6 septate (difficult to observe), filled with guttules, ascospores gather on top of the ostiole in droplets.

Ecology: on dead stem, in mud, of *Phragmites australis*

Studied specimens: VR536

Remarks
The species is collected previously from wood in freshwater habitats and widespread, known from temperate and tropical zones. Our description agrees well with the diagnoses of Conway & Barr (1977) and Hyde (1992). Shearer et al. (1999) introduced *O. commune* which was characterized by ascospores that were consequently 2 μm wide while *O. dolichstomum* was considered to have a more variable (although not discussed) spore-wide (in literature 2-3.5 μm). Molecular data, according Chen et al. (1999), are not available for *O. dolichostomum*. Descriptions of both species are very similar and the creation of the new species *O. commune* seems unnecessary. I regard *O. commune* as a synonym of *O. dolichostomum*. Although spore width in my collection showed very little variability, some variability was noticed and seems natural.
Sphaeria culmorum Crouan in Walker (1972) shows some similarity with *Ophioceras dolichostomum* but has smaller spores as seen by Walker (1972).

Paraphaeosphaeria michotii (Westend.) O.E. Erikss., Cryptogams of the Himalayas 6: 405 (1967).

Ascomata about 200 μm diam., globose, dark brown, immersed, solitary; ostiole central. Peridium textura angularis, thin walled, light brown, with lot of hyphae at the exterior, cells about 7μm wide. Hamathecium filaments abundant, 2 μm wide (but up to 5 μm inflated), septate, constricted at septa. Asci 55-70 x 10-15 μm, cylindrical-clavate, short stipe, clear foot, biseriate, bitunicate. Ascospores 17-22 x 5-6 (7) μm, oblong, rounded at end cells, 2-septate (sometimes a third septum is developed), constricted, straw coloured to dark brown, echinulate, sometimes some small guttules, with uniform slime coat.

Anamorph: *Coniothyrium scirpi* Trail (Webster, 1955: 348): see remark description *Coniothyrium* (coelomycetes).

Studied specimens: VR503; GVR60, 6/01/2000, leaf sheath, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43

Passeriniella obiones (P. Crouan & H. Crouan) K.D. Hyde & Mouzouras, Trans. Br. mycol. Soc. 91(1): 183 (1988)
Synonym: *Passeriniella discors* (Sacc. & Ellis) Apinis & Chesters, Trans. Brit. Mycol. Soc. 47: 432 (1964).

Ascomata 200-300 μm diam., 250-350 μm high, sometimes immersed but often superficial, pyriform, black, brownish, with brown, relatively thick-walled hyphae on the exterior, often flattened on substrate and with a straightened up ostiole; ostiole very variable, short or very long (up to 300 x 70 μm), with periphyses, hyaline. Hamathecium filaments hyaline, cellular. Asci 120-150 x 13-20 μm, cylindrical clavate, thick-walled, bitunicate, 8 spored, biseriate. Ascospores 21-33 x 8-11 μm, curved, ellipsoid to fusiform, 3-septate, constricted; central cells ochraceous brown to dark brown, with several guttules; end cells hyaline, without guttules; no appendages.

Studied specimens: VR445

Remarks
Collected in the entire estuary, from fresh water to mesohaline conditions. Several other genera have similar, peculiar ascospores such as *Savoryella* (Sordariales) (Hyde, 1994), *Chaetosphaerella* (Helminthosphaeriaceae) (Müller & Booth, 1972) and *Chitonospora* (Amphisphaeriaceae) (Eriksson, Svensk bot. Tidskr. 60: 320, 1966).

Phaeosphaeria

Phaeosphaeria spp. (teleomorphs) and their *Stagonospora* (s.l.) anamorphs (see Leuchtmann, 1984) are very common on *P. australis* and are probably one of the most important colonizers involved as endophytes (see Wirsel et al., 2001) and saprotrophs (see this thesis). Their anamorphs are not incorporated in this taxonomic section within Stagonospora s.s. (typified by *Stagononospora paludosa*) but are included within *Hendersonia*. Molecular work confirmed the separation of *Leptosphaeria* from *Phaeosphaeria* (Khashnobish & Shearer, 1996). The former genus seems retricted to Dicotyledons with ascomata mostly with a thick-walled peridium and completely different structure (often superficially), other anamorphs and spores that have different characteristics. *Phaeosphaeria* is supposed to be a parasitic genus of monocots, with

subhyaline to brownish ascospores, mostly highly specialized to its host plant (Shoemaker & Babcock, 1989).

Phaeosphaeria culmorum (Auersw.) Leuchtm., Ber. Naturhist. Ver. Augsburg 26: 60. 1881.

Ascomata 150-200 µm diam., immersed, often in rows aggregated between the nerves of leaf or leaf sheath (every 400 µm an ascocarp); ostiole 30 µm diam., papillate or not, small, with periphyses. Peridium textura angularis, 20 µm thick, cells 7- 10 µm wide, with oil drops in cells; with brown hyphae at the exterior, 2 µm diam. Hamathecium filaments abundant, septate, constricted at septa, 1.5-3.5 µm wide, embedded in slime matrix. Asci 70-90 x 12-16 µm, cylindrical, apex broadly rounded, bitunicate, bi-triseriate, with short stipe, with foot. Ascospores 18-27 (30) x (5) 6-8 (9)µm, broadly fusiform, end cells rounded to conical tapered, 3-septate (sometimes by additional septa in end cells becoming 5-septate), first septum median or submedian, slightly constricted at septa, most at median septum, straight to slightly curved, yellow brown, smooth, with uniform slime coat that swells in water, with small guttules.

Ecology: most often on leaf sheaths (less often on leaf blades) see figure for phenology.

Studied specimens: VR367, VR547; GVR971 (figure), 17/10/2000, on leaf sheath, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43

Remarks
Shoemaker & Babcock (1989) observed no periphyses. We observed periphyses in collection VR547. This species shows lots of similarity with *P. microscopica* but differs in the smooth aspect of the spores and the generaly longer spores. Ascospores of *P. microscopica* are 18-23 x 6-7.5 µm (Leuchtmann, 1984) or 18-24 x 6.5-8(10) µm in Shoemaker & Babcock (1989).
Munk (1957) used *Leptosphaeria microscopica* in synonymy of *L. culmorum*, as a consequence all records of *L. microscopica* by Taligoola (1969) should be interpreted as *L. culmorum* which is a common species on *P. australis* whereas *P. microscopica* probably does not occur on *P. australis*.

Phaeosphaeria eustoma (Fuckel) L. Holm s.l.

Ascomata 90-150 µm, spherical, brown, immersed, with sometimes blue-greenish colouration of the substrate; ostiole small, papillate, with periphyses. Peridium textura angularis, thin (+/- 10 µm thick) (from pale to dark brown), cells 7-8 µm diam. Hamathecium filaments 1,5-2µm wide, scanty, septate, cells 10-15 µm long, cellular. Asci 50-65 x 8-11 µm, cylindrical-clavate, with clear foot, very short stipe, biseriate, gathered in common subhymenium interwoven with physes. Ascospores 17-23 (27) x 4-6 µm, fusiform, with tapered end cells, second cell enlarged

isodiametric, mostly curved (to straight), 3-septate (sequence 2:1:2), primary septum median, slightly or not constricted, with small guttules (living material), pale yellow to ochraceous yellow, rarely with a two-parted slime coat (1 collection).

Ecology: (see figure) seems to be slightly more common in the upper canopy compared to the middle canopy. Mostly found on leaf sheaths but also occurring on leaf blades.

Studied specimens: VR466, VR614; GVR729, on leaf sheath, 19/07/2000, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43

Remark
Ph. eustoma is a complex of species parasitizing on monocotyledons not causing any coloration of the substrate (however we observed a few times a blue-greenish colour!). Ascospores pale yellow to brownish, second cell enlarged, variable in length (16.5-31 x 3.5-6.5). The spores are surrounded by a typical two-parted slime coat: a spherical appendage at the first cell and a basal slime coat surrounding the second till fourth cell (Leuchtmann, 1984). This slime coat proves to be very difficult to demonstrate, I saw it only once. The typical *P. eustoma* when collected fresh has normally a relatively obvious slime coat. So perhaps the majority of our collections could represent a specific variety on *P. australis* (Leuchtmann, pers. comm.). Culture experiments or molecular work could confirm this.

Phaeosphaeria fuckelii (Niessl) L. Holm, Symb. Bot. Upsal. 14(3): 123 (1957).

Ascomata +/- 200 µm diam., pale brown, immersed. Peridium textura angularis with clear oil content of the cells. Hamathecium filaments, 3- 3.5 µm wide, abundant, hyaline, with small guttules, septate, slightly constricted, cellular. Asci 85-105 x 10-11 µm, abundant, cylindrical, slightly clavate, with clear foot. Ascospores 28-32 x 5-5.5 µm, fusiform, 5-septate, fourth cell enlarged, fifth and sixth cell longer than wide, straight to curved, light yellow, with small guttules, slimecoat was not observed.

Studied specimens: GVR400, on leaf sheath, 10/04/2000, Belgium, Scheldt estuary, brackish tidal marsh, Burcht (Schor near Galgenweel), IFBL C4.26.31

Remarks
Characteristic species with 5-septate spores and 4th cell swollen.

Phaeosphaeria graminis (Fuckel) L. Holm, Symb. Bot. Upsal. 14(3): 118 (1957).

Ascomata 250-300 µm diam., spherical, brown with brown-greyish hyphae at the exterior of the fruit body, solitary or with some in short rows aggregated; ostiole papillate, protruding

epidermis, on top of the ostiole spores are gathered. Peridium textura angularis, dark brown to black, thick walled hyphae. Hamathecium filaments 1.5 μm wide, septate, abundant, branched. Asci 115-120 x 10-11 μm, clavate, short stipe (5-10 μm), bi-triseriate. Ascospores 36-44 x 5-6 μm, fusiform, straight to slightly curved, 8-10 septate, slightly constricted at the septa, third cell enlarged, first septum supramedian, finely echinulate, yellow brown, with entire slime coat.

Ecology: leaf sheath, *Phragmites australis*

Studied specimens: VR560

Remarks
Species with wider spores than those of *P. pontiformis* and slightly shorter.
Shows affinities with *P. elongata* which has a different pattern of septation (Shoemaker & Babcock, 1989).

Phaeosphaeria luctuosa (Niessl) Otani & Mikawa, Mem. Nat. Sci. Mus. Tokyo 4: 78 (1971).

Ascomata 250-300 μm diam., spherical, brown to black, losely aggregated, colours substrate black in epidermis, ostiole central. Peridium textura angularis, cells 5-6 μm wide, dark brown, inner excipulum textura angularis with thin-walled cells, hyaline; with many dark brown hyphae at the exterior. Hamathecium filaments 3 μm wide, septate, cells (6) 10-12 μm long, slightly constricted at septa with some small guttules, anastomozing. Asci 105-115 x 10-12 μm, abundant, cylindrical, biseriate, short stipe, obvious foot, two rooted, broadly rounded above. Ascospores 27-36 x 5.5-7 μm, pale yellowish, fusiform, sometimes clearly tapered to the ends, first septum median, becoming 5-septate (sequence 3:2:1:2:3), swollen at third cell but longer than wide, slightly constricted at septa, without guttules or only small ones, germinating at the both end cells; slime coat inconspicuous or absent, uniform, swelling in water.

Studied specimens: VR200, VR741; GVR1226, 27/12/2000, on stem, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43

Remarks
This species resembles immature collections (= phase before formation of longitudinal septa) of *P. vagans* and *P. phragmiticola*. Be sure the specimen is mature (spontaneous expulsion of ascospores from asci).

Phaeosphaeria nigrans (Roberge in Desm.) L. Holm, Symb. Bot. Upsal. 14(3): 112 (1957).

Ascomata ± 250 μm diam., light brown, solitary, immersed. Peridium textura angularis. Hamathecium filaments rather wide up to 3 μm diam., septate, cells 7 μm long, constricted at septa. Asci 60-7-8 μm, cylindrical-clavate, biseriate. Ascospores 24-28 x 3.5 μm, narrowly fusiform, L/W ± 7, second cell enlarged, becoming 5-septate (sequence 2:1:3:2:3), not constricted at the septa, light yellow; slime coat entire, small or absent.

Studied specimens: VR493

Phaeosphaeria phragmiticola Leuchtm., Sydowia 37: 138 (1984).
? non *Leptosphaeria phragmiticola* (P. Crouan) Sacc., Syll. Fung. II: 87 (1883).
Ascomata ± 250 μm diam., spherical, dark brown, immersed, substrate coloured black; ostiole protruding epidermis. Peridium textura angularis, dark brown, thick walled cells with diam. less than 10 μm. Hamathecium filaments abundant, 1.5 μm wide, hyaline, septated, branching, cellular. Asci 100-105 x 9-10 μm, abundant, cylindrical, with clear stipe and foot, biseriate. Ascospores 25-28 x 5.5-6.5 μm, narrow fusiform, with clearly tapering end cells, straight or curved, 5-(6) septate, only constricted at middle cell, central cells with longitudinal septa, yellow brown up to darker brown; slime coat not observed.
Together with anamorph on leaf sheath of *Phragmites*: *Hendersonia*-like conidia, 3-septate, 15-17 x 3.5-4 μm, pale brown-yellow, no slime appendages observed.

Studied specimens: VR157, GVR 935, on stem in litter layer, 9/20/2000, The Netherlands, Het verdronken land van Saeftinghe, Doel, IFBL B4.33.43 (no exsiccatum)

Remarks
We noticed the excistence of *Leptosphaeria phragmiticola* [basionym *Pleospora phragmiticola* P. Crouan, Finist.: 23]. Based on the type description, it is not possible to exclude this species as a synonym for *Phaeosphaeria phragmiticola*, although *Leptosphaeria phragmiticola* seems to be characterized by larger and perithecia which have different shape.

Phaeosphaeria phragmitis (Hollós) Leuchtm., Sydowia 37: 139 (1984).

Ascomata 350-400 μm diam., brown, aggregated in stromatic rows together with anamorph *Septoriella phragmitis* Oud., splitting epidermis longitudinally open; ostiole central. Peridium textura angularis, light brown, cells with guttules. Hamathecium filaments abundant, 2 μm wide, hyaline, anastomozing, cellular. Asci 130-140 x 12-16 μm, cylindrical-clavate, with clear stipe, biseriate. Ascospores 33-43 x 6-8 μm, fusiform, with tapered end cells, straight to curved, smooth, with 5-8 transverse septa, middle cells with longitudinal septa, third cell enlarged,

primary septum median, constricted, light yellow, with small guttules, slime coat entire and swelling in water.

Studied specimens: VR772; GVR3133, 30/09/2002, on stem, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43 (no exsiccatum)

Remarks
This species matures slowly and in the initial state it has a close resemblance to *P. phragmiticola*. All collections of this teleomorph were in association with the anamorph *Septoriella phragmitis* in the same stroma. *P. phragmitis* is characterized by a conspicuous slime coat clearly swelling in water. When mature *P. phragmitis* has much larger ascospores compared to *P. phragmiticola*. Ascomata are scanty on material, more pycnidia of *Septoriella phragmitis*.

Phaeosphaeria pontiformis (Fuckel) Leuchtm**.**, Sydowia 37: 134 (1984).

Ascomata 200-300 μm diam. spherical or somewhat flattened, black, aggregated or solitary, immersed, lifting up epidermis, with lots of dark brown hyphae surrounding ascomata; ostiole protrubing, conical. Hamathecium filaments abundant, hyaline. Asci 90-125 x 11-16 μm, short stipe, with foot, bi-triseriate. Ascospores 38-59 x 3.5-5.5 μm, narrow fusiform-cylindrical, straight or flexuous, 2nd or 3th cell enlarged, 9-13 septate, hardly constricted, smooth, yellow straw coloured, with small guttules, slime appendages sometimes visible at end cells.

Ecology: common species on leaf sheaths in the litter layer

Studied specimens: VR328 VR680; GVR3409, 26/02/2003, leaf sheath, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43 (no exsiccatum) (pictures)

***Phaeosphaeria* sp. I**

Ascomata 400-450 μm diam., spherical, black, immersed; ostiole central. Peridium textura angularis black-brown, with brown hyphae at the exterior; cells 7-9 μm diam., thick-walled. Hamathecium filaments 2-3 μm wide, abundant, septate, cells 10-15 μm long, hyaline, with small guttules. Asci 150-155 x 16-18 μm, cylindrical-clavate. Ascospores 52-60 x 6.5-7.5 μm, narrow fusiform, 10-12 (14) septate, first septum supramedian (0.38), third or fourth cell enlarged, slightly constricted at the septa (not in herbarium), smooth, straw coloured, some small guttules in each cell, with a clear slime coat swelling in water up to 5 μm. A few times a longitudinal septum was seen in fourth cell.

Studied specimens: VR689

Remarks
This taxon belongs to the *P. herpotrichoides* complex (Leuchtmann, 1984). This species assemblage is extremely variable with many intergrading forms. Morphologically our specimen is almost similar to *P. elongata* (Wehm.) Shoemaker & C.E. Babc. (Shoemaker & Babcock, 1989) but our collection differs in having smooth and slightly larger spores.

Phaeosphaeria **sp. II**

Ascocarps 140-350 µm diam., spherical, pale brown, scattered, immersed; ostiole darker at base, slightly papillate. Peridium textura angularis, thin-walled, 10-12 µm, thick, cells pale brown up to 10 µm wide. Hamathecium filaments 2 µm diam., some cells inflated (up to 5 µm), evanescing, hyaline, small guttules, septate, constricted at the septa. Asci 63-100 x 12 –16 µm, cylindrical to oblong, short-stalked, clear foot, bitunicate, 8-spored?, bi-triseriate, at top often tapered, attachted on common subhymenium. Spores (23) 25-35 (40) x 5-7 µm, fusiform, first hyaline becoming very pale yellow and yellow when senescent, 3-septate, first septum median, constricted (sequence 2:1:2), less or not constricted at the other septa, with many small guttules, with slime coat uniform which is sometimes conspicuous in water.

Ecology: on of the early colonizers of leaf sheaths from the basal and middle canopy (see figure).

Studied specimens: VR381, VR783; GVR235, on leaf sheath, 12/02/2000, Belgium, Scheldt estuary, Het Kijkverdriet, Steendorp (Temse), 25/04/2001, IFBL C4.54.11 (no exsiccatum); GVR451, on leaf sheath, 10/04/2000, Belgium, Scheldt estuary, Tielrode schor, Tielrode, IFBL C4 52 41 (no exsiccatum); GVR708, 19/07/2000, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43 (no exsiccatum)

Remarks
This species is relatively common on *P. australis* in the Scheldt estuary and appears to be very close (identical?) to *P. juncicola* (Rehm) L. Holm or in the narrow species concept of Shoemaker and Babcock (1989) we could name it more likely *P. fetanensis* Shoemaker & C.E. Babc. We should compare our collections with specimens of the mentioned species before taking a decision.

Phaeosphaeria **sp. III**

Ascomata 150-200 µm diam., spherical, immersed, globose to slightly compressed, dark brown; ostiole central, protruding, not clearly developed. Peridium textura angularis, brown to greyish

brown, cells 6-10 µm diam., not thick-walled; with many hyphae at exterior, pale brown 2 µm diam. Hamathecium filaments 1.5-2 µm diam., sometimes unclear, constricted at septa, embedded in slime matrix, cellular. Asci 65-80 x 8-12 µm, cylindrical, short stalked but clear foot. Ascospores 20-30 x 4-6 µm, fusiform to slightly oblong, pale yellowish becoming ochraceous, mostly curved, 3-septate (sometimes 1-2 additional septa), slightly constricted, all cells subequal, becoming soon finely echinulate, primary septum supra-median, with guttules, slime coat uniform.

Ecology: most common on leaf blades in the middle canopy. Also frequently on the middle height leaf sheaths (see figure).

Studied specimens: VR475, VR498; GVR974, on leaf sheath, 17/10/2000, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43 (no exsiccatum); GVR3149, on leaf sheath; 30/09/2002, ibid

Remarks
These collections have affinities to the *P. triglochinicola* species assemblage (Leuchtmann, 1987). Typical are the supra-median primary septum and the finely echinulate spores when mature. However our specimens seem to be different form *P. triglochinicola* by the shape and size of the ascospores and from *P. juncophila* in the fact that spores are soon (in ascus) finely echinulate. Furthermore our collections show resemblance to *P. kukutae* and *P. oryzae*. We should compare our collections with above mentioned species. Seems to differ from *P. eustoma* s.l. by the echinulate, larger spores with uniform sheath and larger asci.

***Phaeosphaeria* sp. IV**

Ascomata ± 80-100 µm diam., immersed, pale to deep brown; peridium textura angularis; ostiole protruding, darker, not papillate. Hamathecium present, cellular. Asci bitunicate, no measurements. Ascospores 10-12.5 x 2-2.5 µm, subhyaline, fusiform, 2-septate, central cell enlarged, basal cell longer, both upper and basal cell tapered, with few small guttules.

Studied specimens: no material in herbarium

Remarks
This is a very small species with few asci and ascospores available. It was seen several times but we were not able to study it properly. No herbarial specimen or a permanent slide was made. However the species is well recognizable by the small ascospores which are only 2-septate.

Phaeosphaeria vagans (Niessl) O.E. Erikss., Ark. Bot. 6: 430 (1967).

Ascomata 300- 350 μm diam., spherical to flattened, dark brown, substrate may color brown near the ascomata, ostiole undifferentiated, perforation in epidermis. Peridium textura angularis, dark brown, with small cells (+/- 5 μm diam.), cells rather thick-walled. Hamathecium filaments, abundant, 1-1.5 μm wide, slender, hyaline, septate. Asci 90-130 x 13-15 μm, clavate, with short stipe and foot, biseriate. Ascospores 25-36 x 6-8 μm, ellipsoid to fusiform, 5-6 transverse septa, middle cells with longitudinal septa (variable number!), light yellow-brown, no guttules, slime coat entire, swelling in water.

Studied specimens: VR506

Remarks
This species seems uncommon on reed and could be confused with *P. luctuosa* which has no longitudinal septa and narrower spores. Furthermore, it could be confused with taxa having also longitudinal septa in the mature spores such as *P. phragmiticola* which has spores with a typical fusiform shape and *Pleospora abscondita* which seems more common on *P. australis*. The latter is characterized by larger ascomata, the yellow-brown peridium and the lighter coloured spores with rounded end cells.

Phomatospora berkeleyi Sacc., Nuovo Giorn. Bot. Ital. 7: 306 (1875).

Ascomata 90-150 μm diam., immersed, spherical, light yellowish brow peridium, becoming dark near the ostiole, often with a yellow colouration of the substrate; peridium textura epidermoidea; ostiole protruding, fine, black, with periphyses. Hamathecium filaments absent. Asci 53-90 x 5-6 μm, cylindrical, unitunicate, with refractive apical apparatus, IKI^-, 8-spored, uniseriate. Ascospores 6-10 x 2-3 μm, cylindrical, hyaline, aseptate, 2 guttules, thin-walled, finely striate, sometimes with two polar slime caps about 2 μm wide.

Studied specimens: VR22, VR488

Remarks
Phomatospora berkeleyi is a polyphageous species common on *Phragmites* and recorded from a large range of host plants. Our collections are very similar to the type collection investigated by Fallach & Sheaerer (1998).

Phomatospora cf. dinemasporium Webster, Trans. Brit. Mycol. Soc. 38: 364 (1955).

Ascomata 200-250 μm diam., light brown, spherical, immersed, often gregarious in short rows; ostiole 40 μm diam., black, with thick-walled cells on the basis. Peridium textura epidermoidea. Hamathecium filaments not seen. Asci 110-160 x 6 μm, cylindrical, unitunicate, with refractive

apical apparatus, IKI$^-$, 8-spored, uniseriate. Ascospores 12-15 x 4-5 μm, cylindrical, hyaline, striate, aseptate, 4 guttules (2+2) when fresh.

Ecology: meanly on stems, also leaf sheaths

Studied specimens: VR145, VR270

Remark
Phomatospora is in need of a taxonomic revision. *Phomatospora dinemasporium* is a doubtful species and whether the type species is congeneric with *Phomatospora* remains to be sorted out. It is strange in several ways: (i) the species was decribed to have a *Dinemasporium* anamorph (Webster, 1955) whereas *Phomatospora* species were demonstrated to have *Sporothrix* anamorphs (Rappaz, 1992), (ii) no apical apparatus was mentioned in the type description of Webster (1955), (iii) no striation and no appendages were mentioned on the ascospores, (iiii) the normaly rather clear textura epidermoidea is not mentioned. To sort this out the type collection should be studied [Type: Specimen No. 1163 Mycological Herbarium, University of Sheffield]. Although the incomplete diagnoses, the species was included in Ellis & Ellis's handbook and since the name was used for *Phomatospora* collections recorded on grasses with larger ascospores (like ours). However our collection has larger asci and wider spores compared to the type collection.
Phomatospora arenaria Sacc., E. Bommer & M. Rousseau recorded on *Ammophila* has larger spores (13-17 x 4-5 μm) compared to *P. berkeleyi* and could be a good name for our collections and possibly a synonym for *P. dinemasporium*. *Phomatospora* cf. *ovalis* (Pass.) Sacc. (see Munk (1957): 177) is another similar species.
I refrain to name our collection to a specific species, pending further studies. Anyhow our collections are truly congeneric with *Phomatospora*.

Phomatospora phragmiticola O.K. Poon & K.D. Hyde, Bot. Mar. 41: 148 (1998).

Ascomata 130-170 μm diameter, immersed, subglobose, solitary or aggregated; peridium brown-yellowish with thickened and black near the ostiole, textura epidermoidea (surface view); ostiole short papillate, black. Hamathecium filaments ca 80 μm long, septate, free, hyaline, tapered near the top. Asci 80-108 x 5-7 μm, cylindrical, short pedicel, with clearly a refractive apical apparatus. Ascospores (8)-10-11-(12) x 3-3.5 μm, ellipsoid, striate, hyaline, aseptate; slime coat initially entire, becoming polar appendages which appear to be attached with a short stipe and eventually appearing bifurcate; two large polar guttules with sometimes additional small ones.

Studied specimens: GVR935, 20/09/2000, on stem, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43 (no exsiccatum but permanent slide available)

Remarks
Several species were compared by Poon & Hyde (1998) and especially *Phomatospora bellaminuta* Kohlm., Volkm.-Kohlm. & O.E. Erikss. (Kohlmeyer et al., 1995) seems close but the latter species would differ in the gelatinous caps that do not stain in methylene blue and asci with mainly biseriate ascospores. The authors did not mention *Phomatospora aquatica* Minoura & Muroi (Minoura & Muroi (1978). Judged from the type description, the latter species is very close to *P. phragmiticola* and could be a synonym. Type species of those two species should be compared.

Phomatospora **sp. I**

Ascomata 300-450 µm diam., spherical to flattened, light brown-yellow, darker at the ostiole, substrate coloured dark by accumulation of amorph material, solitary or sometimes in short rows; ostiole 35 x 25 µm, dark brown, papillate, with periphyses. Peridium textura epidermoidea, thin-walled. Hamathecium filaments rare, filiform. Asci +/- 100 x 5-6 µm, cylindrical, narrow, short stipe, with a refractive apical apparatus, uniseriate. Ascospores 12-14.5 x 4-4.5 µm, cylindrical-oblong, aseptate, striate epispore, hyaline, 4 (2+2) large guttules and several small ones; appendages bipolair, up to 4 µm long, (becoming?) dendroïd, disappearing in cotton blue.

Studied specimens: VR540

Remarks
This species could not be named and has obvious and typical slime appendages in fresh condition.

Phomatospora **sp. II**

Ascomata 350-400 µm diam., spherical, solitary, immersed; ostiole weakly papillate, protruding. Peridium textura epidermoidea, yellow-brown, dark brown and thicker near ostiole and becoming a textura angularis. Hamathecium filaments abundant but evanishing, septate, branching. Asci +/- 110 x 5 µm, cylindrical to slightly fusiform, narrow, with refractive apical apparatus, chitinoid (=colouring in cotton blue), released from hymenium and evanashing at the base, with a guttule under the apical apparatus visible in cotton blue, unitunicate, uniseriate. Ascospores 10-13 x 3 µm, narrow, cylindrical-oblong, striate, aseptate, two distal guttules, no sheath observed.

Studied specimens: VR673 (GVR1500); GVR 2559, 31/01/2002, stem standing middle height, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43 (figure, slide; no exsiccatum)

Remarks
These collections seem related to *Phomatospora berkeleyi* which has smaller spores. Perhaps these collections could be recognized as a longspored variety of *Phomatospora berkeleyi* (➔ *Phomatospora berkeleyi* var. *longispora*?). A seemingly large variability within *P. berkeleyi* was noticed by Scheuer (1988) and perhaps our species concept is to narrow. However before proven otherwise the narrower species concept is applied.

***Phomatospora* sp. III**

Ascomata 300-350 μm diam., spherical, immersed, light brown, darker ostiole, pale yellow colouration of substrate near fruit bodies (as often seen with *Phomatospora berkeleyi*). Peridium textura epidermoidea, more textura angularis near ostiole, thicker walled (almost carbonaceous) at ostiole. Hamathecium filaments abundant but evanishing, septate, hyaline,with small guttules. Asci 130-140 x 6 μm, cylindrical, dehiscent, base evanishing, narrowing at base and forming an unclear stipe, refractive apical apparatus becoming more obvious in cotton blue, unitunicate, uniseriate. Ascospores 13-16 x 5,5-6 μm, ellipsoid-oblong, aseptate, striate, hyaline, two large guttules and several small ones, with two conspicuous appendages forming large stipate caps.

Studied specimens: VR674

Remarks
This species (variety?) seems related to *Phomatospora phragmiticola* (see above) which has similar appendages. However, *Phomatospora* sp. III has larger ascospores and asci.

***Phomatospora* sp. IV [*Phomatospora hypersaprotrophica* nom. prov.]**

Ascomata 100-250 wide x 100-200 μm high, light to dark brown, immersed, aggregated, hypersaprotrophical in old perithecia of e.g. *Massarina arundinaceae* or solitary on nearly decomposed leaf sheaths half-immersed straight or almost horizontaly on substrate with lateral ostiole, sometimes clypeoid structures present near ostiole (seen with the hypersaprotrophical collections; ostiole mostly well developed up to 200 μm long, 40 μm wide, dark coloured at base, becoming paler on top, few specimens with two ostioles. Peridium textura epidermoidea, brightly yellow-brown, +/- 7 μm thick. Hamathecium filaments relatively long persisting eventually vanishing. Asci 110-130 x 5,5-7 μm, cylindrical, long stipe, unitunicate, with strong refractive apical apparatus which colors in cotton blue in lactic acid and visible in KOH,

uniseriate, eventually vanishing. Ascospores 15-22 (26) x 3,5-5 μm, narrowly fusiform, one side somewhat convex, straight or curved, becoming 3-septate, hyaline, with several small guttules in each cell.

Ecology: on stem and leaf sheath of *Phragmites australis*

Studied specimens: VR316, VR107, VR253, VR799

Remarks
This species needs some further study. It is peculiar in *Phomatospora* because of the very long ostiole and the non-striate, septate ascospores. It seems related to *Phomatospora radegundensis* (Scheuer 1988) and to *P. admontensis* Nograsek (Nograsek & Matzer, 1991) but our specimens show narrower spores and are predominantly 3-septate. The same species was also collected by C. Scheuer (pers. comm.) and is most probably an undescribed species. Compare with Asco sp. incertae sedis II.

Pleospora abscondita Sacc. & Roum., Reliq. Lib. Ser. II, n. 157. t, ?, f. 11 (1881).

Ascomata 350-450 μm diam., spherical, often flattened, black to dark brown, immersed, lifting the epidermis round the fruit body giving the epidermis a white coloration, very rudimentary clypeus formed by loosely grouped brown hyphae, fruit body easily released from substrate, mycelium white and loosely covering ascomata; ostiole not papillate, as a circular perforation in the epidermis. Peridium textura angularis, thin-walled, brown; cells 5-9 μm diam., guttulate. Hamathecium filaments 1-2 μm wide, hyaline, septate, abundant, cellular. Asci 110-130 x 15-20 μm, cyilindrical, stipe 10-30 μm long, biseriate. Ascospores (25) 28-35 x 10-13 μm, subfusiform, sometimes apically broader and basally attenuated, end cells rounded, 5-septate and with one to several longitutinal septa (end cells without longitudinal septa), light brown, with entire slime sheath.

Ecology: on one year old, still standing, median height, dead, leaf sheaths of *Phragmites australis*.

Studied specimens: VR364, VR533, VR685

Remarks
This species has larger ascospores than *Lewia infectoria* and *Phaeosphaeria vagans* with a light coloured peridium and light coloured ascospores which are rounded at the apex. Crevelli (1983) pointed out that the *Pleospora abscondita* sensu Wehmeyer is synonym of *Massariosphaeria automnalis* (see Leuchtmann, 1984).

Pleospora herbarum (Pers.) Rabenh. ex Ces. & De Not., Comm. Soc. crittog. Ital. 1: 217 (1863).

This plurivorous species (see Dennis, 1980; Ellis & Ellis, 1997) was rarely encountered on *P. australis* and had rather small perithecia 150- 200 μm diam. Peridium textura angularis, dark brown cells ± 5 μm diam., with oil drops. With only 7 asci, bitunicate, oblong, short stipe. Hamathecium filaments cellular, septate, cells 8-12 μm long, hyaline. Ascospores 32-38 x 12-17 μm, oblong, cylindrical, end cells rounded, smooth, bright yellw-brown, with 6-7 transverse septa and 1-5 longitudinal septa, with a gelatinous sheath entire, 3-5 μm wide.

Studied specimens: GVR175, 6/01/2000, Belgium, Scheldt estuary, brackish tidal marsh opposite to Bayer, near Liefkenshoekfort, IFBL B4.54.42 (no exsiccatum)

Remarks
This species was interpreted with a wide species concept and it would not come as a surprise that this taxon encompasses several cryptic taxa.

***Podospora* sp. I [*Podospora gigacommunis* nom. prov.]**
Etymol.: referring to the large size of the ascospores and 'communis' refers to *Podospora communis* which shows resemblance to our collection.

Ascomata 650-1000 μm diam., spherical, olive green-brown, solitary, superficial, young perithecia with long flexible pale brown hyphae, 2,5 μm wide, septate, hyphae disappearing when perithecia overmature; ostiole black, rounded not well developed. Peridium outer layer textura angularis, cells 4-6 μm diam. Hamathecium filaments present, hyaline, septated, evanishing. Asci 350-480 x 60-75 μm, clavate, thick-walled at the apex, unclear apical ring, unitunicate, 8-spored, biseriate. Ascospores 57-62 x 33-37 μm, elliptical, when young clavate, thin-walled, first hyaline later dark brown, filled with small guttules; primary appendage (pedicel) short 18- 20 x 10-12 μm, hyaline, aguttulate, cylindrical; apical secondary appendage 30-100 μm long, clearly striate, basal secondary appendage up to 40-70 μm long with smaller base, first coherent later splitting into different parts (up to 5).

Studied specimens: VR352

Remarks
Compare this collection with *Podospora communis* (Speg.) Niessl, Hedwigia 22: 156 (1883) which has according to Saccardo I: 231 much smaller ascospores and asci.
Very large spores! Not known for this genus!
This species was recently also collected on *P. australis* and other monocots by B. Declercq (pers. comm.).

***Podospora* sp. II**

Ascomata 200 µm diam., 300 µm high, olive-green, pyriform, superficial; ostiole clear, blunt, black. Peridium thin-walled, membranous. Hamathecium filaments abundant, hyaline, septate. Asci 220-235 x 60-72, clavate, thick-walled at apex, unitunicate, with unclear apical ring, 12 asci in one ascoma, **16-spored**. Ascospores 31-37 x 23-30, broadly ellipsoid, first hyaline later dark brown, thin-walled, filled with small guttules, entire slime sheath; primary appendage (pedicel), soon differentiated in ascus, 10-13 x 2 µm, hyaline, eguttulate, cylindrical; secondary appendages absent.

Ecology: on leaf blade.

Studied specimens: No herbarium material, only a drawing was made, master thesis (Van Ryckegem (1999) as no 490, 14/03/1999, Belgium, Oostpolderkreek (Sint-Laureins, Bentille), IFBL C2.18.14)

Pseudohalonectria adversaria Shearer, Can. J. Bot. 67(7): 1945 (1989).

Ascomata 250 - 350 µm diam., spherical, black, substrate sometimes showing yellow colouration, immersed, solitary or in short rows; ostiole protruding epidermis, short. Peridium textura angularis to prismatica, 13 µm thick, orange brown to dark brown. Hamathecium filaments 140 x 4 µm, longer than asci , septate, cells 20 µm long, slightly constricted at the septa, tapering to point. Asci 100-130 x 9-12 µm, cylindrical, short stipe, dehiscent; with apical apparatus thimble-shaped and 1,5 µm high by 2,5-3 µm wide, apical apparatus coloured in cotton blue (chitinoid), spores shooting out in water, biseriate. Ascospores 37-43 x 4-6.5 µm, narrowly fusiform, smooth, straight to slightly curved, 3-5 septate, filled with small guttules.

Ecology: on submerged, old stems in freshwater conditions

Studied specimens: VR625

Remarks
Our collections fit well with the type description of Shearer (1989).

Pseudohalonectria aff. falcata Shearer, Can. J. Bot. 67(7): 1945 (1989).

Ascomata 200-250 wide, 350-450 µm high, pale yellow-brown, lying horizontally on substrate with raising ostiole protruding epidermis. Peridium inner layers textura angularis with lots of guttules, outer excipulum unclear textura prismatica to epidermoidea, yellow brown. Hamathecium filaments at base up to 10 µm wide, up to 180 µm long, tapering. Asci 125-195 x

11-13 μm, cylindrical-fusiform, short stipe, dehiscent and spores often liberated by breacking up of the basal part of ascus, with thimble shaped apical apparatus 2.5 μm wide by 1-2 μm high visible in cotton blue, multiseriate and often spiralizing spores. Ascospores 135-175 x 3-5 μm, scolecospore, tapering to ends, smooth, bended or straight, 6-9 septate but difficult to see, multiguttulate, germinating at both ends.

Studied specimens: VR632, VR707

Remarks
Differs from the type collection by (1) asci which show a thimble shaped apical apparatus (*P. falcata* has a flattened apical apparatus fide Shearer, 1989) and (2) smaller and not clearly falcate ascospores as presented by Shearer (1989).

Rosellinia musispora Van Ryck. & Verbeken, Sterbeeckia 19: 3 (2000).

Subiculum weakly developed, brown-black, felty. Stromata 700-850 μm diam., 400-600 μm high, superficial, uniperthiciate, solitary or 2-3 stromata aggregated, slightly flattened to spherical, black, smooth; ostiole pointed to papillate; peridium 50-70 (100) μm thick, carbonaceous. Hamathecium filaments hyaline, filiform, soon vanishing. Asci 160-190 x 6.5-7 μm, unitunicate, cylindrical, tapering in a short stipe (26 μm long), IKI^+, with large apical apparatus 6 x 4 μm. Ascospores 22-30 x 4.5-7 μm, fusiform, slightly curved to straight, with rounded ends, deep brown, aseptate; clear germ slit, almost over the entire spore length, straight to weakly sigmoid; slime coat entire, 2-3 μm; no appendages.

Studied specimens: VR341 (Holotype), VR304

Remarks
See Van Ryckegem & Verbeken (2000) for a full discussion of this taxon.

Savoryella lignicola E.B.G. Jones & R.A. Eaton, Trans. Br. Mycol. Soc. 52(1): 162 (1969).

Ascomata ± 250 x 150 μm, solitary, partly immersed, dark brown, with well defined ostiole; peridium textura epidermoidea-angularis. Hamathecium filaments not observed. Asci ± 120-150 x 20 μm, with refractive apical apparatus, IKI^-, spores biseriate. Ascospores 25-31 x 7-9 μm, ellipsoid, smooth, 3-septate, central cells brown, end cells hyaline, constricted; no slime coat was observed.

Studied specimens: Sweden, collection no. 02-215 (pers. herbarium?), leg. S.-A. Hanson (sub cf. *Magnaporthe* sp. collection sent by T. Læssøe.

Remarks
This species is different form *Passeriniella* (see above) in having unitunicate asci with a clearly refractive apical apparatus. The collection fits well with the type description. The species seems to be very wide spread and is found both in temperate and tropical areas (Hyde, 1994).

Schizothecium hispidulum (Speg.) N. Lundq., Symb. Bot. Upsal. 20: 254 (1972).

Ascomata ± 200 μm diam., 500 μm high, pyriform, cylindrical, dark brown, granular, at the base with brown hyphae; peridium thin-walled, olive-greenish under the microscope; hairs agglutinate, pale brown, with pointed end cell; ostiole flattended, black, composed out thick-walled cells. Hamathecium filaments not described. Asci 170-250 x 30-40 μm, clavate, without apical apparatus, unitunicate, 8-spored, IKI^-, spores bi-triseriate, uniseriate at base. Ascospores 23-28 x 16-18 μm, ellipsoid, at first hyaline, later dark brown at first with a blue tint, filled with small guttules; primary appendage (pedicel) 10-15 x 2 μm, formed in an initial phase of spore maturation, eguttulate; no secondary appendages.

Ecology: on rotten, humid reeds preferably in eutrophic sites.

Studied specimens: VR128

***Schizothecium* sp.**

This specimen was not studied in detail; it differs from the previous taxon by it's larger ascospores (35-45 x 21-27 μm) and a short pedicel of 10-12 x 2 μm. No secondary appendages were observed.

Studied specimens: VR322

***Sphaerodes* sp.**

Ascomata 200-250 μm diam., spherical, dark brown when growing in old perithecia, light brown when immersed in reed, ostiole not well observed but presence is suggested as glistening, dry, spore mass gathers on top of the fruitbodies. Peridium textura angularis, thick-walled dark brown to yellow when immersed. Hamathecium filaments absent or evanescent. Asci 80-90 x 45-50 μm, (55 x 25 μm with ascospores still immature), J^-, broadly clavate, irregular shaped, thin-walled, 4-spored, deliquescent. Ascospores 31-40 x 16-20 μm, ellipsoid, truncate at ends, aseptate, thickened around two distinct apical germ pores, dark brown, smooth, with many small guttules (forming one large guttule when dead).

Substrate: possibly sometimes hypersaprotrophic on old perithecia of *Massarina arundinaceae* on stems of *Phragmites australis*.

Studied specimens: VR118

Remarks
Spores resemble well those of *Corynascella humicola* see von Arx (1975: 24). But in contrast to first observations, this species has no cleistothecial ascomata and no textura epidermoidea which makes *Corynascella* impossible.
Stands close to *Melanospora* Corda, *Persiciospora* Cannon & Hawksworth and *Sphaerodes* Clem. (Cannon & Hawksworth, 1982).

- differs from *Melanospora* by the clear truncate, tuberculate pores. In contrast *Melanospora* is characterized by depressed germ pores.
- differs from *Persiciospora* by the smooth, non ornamentated ascospores and clearly truncated ascospores.
- Close to *Sphaerodes* on basis of the pores but our species shows no reticulate ascospores (perhaps EM) and is perithecoid which is rare in this genus. *S. fimicola* shows an ostiole. *S. fimicola* differs e.g. in having superficial perithecia, smaller (14-26 x 10-17 µm) and coarsely reticulate ascospores (see also von Arx, 1981).
- Check out next species: *Melanospora singaporensis* Morinaga et al. 1978: 142 Trans. Mycol. Soc. Japan 19: 135-148; and *Microthecium ellipsosporum* Takada 1973: 527 Bull. National. Science Museum, Tokyo 16: 521-535. Those species could perhaps belong to *Sphaerodes* as suggested by the typical raised annulus around the pore.
- Check out: *Sphaerodes manginii* (Vincens) Arx, [description: Bull. Soc. Mycol. Fr. 33: 67 ?1917]
- not treated in von Arx et al. (1988). Beih. Nova Hedw. 94: 1-104.
- not in Mycological Research 104: 887.

This species was also recovered from *Phragmites* by Taligoola (1969) and provided with a short description.

Wettsteinina cf. niesslii E. Müll., *Sydowia* **4**: 204 (1950)

Ascomata 300-500 µm long, 350-400 µm broad, spherical to cylindrical-oblong, variable, immersed, gregarious but not confluent, no clypeus observed; ostiole central, round, filled at first with hyaline cells, protruding, slightly elevating epidermis. Peridium textura angularis to textura prismatica near ostiole, pale yellow brown, thin-walled. Hamathecium filaments cellular, abundant, 3 µm wide, septate, cells 10-15 µm long, weakly constricted, with small guttules, anastomozing. Asci 95-130 x 13-15 µm, cylindrical, short stipe, foot not rooted, adhered on a common subhymenium, bitunicate, spores bi-triseriate. Ascospores 38-45 x 6-8 µm, (mean 42 x 6.5 µm), rather variable in length, fusiform, 3-5 septate, slightly constricted,

smooth, hyaline, becoming pale brown when senescent, with guttules, in end cells mostly less guttules, with two-parted sheath attached at both end cells.

Studied specimens: VR688, VR734 (GVR 2248)
Another collections GVR 1108 (only slide) looks superficially like this species but appears to have larger spores and trabeculae.

Remarks
Our collections seem close to the species decribed by Müller (1950) as *Wettsteinina niesslii*. Scheuer (1995) pointed out that Müller's collection on *Phragmites* is not conspecific with the type of *Wettsteinina niesslii* from *Carex* and is probably an undescribed and related species. Notwithstanding the resemblance there are some differences such as the slime coat, which seems in fresh conditions to be two parted. It should be noted that slime coat characteristics tend to be only a valuable characteristic if fresh material is observed as striking morphological differences can be observed between fresh and herbarium material (see Baral, 1992).
Further study is needed to name this species (and our collections should be compared thoroughly). Anyhow I feel the hamathecium characteristics are atypical for *Wettsteinina* and both our specimens and *Wettsteinina niesslii* (as described by Scheuer (1995)) should be transferred to another genus, perhaps *Massarina*.

Wettsteinina moniliformis Van Ryck. & Aptroot, Nova Hediwigia 73: 164 (2001).

Ascomata 100-150 μm diam., 90-120 μm high, globose to ovoid, aggregated into linear rows, black, immersed in basal stroma; ostiole 35-40 μm high, and up to 60 wide, papillate, black. Stroma up to 350 μm thick and 2 cm long, lenticular, with longitudinal orientation on stems, black, up to 7 rows ascocarps aggregated. Peridium 20-25 μm, textura angularis- prismatica with cells up to 5-10 μm diam. Hamathecium filaments numerous, 4-6 μm diam., septate, cells 6-10 μm long, moniliform, branching, with some small guttules. Asci 61-66 x 13-16 μm, numerous, broadly fusiform with 8 bi-triseriate overlapping ascospores. Ascospores 19-23 x 7-9 μm, broadly fusiform with rounded ends, 1-septate becoming 3-septate (sequence 2:1:3), constricted at first septum, apical cell little enlarged, first smooth and hyaline, with many small guttules, brown and verrucose when old; slime sheath uniform, indistinct, narrow.

Studied specimens: VR561 (holotype)

Anamorphic fungi

The anamorphic taxa, deuteromycetes or mitosporic fungi are artificially divided into the coelomycetes and the hyphomycetes. Coelomycetes are characterized by conidiomata were the conidia are formed within a cavity formed by fungal tissue or a combination of fungal tissue and host tissue. For example an acervulus or pycnidium. Hyphomycetes comprise species which have freely exposed conidiogene cells.

Coelomycetes

Aposphaeria sp.

Conidiomata pycnidial ± 100 µm diam., spherical, globose, black, aggregated in small groups, superficial; ostiole central, not papillate. Peridium textura angularis, tick-walled cells, 4 cell layers, about 10 µm thick. Conidiophores 8-10 x 1.5-3 µm, hyaline, composed out of 2-3 cells, conidiogene cells sometimes integrated. Conidiogene cells 3-4 x 2-3 µm, short cylindrical, enteroblastic (not very refractive), hyaline.

Ecology: most often on stems in the litter layer

Studied specimens: VR606

Remarks
Very inconspicuous in the field. *Aposphaeria*-like anamorphs (~*Pleurophomopsis*, *Asteromella* see Sutton, 1980) are reported for several teleomorphic genera such as *Massariosphaeria* (Leuchtmann, 1984), *Trematosphaeria* (Boise, 1985), *Lophiostoma* (Leuchtmann, 1985).

Ascochyta* cf. *arundinariae Tassi, Atti Accad. dei Fisiocritici Siena, Série 4 8: 65 (1896).

Conidiomata 100-120 µm diam., spherical, light ochre brown, immersed; ostiole darker than the rest of the pycnidium, +/- 12 µm diam. Peridium textura angularis, cells 5-7 µm diam., thickness 10-15 µm, light yellow. Conidiogene cells 6-7 µm wide x 6-8 µm high, small, inconspicuous, ampulliform, hyaline, forming inner excipulum, enteroblastic. Conidia 10-15 x 3-3.5 µm, cylindrical-oblong, rounded ends, straight to curved, hyaline, with mostly one sept, occasionally aseptate or 2-3 septa, slightly constricted (fresh!), with some small guttules near the septa.

Ecology: on leaves (blades and sheaths) in the basal canopy of standing culms.

Studied specimens: VR400

Remarks
This species keys out with Punithalingam (1979) as *Ascochyta arundinariae* Tassi. However this species is characterized by more fusiform conidia.

Ascochyta* cf. *leptospora (Trail) Hara, Diseases of the Rice Plant (Japan): 178 (1918).

Conidiomata 250 µm diam., spherical, light brown, solitary; ostiole central, weakly papillate. Peridium textura angularis, rather compressed cells, with many hyphae at the exterior, olive-brown. Conidiogene cells 5-6 µm high x 5 µm wide, ampulliform, hyaline, forming inner layer of excipulum, no periclinale thickening was observed. Conidia 13-15 x 2.5-3 µm, cylindrical to slightly fusiform, straight to curved, 1-septate, not to slightly constricted, hyaline, 2-3 guttules each cell.

Ecology: on leaf sheaths in the middle and top canopy of standing dead culms.

Studied specimens: GVR 1464, 23/03/2001, The Netherlands, Het verdronken land van Saeftinghe, Doel, IFBL, B4.33.43, and GVR3177, 30/09/2002, ibid, no herbarium collection only slide

Remarks
Differs from *Ascochyta* cf. *arundinariae* Tassi by the generally longer, smaller and more fusiform conidia. Many species have been described within the genus *Ascochyta* and several look similar. The following two species were reported from *Phragmites* and based on the descriptions both could fit. ***Ascochyta leptospora*** (Trail) Hara (Punithalingam (1979): 105). "Conidia cylindrical, mostly straight, sometimes slightly truncate or rounded, usually hyaline but occasionally very faintly pale yellow when the conidial contents have been released to the exterior, (13-)14-16(-18) x 2.5 -3 µm, median septate, rarely 2 septa."
Ascochyta arundinis Fautr. & Lamb. (Punithalingam (1979): 185). original description of this species " Périthèces nombreux, alignés dans les stries de la feuille. Spores cylindriques, atténuées aux extremites, hyalines, uniseptées, 16 x 4 µm." (➔ species with uncertain identity: type locality is unknown and should be located and examined to figure out whether this is an *Ascochyta* species or not (Punithalingam, 1979)).

Camarosporium feurichii Henn., Kgr. Sachsen ges. Sphaerioidaceae: 433 (1904).
= *Hendersonia phragmitis* Desm. Not. XXI, nr. 70 [date?].
=? *Camarosporium phragmitis* Brun., Revue Mycologique 1886: 142.

Conidiomata 150-200 µm diam., dark brown, spherical, immersed, substrate sometimes whitish around conidiomata; ostiole 60 µm wide, 60-70 µm high, central, papillate. Peridium textura angularis, unclear, thin-walled, yellow brown, cells 5-10 µm diam., inner excipulum hyaline.

Conidiophores 7-20 µm long, mostly 1 or 2 cells, sometimes sparsely branched, cylindrical or somewhat swollen, irregular, hyaline. Conidiogene cells 7-15 x 3-4 µm, doliiform to lageniform, conidial ontogeny holoblastic, conidiogene cells percurrent enteroblastic, annellidic, hyaline. Conidia 14-18 x 6-8 µm, shape somewhat variable, ovoid, cylindrical to clavate, first hyaline becoming dark brown, echinulate, 3 transversal septa, and 0-1-2 longitudinal septa, slightly constricted at septa.

Ecology: on leaf sheaths in the middle canopy of standing dead culms.

Studied specimens: VR348, VR453

Remarks
The conidia are definitely echinulate at mature stage, a feature not previously noticed by other workers. Species shows similarity with *Hendersonia culmiseda* but differs by the echinulate and longitudinale septated conidia and the shape of the conidiogene cells.
For a recent description see Scheuer & Chlebicki (1997). Saccardo, Syll. Fung. XVIII: 375, mentions conidia with 3 transverse septa, 10-15 x 4-6 µm. Ellis & Ellis (1997), give for the conidia: 15-17 x 5-6 µm.
Two sheets out Desmazières Plantes Cryptogames BR (BR-MYCO 118023-71), labeled with *Hendersonia phragmitis* Desm. [as 'phragmitidis'] contained pynidia and conidia typical for *Camarosporium feurichii.*
Two species are known from *Phragmites* in literature: *C. feurichii* (see above) and *C. phragmitis* Brun. The latter is described shortly in Saccardo X: 347: 3-septate conidia, 18-22 x 7-8 µm. and the type description in Revue Mycologique 1886: 142 shows the remark "Peut-être est-ce un état avancé de *Hendersonia phragmitis* Desm.".
A new combination for the name should be made.

***Camarosporium* sp.**

Conidiomata 110-200 µm diam., spherical, dark brown, immersed, mostly aggregated near the top of leaf sheath; ostiole central, papillate, sometimes somewhat darker, thicker peridium and thicker walled cells near ostiole. Peridium textura angularis, light brown to yellow, thin-walled cells 7- 10 µm diam., inner excipulum hyaline. Conidiogene cells 5-8 µm wide x 7-10 µm high, ampulli-doliiform, hyaline, discrete, holoblastic, no proliferation observed. Conidia 23-27 x 7-10,5 µm, broadly ellipsoid, brown, eventually 5 transverse septa (sequence 3:2:1:2:3), all but end cells with longitudinal septa (up to 2-3 each cell), constricted at septa, no guttules, smooth, no appendages.

Ecology: on leaf sheaths in the middle canopy of standing dead culms.

Studied specimens: VR365, VR545

Remarks
Camarosporium sp. differs from *C. feurichii* by the lighter coloured conidia, 5-transverse septa, more longitudinal septa, no echinulation and generally smaller pycnidia.

Coelomycete sp. I

Conidiomata variable in size 100-600 µm diam., spherical, immersed, solitary, light brown, central ostiole. Peridium textura angularis?, yellow brown, with hyphae at the exterior. Conidiogene cells 10-6 x 4-5 µm, doliiform, enteroblastic, hyaline, smooth. Conidia 11-14 x 2,5-3 µm, fusiform, 1-septate, slightly constricted, light yellow, no guttules.

Ecology: leaf sheaths middle canopy of standing dead culms; occasionally on leaf sheaths in the litter layer.

Studied specimens: GVR49, 6/1/2000, The Netherlands, Het verdronken land van Saeftinghe, Doel, IFBL, B4.33.43, (slide); VR532

Remarks
Thin walled pycnidial fungi with brown-yellow, 1-septated conidia and enteroblastic mode of conidiogenesis are mostly referred to *Pseudodiplodia* (P. Karst.) Sacc. (Sutton, 1980). The type species of this genus proved to have annellidic conidiogenesis Buchanan (1987). *Pseudodiplodia* is in urgent need of a revision. In the sense of Punithalingam (1979), this species would be included in the genus *Ascochyta*, but when using the key no appropriate species name is available for this species.

Coelomycete sp. II

Conidiomata 150-350 µm diam., spherical, light brown to ochre yellow eventually dark brown, immersed, often causing yellow coloration of substrate; ostiole protruding epidermis, darker coloured, with periphyses. Peridium textura angularis, thin walled, cells about 7 µm diam., with hyphae at exterior. Conidiogene cells 4-5 µm, very small, ampulliform, with porus which is little refractive (mode of conidiogenisis unsure), formed from inner layer excipulum. Conidia 15-28 x 3-4 µm, subhyaline to light brown (pinkish to brownish in mass), fusiform, first 1-septate, becoming 3-septate (sequence 2:1:3), not or slightly constricted, aguttulate or some small guttules, no appendages in kongored.
Some collections with microconidia 1.5-2 µm, cylindrical-globose, hyaline.

Ecology: on dead leaves (blades and sheaths) in the canopy.

Studied specimens: GVR109, 6/01/2000, Belgium, freshwater tidal marsh of the Scheldt estuary, Het Kijkverdriet, Steendorp (Temse), IFBL C4.54.11, (no herbarium collection); VR388; VR497; VR550; VR701

Remarks
This species is more abundant in the freshwater tidal system than in the brackish part. If placed in a form-genus this species would fit into *Stagonospora* s.l. This species appears like an immature *Hendersonia culmiseda* but it remains subhyaline and has more fusiform conidia.

Coelomycete sp. III

Conidiomata 250-300 µm diam., spherical, light brown, solitary, immersed, sometimes colouring substrate lighter and with margins of mycelia clearly visible (reaction zones); ostiole +/- 30 µm, protruding, with periphyses, peridium cells near ostiole darker, with thicker wall. Peridium textura angularis, thin walled (12 µm thick), many light brown hyphae at the exterior. Conidiophores 10-30 µm x 3-4 µm, 2-4 septa, constricted, branched, hyaline. Conidiogene cells enteroblastic, phialidic, integrated, doliiform, ampulliform. Conidia 8-11 x 1-1.5 µm, filiform, bacilliform, aseptate, smooth, straight or slightly curved, hyaline.

Ecology: in middle and upper canopy on standing moribund and dead leaf sheaths during late summer, autumn and winter.

Studied specimens: VR386 (GVR114), VR527

Remarks
Determination of this common taxon did not work out.
In Sprague (1950) some *Septoria* sp. are listed with aseptate, hyaline spores but none of these are similar to our taxon.

Coelomycete sp. IV

Conidiomata 500 µm long x 300 µm wide, immersed, splitting the epidermis open, solitary, not stromatic, pinkish inside when cut open. Conidiophores absent or 1-2 short cells. Condiogene cells, 6-9 x 3-4 µm, lageniform, enteroblastic (?) no clear periclinal thickening, annellidic, hyaline with the collars relatively wide from each other. Conidia 12-13 x 2-3 µm, cylindrical, rounded ends, straight, with small guttules, 0-3 septate (often 1 septate), guttulate.

Studied specimens: VR481 (GVR 412)

Remarks

This species shows affinities with *Stagonospora montagnei* (= *Stagonospora graminella* var. *lophiodes*) (see Castellani & Germano, 1977) but has smaller conidia. The anamorph of *Massarina lacustris* as described by Leuchtmann (1984) shows similarity to our collection. Compare with *Cytoplacosphaeria rimosa* (below)

Coelomycete sp. V

Conidiomata 250- 300 μm long and slightly smaller, unilocular, dark brown, immersed; ostiole not observed. Peridium textura angularis. Conidiophores 10-15 μm long, hyaline, branched some parts of conidiomatum without and conidiogene cells forming inner excipulum. Conidiogene cells 5-9 x 3-5 μm, ampulliform- lageniform, integrated or terminal, hyaline, holoblastic, proliferation percurrently, 0-4 annellations. Conidia 11-19 x 2.5-3 μm, fusiform, straight, 1-septate, not constricted, hyaline, no appendages, smooth, with some small guttules in each cell.

Studied specimens: GVR 1114, 20/09/2000, on leaf sheath in upper canopy, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43 (no exsiccatum), drawing

Remarks
Differs from Coelomycete sp. III in having larger conidia, consequently being 1-septate, the presence of short condiophores. Differs from *Cytoplacosphaeria rimosa* s.l. in the shape of the conidia which is typical for *C. rimosa* somewhat constricted.

Coelomycete sp. VI

Conidiomata ± 300 μm diam., immersed, light brown, peridium textura angularis; ostiole central, not papillate. Conidiophores absent. Conidiogene cells 4-5 μm doliiform, holoblastic(?), hyaline, forming the inner excipulum. Conidia 12-17 x 10-14 μm, subglobose to ellipsoid, ovoid, sometimes slightly irregular, hyaline, with multiple guttules, aseptate.

Studied specimens: GVR3249, 11/28/2002, on leaf sheath in canopy, Belgium, tidal marsh Scheldt estuary, Burcht (Schor bij het Galgenweel). No herbarium material only pictures and slide.

Remarks
We could not come to an appropriate genusname with Sutton (1980).

***Coniothyrium* sp.**

Conidiomata +/- 200 µm diam., spherical, black, solitary, immersed, thin walled; ostiole circular, central. Conidiogene cells small discrete, conidiogenese not observed. Conidia 5-6 x 2-3 µm, flattened, disc-shaped, brown, thick-walled, verrucose.

Studied specimens: description based on one observation: Van Ryckegem (1999) no179, 14/10/2001, on stem, Belgium, Molenkreek (Sint-Magriete), IFBL B2.57.22, no exsiccatum

Remarks
The genera *Coniothyrium*, *Sphaeropsis* and *Microsphaeropsis* show high resemblance and are in need of a revision. We could key these Coelomycetes with brown, aseptate conidia out as follows (based on Sutton, 1980):

1. Conidiogenesis holoblastic ..... ➔ 2
1. Conidiogenesis enteroblastic, with descrete conidiogene cells, conidiomata thin-walled, immersed, pycnidial ..... ➔ ***Microsphaeropsis***

2. Conidiogene cells, descrete, ampulliform; conidia thick-walled, rather small and generally verrucose. .....➔ ***Coniothyrium***
2. Conidiogene cells lageniform; conidia large, ornamentation on the inner side of the spore
..... ➔ ***Sphaeropsis***

Many species formerly placed in *Coniothyrium* probably belong in *Microsphaeropsis* (Sutton, 1980). *Coniothyrium* is said to by the anamorph of *Paraphaeosphaeria* spp. (see above) (Webster, 1955). However based on the description by Webster (1955) the anamorph appears to be closer to *Microsphaeropsis* as it has small, smooth conidia and thin-walled pycnidia. Our specimen fits best in *Coniothyrium* sensu Sutton (1980).

Cytoplacosphaeria rimosa (Oudem.) Petrak, Ann. Mycol. 17:79 (1919).

General description: ***sensu lato***

Conidiomata 300-350 µm long, aggregated up to 1-2 mm long, stroma development variable, immersed, solitary or aggregated, black, epidermis lifted and splitting open along the longitudinal axes of the stem, in leaf sheaths, black, thick-walled, with many hyphae at the exterior, flattened, solitary, no stroma; ostiole central, circular, conidia discharged as cirri, with periphyses. Peridium textura angularis, yellow at bottom and side, black and thicker walled at

the top of conidioom. Conidiophores absent or up to 30 µm long, septated, hyaline, branched with lateral and apical conidiogene cells, from the inner excipulum, variable. Condiogene cells enteroblastic, cylindrical, lageniform, some ampulliform, variable!, some collections percurrent with 1-4 flared annulations, terminal or lateral, some cells form new conidiogene cells on top tending to form conidiophores with a nodulous appearance (? in a late phase), hyaline, smooth. Conidia 11-23 x 3-4 µm, cylindrical to fusiform, often constricted in the middle, rounded or tapered, straight often bended, 0-3 septate, generally 1 median septum, with small guttules.

Ecology: on stems in litter layer, less on leaf sheaths.

Studied specimens: VR194, VR195, VR205, VR486, VR553; GVR950, 20/09/2000, stem, Belgium, Scheldt estuary, Het Kijkverdriet, Steendorp (Temse), 25/04/2001, IFBL C4.54.11 (no exsiccatum); GVR926, 20/09/2000, leaf sheath, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43 (no exsiccatum), drawing; GVR1443, 23/03/2001, on stem, ibid., (no exsiccatum), drawing

Remarks
Whether this wide species concept is correct remains to be sorted out as more collections are examined in detail. Possibly several species could be involved.

Following literature this species should differ from *Placonemia dothideoides* (Mont.) Petrak by the more thin-walled conidiomata, the somewhat larger conidia which can become 2-3 septate. These 2 species should be investigated on their variability in condiomata structure and conidia but I have the feeling they are very close to each other and in our wide concept they merge together. Collection VR205 could be closer to *Placonemia dothideoides* (Mont.) Petrak in the sense of Sutton (1980). VR486, 553 respond to the description given by Sutton (1980) for *Cytoplacosphaeria rimosa*.

Following the key provided by Sutton (1980), one wile not easily key out to *Cytoplacosphaeria* because of a mistake in the key. The genus is keyed out with conidia regarded as being multi-septate (while most conidia are 1-septate) and you should follow the step stating: 'conidiophores present', while in the description and in the illustration of the genus they are stated to be absent. [however in our concept they can be present!]

Cytoplacosphaeria and *Placonemia* are regarded to have ampulliform conidiogene cells, without conidiophores (or some small basal cells only). But our collections with similar conidia show a high variability in condiogene cell structure. The conidiogene cells were lageniform to ampulliform and sometimes integrated or terminal on conidiophores. Some cells showed clearly annellations caused by percurrent conidiogenesis. (VR484) answers to this variability. Perhaps *Cytoplacosphaeria* shows different ways of conidiogene cell formation depending on

ecological circumstances? Or when becoming (over)mature the species seems to show percurrent condiogenesis with catenate conidiogene cells.

VR195 was first determined as *Ascochyta* sp. and fits *Diplodina* sp. sensu Barnett & Hunter (1987) but is here include in our wide concept of *Cytoplacosphaeria rimosa*:
Conidiomata 200-150 x 150-100 µm, flattened, dark brown, relatively thick walled, immersed in stem; ostiole central, 15 µm diam. Peridium textura angularis, dark brown, light brown hyphae at the exterior, cells +/- 5µm diam. Conidiophores up to 15 µm, sometimes present, few cells bearing several conidiogene cells, smooth, hyaline. Conidiogene cells 10-6 x 5 µm, enteroblastic, phialidic, lageniform to ampulliform, periclinal thickening. Conidia 14-21 x 2,5-3 µm, fusiform, straight to slightly curved, 1-septate, not to slightly constricted, smooth, hyaline, several small guttules.
Collection VR481: (=Coelomycete sp. IV) shows clearly 1-3 septate conidia with rounded ends and ampulliform to lageniform conidiogene cells and is regarded as a separate species.

Dinemasporium graminum (Lib.) Lév., Annls Sci. Nat., Bot., sér. 3 5: 274 (1846).
= *Dinemasporium strigosum* (Pers. ex Fr.) Sacc., Michelia 2: 281 (1881).

Conidiomata ± 350 µm, superficial, black, at first spherical but spreading open and becoming cupulate with hairs at the margin. Conidiophores up to 23 µm long, unbranched, hyaline. Conidiogene cells slender, lageniform, cylindrical, tapered, enteroblastic, minute channel with periclinal thickening. Conidia 9-12 x 1.5-2 µm, fusiform, allantoid, hyaline, biguttulate, thin-walled, with a single unbranched setula at each end about 5-6 µm long. Hairs up to 350 µm long 5-6.5 µm wide, dark brown, septated, pointed.

Ecology: on several grass species, not common on reed

Studied specimens: VR11

Remarks
Phomatospora dinemasporium is regarded (doubtfull, see *P. dinemasporium*) as teleomorphic state (Webster, 1955).

***Diplodina* sp.**
(no figure)

Conidiomata 200-400 µm long, solitary or grouped in stromata up to 2 mm long, immersed becoming erumpent, spitting the epidermis open in the length-axes of the stem, black; acervular. Conidiophores 20-80 µm, hyaline, sometimes branched at base, 0-3 septa. Conidiogene cells enteroblastic, phialidic, hyaline, smooth, a conspicuous apical aperture,

collarette and channel minute, periclinal wall thickened. Conidia 14-19 x 3-4 μm, fusiform, 1-septa, slightly constricted, hyaline, with small guttules at ends and at the septa.

Studied specimens: VR87 (see Van Ryckegem, 1999 no 123).

Remarks
Conidia of this species resemble those of *Ascochyta* spp. However different conidiogene cells and the presence of slender conidiophores are arguments to give this collection a separate status and place them in the genus *Diplodina* (sensu Sutton, 1980). Collected once during study.

***Hendersonia – Stagonospora* remark:**

Hendersonia and *Stagonospora* like anamorphs are one of the major taxonomic challenges in mycology. Those form-genera are extremely diverse and seem to be important on several hosts as endophytes and are important decomposers in several ecosystems. They have been linked to multiple teleomorphs (e.g. *Phaeosphaeria*, *Mycosphaerella*, *Massarina*, *Massariosphaeria*, *Leptosphaeria*, …) and no modern revisions are available.
The determination of *Hendersonia* and *Stagonospora* species on *Phragmites* is a hell's job since many species have been described and it seems that for a lot of species several names are available. Furthermore few morphological characters are available and the original descriptions often delineate species based on conidial size. *Hendersonia* [more than 1000 names!] is rejected in favour for *Stagonospora*, since the type (*Hendersonia paludosa* Sacc. & Speg.) belongs to the traditionally hyaline-spored *Stagonospora* (Wakefield, 1939). However, many mycologists stayed using the name *Hendersonia* in a Saccardian sense characterized by the brown-coloured phragmoconidia. All of these species should be replaced in other appropriate genera such as *Seimatosporium*, *Phaeoseptoria*, *Sonderhenia*, *Sclerostagonospora*, *Septoria*, *Septoriella* and *Stagonospora* and probably some new genera should be created to accommodate all those former *Hendersonia* species.
However during our surveys of fungi on *P. australis* we stayed using the name *Hendersonia* for some taxa. Those taxa were characterized by brown phragmoconidia without appendages (however *H. culmiseda* proved to possess small slime caps) and with holoblastic, ampulliform conidiogene cells.

During our work on phragmiticolous fungi we interpreted *Stagonospora* in a narrow sense (*sensu* Sutton, 1980) with hyaline, phragmoconidia and holoblastic often annellidic conidiogene cells. Cunfer & Ueng (1999) published an incomplete review paper on *Septoria* and *Stagonospora* species on cereals (e.g. they don't cite important taxonomic work (Sutton, 1977; 1980) using fungal morphology to distinguish between those genera based by detailed observation of conidiogenesis.

One type was investigated: *Hendersonia neglecta* Westend. **(BR-Myco 118004-52)** and is discussed below.

Hendersonia neglecta Westend., Herb. crypt. fasc. 25, n° 1224 [DATE?]
See Kickx Fl. Crypt. Flandres I: 390 (1867).

Conidiomata 190-260 µm diam., spherical, brown-black, solitary, immersed; ostiole protruding weakly papillate. Peridium textura angularis, thin-walled (10-15 µm); cells 6-9 µm at broadest part, light brown, thin-walled. Conidiophores absent. Conidiogene cells 4-5 µm wide, 3-4 µm high, ampulliform, forming inner layer wall, hyaline, probably holoblastic. Conidia 19-29 x 4-5 µm, cylindrical to slightly fusiform, rounded ends, 3-septate, slightly constricted, smooth, aguttulate, with apical slime appendage up to 5 µm long and staining in kongo red.

On leaf of *Phragmites australis*
Together with *Pseudoseptoria donacis*.

Remarks
According to the description of Westendorp this species had hyaline conidia, however they are light yellowish and hence don't fit in *Stagonospora sensu stricto*. This species is identical or belongs to *Septoriella* sp(p). (see below) a very common species complex (or is it one variable taxon?) on *P. australis*.

Hendersonia* aff. *culmiseda Sacc.

No good description is made of this species (?) so far.
No figures

Studied specimens: VR640

Remarks
Conidia 3-septate, resemble those of *Hendersonia culmiseda* if young but are larger, lighter coloured and have no appendages in Kongored (the value of this feature should be checked). When older they become more elongated and look more like *Septoriella* sp(p). but without appendage, up to 7 septate.

Hendersonia culmiseda Sacc., Syll. Fung. 3 (1884).

Conidiomata 200-450 µm, spherical to somewhat compressed, black, immersed, solitary or in short rows breaking the epidermis open; ostiole papillate, build out thicker-walled cells, darker than conidiomatum; periphyses short, hyaline, 0-1 septate, endcell slightly swollen in ostiolar channel. Peridium textura angularis +/- 10-15 µm thick out of 5-6 cellayers, near ostiole textura prismatica, cells dark brown, slightly thick-walled; with some hyphae at the exterior. Conidiogene cells forming inner layer of conidiomata, 5-7 x 3-5 µm, ampulliform, holoblactic,

annellidic (! but very difficult to observe). Conidia 12-19 x 4-7 μm, ellipsoid, tapered near ends, dark brown, in mass almost black, 3-septate slightly constricted, aguttulate, with 1 clear apical appendage and sometimes a small basal appendage visible in kongo red.

Ecology: very common species, especially on leaf sheath but occasionally also on leaf and stem. Dominant on leaf sheath in the upper canopy.

Studied specimens: VR144, VR368, VR751

Hendersonia sp. I

Conidiomata ± 150 μm diam., spherical, brown, immersed, solitary; ostiole central, ± 20 μm diam. Peridium textura angularis 12-15 μm thick, cells 8-10 μm diam., many hyphae at the exterior. Conidiogene cells 7-8 x 6-7 μm, ampulliform, hyaline or subhyaline, ontogeny holoblastic. Conidia 38-56 x 4.5-7 μm, cylindrical often tapered at the top, 5-8 septa mostly 7, constricted, septa pigmented yellow-greenish (seen with a lot of light), multiguttulate, very light straw-colour, smooth, no appendages.

Ecology: occassionaly on leaf and leaf sheath in middle and upper canopy.

Studied specimens: GVR882, 20/09/2000, on leaf sheath, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43 (no exsiccatum), drawing

Remarks
This species is characterized by the generally wide conidia which are light coloured.

Hendersonia sp. II

Conidiomata ± 350 μm diam., spherical, brown, with central ostiole. Peridium textura angularis, light brown. Conidiogene cells ± 5 μm diam., ampulliform, hyaline, conidiogenesis could not be observed. Conidia 23-26 x 2-2.5 μm, filliform-cylindrical, straight or curved (rather variable), 3-septate, light brown, in mass orange brown, aguttulate or with some small guttules near the septa, no slime appendage was observed.

Studied specimens: VR511; GVR1634, 25/04/2001, Belgium, Scheldt estuary, Het Kijkverdriet, Steendorp (Temse), 25/04/2001, IFBL C4.54.11 (no exsiccatum)

Remarks
Because of the narrow, coloured spores this deuteromycetes could be placed within *Phaeoseptoria*. It has affinities to the anamorph of *Phaeosphaeria eustoma* (Leuchtmann, 1984) and it fits nearly perfect for the anamorph of *Phaeosphaeria pontiformis* (Webster & Hudson, 1957).

Hendersonia **sp. III**

Conidiomata 300-350 µm diam., solitary or with 2-3 conidiomata together, immersed, dark brown; ostiole like perforation in epidermis ± 25 µm diam.; peridium textura angularis, light brown, thin-walled, with hyaline hyphae (1.5 µm wide) at the exterior. Conidophores absent. Conidiogene cells ± 5 µm, ampulliform, holoblastic (?), hyaline, formed from the inner cells of peridium. Conidia 32-45 x 5-5.5 µm, truncate near base, tapered to the top, widest part submedian, straight or slightly bended, 7 septate, slightly constricted, no appendages.

Studied specimens: VR412, GVR401, on leaf sheath, 10/04/2000, Belgium, Scheldt estuary, tidal marsh Burcht, IFBL C4.26.31

Remarks
This taxon has conidia with a typical shape and similar anamorphs are figure by Leuchtmann (1984) as anamorphs for *Phaeosphaeria* spp. Perhaps this taxon is the anamorph of *Phaeosphaeria culmorum* (see above).

Microdiplodia sp.

Conidiomata 250-400 µm long x 150-200 µm wide, lenticular, orientated in length of the substrate, black, immersed; ostiole circular, unclear, not papillate. Peridium textura angularis, cells 6-9 µm wide, light brown. Conidiophores absent. Conidiogene cells 5-10 x 5 µm, ampulliform to doliiform, hyaline, conidiogenisis holoblastic? Conidia 11-15 x 4-5.5 µm, ellipsoid, rounded ends, 1-septated, constricted, brown to chestnut brown, aguttulate, with terminal small appendage as a fine drop not staining in kongo red.

Ecology: leaf blades, leaf sheaths (most often) and stems in a standing dead position. Recorded during winter.

Studied specimens: VR450; GVR3384, 26/02/2003, leaf sheath, Belgium, Scheldt estuary, tidal marsh Burcht, IFBL C4.26.31

Remarks
The genus *Microdiplodia* Tassi, Bull. Lab. Ort. Bot. Siena 5: 29 (1902) was originally introduced for stem- or branch- inhabiting species with small, brown, 1-septate conidia; in constrast to

Diplodia with large conidia. No lectotype is designated (Sutton, 1977) and no recent revision is available.

Our collections are close to:
➔ *Diplodia beckii* Bäuml., Myc. Not. p. 1. (Saccardo, 1892 (X: 291)) "10-13 x 4-4.5 μm".
➔ *Microdiplodia machlaiana* I. Reichert, Engl. Bot. Jahrb. 56, p. 714 tab. III, fig. 5, 1921 in Saccardo, 1972 Suppl. (XXVI: 1074) "sporis ovoideis uniseptatis, fuscis, 7-10 x 3-5 μm".

Microsphaeropsis arundinis (J. Ahmad) B. Sutton, The Coelomycetes: 423 (1980).

Conidiomata 200 -250 μm diam., spherical, brown, immersed, solitary; ostiole central, weakly papillate, cells around ostiole darker brown compared to the rest of peridium. Peridium textura angularis, light brown, thin walled 13-15 μm, 5-6 cell layers, smooth at the exterior (few hyphae), cells 6-8 μm diam. Conidiogene cells 5-6 μm, enteroblastic, ampulliform, inconspicuous, (sub)hyaline, channel very narrow, periclinal thickening present. Conidia 3-5,5 x 1,5- 2,5 μm, cylindrical, rounded, light brown, aseptate, 1-2 guttules at the ends.

Studied specimens: VR496

Remarks
Fisher et al. (1986) have shown that antibiotic activity is particularly high in species of the genus *Microsphaeriopsis*. Many species are considered to be endophytes.

***Microsphaeropsis* sp. I**

Conidiomata about 200 μm diam., spherical, yellow-brown, immersed; ostiole 30 μm long, 25 μm innerdiam., slightly papillate, cells near ostiole darker brown and globose. Peridium textura angularis-globosa, near ostiole more textura prismatica, light yellow, individual cell subhyaline, 5 μm diam., at the exterior many hyphae and amorphous segregations were seen. Conidiogene cells 6 x 5-5.5 μm, enteroblastic, ampulliform, hyaline, forming inner layer of excipulum. Conidia 6-8.5 x 3-3.5 μm, rounded ellipsoid, aseptate, smooth, light yellow, brown in mass, with terminal some small guttules.

Ecology: on leaf sheaths of middle height canopy.

Studied specimens: GVR235, 12/02/2000, leaf sheath, Belgium, Scheldt estuary, Het Kijkverdriet, Steendorp (Temse), IFBL C4.54.11 (no exsiccatum)

***Microsphaeropsis* sp. II**

Conidiomata about 300 µm diam., flattened to spherical, black, immersed. Peridium textura angularis, light brown, cells thin-walled. Conidiogene cells 7 x 5 µm, small, ampulliform, hyaline, mode of conidiogenesis could not be observed. Conidia 10-11.5 x 4-5.5 µm, ovoid-ellipsoid, ochre-brown, aseptate, smooth, no guttules or some small ones.

Studied specimens: GVR176, 6/01/2000, on leaf sheath, Belgium, Scheldt estuary, brackish tidal marsh opposite to Bayer, near Liefkenshoekfort, IFBL B4.54.42, no exsiccatum, drawing

Remarks
This species is perhaps a *Sphaeropsis* sp. with small spores?

Neottiosporina australiensis B.Sutton & Alcorn, Austr. J. Bot. 22: 526 (1974).

Conidiomata 120-350 µm diam., spherical, pycnidial, brown-black, immersed, solitary or aggregated in rows or small groups on leafs and leaf sheaths, on stems often forming thight groups in pseudostroma; ostiole central becoming erumpent and sometimes slightly papillate. Peridium textura angularis, thin-walled; often amorphous, black-brownish thickenings present, cells black-brown, rather thick-walled, with hyphae at the exterior. Conidiophores absent. Conidiogene cells 4-5 µm, ampulliform- doliiform, conidial ontogeny holoblastic, hyaline, smooth. Macroconidia 25-34 x 6-9,5 µm, oblong, basally often truncate, 3-septate, constricted, hyaline, multiguttulate; appendage apical, cap-like, funnel shaped expanding in water up to 8 µm long. Microconidia 4-5 x 2-3 µm, restricted to ostiolar channel, rare, subcylindrical, hyaline.

Ecology: common on leaf sheaths on standing dead culms and in the litter layer.

Studied specimens: VR215, VR508

Remarks
Was observed in close relationship with *Massarina fluviatilis* Aptroot & Van Ryck. Studied materiaal: Oostpolderkreek, 14/03/99, Van Ryckegem 251 (GENT), Van Ryckegem 508 (GENT).

We investigated the type material of *Stagonospora simplicior* Sacc. & Briard (BR-MYCO 118003-51 1887) and this species proves identical and hence an older name for *Neottiosporina australiensis* B. Sutton & Alcorn.
Therefore a recombination should be made: ***Neottiosporina simplicior*** (B. Sutton & Alcorn) Van Ryck.

Bas. *Stagonospora simplicior* Sacc. & Briard in Roum., Fungi Gall. No. 4086; Syll. X., p. 336 (1892). (check basionym reference)

Description of the type material:

Conidiomata 150 μm diam., spherical, pycnidial, black, immersed, in rows on leaf sheath of *Phragmites australis*, lifting up the epidermis; ostiole central, 40 μm outer diam. Peridium textura angularis with smaller more thick-walled cells near the ostiole. Conidiophores absent. Conidiogene cells ampulliform, small, conidiogenese not observed. Conidia 28- 32 x 8-9,5 μm, oblong, basal cell often truncate, 3-septate, constricted, with contents but guttulation dis-appeared, appendages often lost but some still show a typical funnel shaped appendage.

Type is well preserved.

***Phaeoseptoria* sp.**

Conidiomata ± 250 μm diam., black-brown, solitary, immersed, mostly on margin of leaf sheath; ostiole central, not papillate. Peridium textura angularis, thin-walled; cells ± 10 μm diam. Conidiogene cells, ± 5 x 5 μm, ampulliform, hyaline, polyblastic, ontogeny enteroblastic. Conidia 65- 100 (140) x 3-4 μm, filliform, tapered at apex, 7-11 (14) septate, mostly 10 septate, not constricted, light brown, guttulate, with small apical appendage.

Ecology: in a basal height standing position on moribund and newly died leaf sheaths.

Studied specimens: VR581

Remarks
Species close to *P. festucae* Sprague and *P. phalaridis* (Trail) Sprague (Punithalingam, 1980).

***Phialophorophoma* sp.**

Conidiomata 200-250 μm diam., spherical, greyish often with a sea-green tint in the peridium and in the surrounding substrate; ostiole central, not papillate, protruding. Peridium textura angularis, up to 15 μm thick. Conidiophores 15-20 μm long, filliform, up to 5-septa, branched, hyaline. Conidiogene cells phialides, enteroblastic, integrated or terminal, hyaline. Conidia 4-5 x 1.5-2 μm, bacilliform, aseptate, hyaline, aguttulate, thin-walled.

Ecology: on leaf sheaths in a middle height position in the canopy. Often associated with *Stictis* sp.

Studied specimens: GVR389, 10/04/2000, Belgium, Scheldt estuary, tidal marsh Burcht, IFBL C4.26.31, no exsiccatum, only drawing

Remarks
Our specimens were placed in the genus *Phialophorophoma* Linder based on microscopically features such as conidiophores, conidiogene cells and conidia. However, based on the conidiomatal characteristics this species is not congeneric with the monotypic *Phialophorophoma* Linder as described by Barghoorn & Linder (1944), Sutton & Pyrozynski (1963), Sutton (1980) and Kohlmeyer & Kohlmeyer (1979).

Our species shows close resemblance to type species *Phialophorophoma littoralis* Linder but this species has 1) conidiomata which are dark brown, subcarbonaceous and 2) a different ecology on marine drift wood (Kohlmeyer & Kohlmeyer, 1979), our specimen are mostly terrestrial collections (on the upper part of the leaf sheaths of *Phragmites australis*) although in an estuarine habitat.

***Phoma* sp. I**

Collections assigned to this species were recognized by the small conidia 3-5 x 1.5-2 μm, with one guttule at both ends and with lageniform conidiogene cells.
Determinations were sometimes arbitrary done studying only the conidial size, so perhaps several species are gathered in *Phoma* sp. I

Conidiomata 350-450 x 100-150 μm on stems, elongated along the length of the substrate, greyish-brown, immersed, solitary, (collections on leaf sheaths smaller and globose); ostiole not observed. Peridium textura angularis, thin walled, with refractive droplet in each cell. Conidiophores sometimes 1-3 basal cells which could be interpreted as conidiophores together up to 15 μm long, hyaline, with some basal branches. Conidiogene cells 8-10 x 3-3.5 μm, lageniform, with variable neck-length, hyaline, percurrent enteroblastic (?) (Difficult to observe), conidiogene cells could be superimposed on each other. Conidia 3-5 x 1.5-2 μm, elliptical, oblong, aseptate, smooth, with one guttule at both ends.

Ecology: preferable on stems in litter layer both in fresh and brackish water, on old stems (well decomposed).

Studied specimens: VR624; GVR940, 20/02/2000, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43 (no exsiccatum), drawing; GVR3337, 26/02/2003, stem, ibid., picture.

***Phoma* sp. II** and ***Phoma* sp. IIa**

Phoma sp. II is a species-complex of *Phoma*-like coelomycetes characterized by:

Conidiomata small ± 100-150 µm, spherical, yellow-brown. Peridium textura angularis. Conidiogene cells ampulliform, small, conidiogenisis enteroblastic but difficult to observe. Conidia 5-7 x 2.5-3.5 µm, hyaline, oblong-cylindrical.

➔ *Phoma* sp. II: biguttulate (one at each end)
➔ *Phoma* sp. IIa: aguttulate.

Studied specimens: Phoma sp. II: GVR277, 12/02/2000, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43, (no exsiccatum), drawing; GVR3221, 30/09/2002, on standing dead stem from previous growing season, ibid., (no exsiccatum), pictures;
Phoma sp. IIa: GVR1125 culture (dried) (no pictures).

Remarks
The guttulation pattern was not noticed from the start, so the separation of the two forms is inconsequent, it is also unclear if the guttulation pattern is a good characteristic to separate two species.
Phoma sp. II proved to be the microconidial form of *Stagonospora vexata* (see below) and can be found associated together with the macroconidia in the same pycnidia. However solitary pycnidia with only the *Phoma*-state were also found.
A Phoma sp. IIa was cultured on CMA and large multicellular chlamydospores were formed. This taxon belongs to the section *Peyronellaea* and determination with Boerema (1993) leads to *Phoma glomerata* (Corda) Wollenw. & Hochapf. Although our taxon showed some hyaline to light brown hyphae on the outside of the peridium, it looks very similar in all other aspects.

Phoma sp. III

Conidiomata variable, on stems often aggregated in rows, with ostioles every 200 µm but united into one large lireate pycnidium, sometimes also solitary on stems; on leaf sheaths 150-500 µm diam., spherical, solitary, elongated in the length of the substrate, sometimes compressed; ostiole central short. Peridium regular textura angularis, thin-walled, at first (on green substrate) subhyaline but becoming melanized on moribund or dead substrate, smooth; cells rather large, up to 10-15 µm diam. Conidiogene cells 4-7 µm, ampulliform, hyaline, forming inner textura, enteroblastic. Conidia 7.5- 10 x 3-4 µm, ellipsoid, aseptate, hyaline, smooth, sometimes a few small guttules at the ends.

Ecology: dominant species our best studied site in basal and middle canopy on moribund and newly died leaf sheaths (see picture).

Studied specimens: VR565; GVR994, 17/10/2000, leaf sheath, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43, (no exsiccatum), drawing and dried culture

Remarks
Very common species, probably conspecific with *Phoma arundinaceae* (Berk.) Sacc. often cited on *P. australis* and perhaps identical to *Phoma rimosa* Westend.
Before the spores germinate they become larger up to (15-18 x 7-10 μm), germination tubes with enlarged, buliform cells just outside the conidium.
Phoma is often cited as anamorphic state of *Didymella* (Kirk et al., 2001).

***Phoma* sp. IV**

Conidiomata 200-300 μm diam., spherical to somewhat elongated, immersed, brown, olive-green, immersed; ostiole central, circular, protruding, becoming papillate. Peridium textura angularis, thin-walled (3-4 cellayers), often with many hyphae at the exterior, light brown; cells 6-12 μm diam., thin-walled. Conidiogene cells 3-6 μm diam, ampulliform to slightly lageniform, conidiogenesis difficult to observe, seems holoblastic (no refractive periclinar thicknings) under light microscope. Conidia 9-12 x 1.5-3 μm, cylindrical-oblong, aseptate, smooth, hyaline, with small guttules (becoming 1 when senescent) at the ends.

Ecology: on dead basal leaf sheaths during the winter (November – February) in Scheldt estuary.

Studied specimens: VR444, VR457, VR609

Remarks
If this species proves to be holoblastic it should be removed from *Phoma*.

Pseudorobillarda phragmitis (Cunnell) M. Morelet, Bull. Soc. Sci. Nat. Archéol. Toulon & Var. 175: 6 (1969).

Conidiomata up to 500 μm, spherical, immersed, brown; peridium with outer layers brownish green, textura angularis to globosa, cells 5-8 μm, the inner excipulum more prismatic, brownish-yellow, larger, ± 35 x 12 μm; contents of pycnidium appeared orange-yellow when cut open; ostiole central, circular, weakly papillate. Conidiophores unbranched, hyaline, one-celled. Conidia 13-20 x 3-4 μm, hyaline, 0-1 septate, guttulate; setulae three, 8-14 μm long. Paraphyses not seen.

Studied specimens: VR76

Remarks
This species was only collected once and the collection appeared somewhat immature. Septate conidia were only observed for few conidia (see Nag Raj et al. 1972).

***Pseudorobillarda* sp.** [*Pseudorobillarda appendiculata* Van Ryck. nom. prov.]
Etymology: referring to the specific appendages

Conidiomata 250-400 x 100-120 µm, lenticular orientated in the length of the substrate, greyish-black, immersed, smooth; ostiole central, not papillate. Peridium textura angularis, greyish-brown, thin-walled (5-10 µm), cells 8-10 µm diam. Paraphyses present, up to 45 µm long, enlarged at base, not septated (difficult to observe), with small guttules, flexible. Conidiogene cells 8-10 x 2.5-3.5 µm, ampulliform-lageniform, short cylindrical neck with sometimes up to 3 annellations, holoblastic with proliferation enteroblastic percurrent lacking periclinal thickenings. Conidia 19-22 x 3.5-4.5 µm, cylindrical, oblong, straight, sometimes slightly indented in the middle, 0 (mostly)- 1 septate, hyaline, full with small guttules; appendages flexible, 4-6, extracellular, about as long as conidia, attached to conidia by small fibril, forming and enlarged part just above fibril, ontogeny of the appendages could not be determined.

Ecology: on stems in freshwater habitats, submerged conditions

Studied specimens: VR618

Remarks
Appendages are atypical for the genus *Pseudorobillarda* (Punithalingham, 1986) but no other appropriate genus seems available (Nag Raj, 1993).

Pseudoseptoria donacis (Pass.) B. Sutton, Mycol. Pap. 141: 169 (1977).
Conidiomata ± 100 µm, very small, solitary or in small groups aggregated, spherical, light brown to black; peridium textura angularis, brown-black, cells ± 10µm diam. Ostiole small papillate. Conidophores absent. Conidiogene cells ± 4-5 µm, ampulliform, annellidic (difficult to see!). Conidia 16-24 x 3-4 µm, lunulate, aseptate, hyaline, some small guttules.

Ecology: on leaves and leaf sheaths in the upper canopy of reed vegetations. On moribund and senescent plant parts.

Studied specimens: VR615 (+ dried culture)

Remarks

We cultured this coelomycetes from a single conidie and did a peculiar observation (see pictures). In culture we could not observe pycnidia, only primordia or plectenchymatic stroma (?). However, a hyphomycete developed which formed nearly identical conidia and seems identical to the description of *Microdochium phragmitis* Sydow (Sutton et al., 1972). This latter species is considered to be an endophyte and was isolated from leaves (and other plant organs) by Wirsel et al. (2001) and proves to be a common species. However, I never saw *M. phragmitis* sporulating on *P. australis* in the field (probably you can induce it easily when leaves are incubated in moist conditions). If this observation proves to be true, it seems that *Pseudoseptoria donacis* is another form (better adapted to sporulate in the upper canopy?) of *M. phragmitis*. Confirming this data by molecular work would be interesting.

***Septoria* sp.**
(no pictures)

Conidiomata ± 200 µm, immersed, subhyaline fruit bodies, spherical; ostiole central inconspicuous; peridium textura intricate. Conidiophores absent. Conidiogene cells ± 10 x 1 µm, slender, branching near top, polyblastic, refractive apical aperture. Conidia 70-100 x 1-2 µm, scolecospores, filliform, attenuated, truncate at base, 9-11 septate, with small guttules near septa.

Studied specimens: VR745

Remarks
This species is considered to be a typical *Septoria sensu* Sutton (1980).

Septoriella phragmitis Oudem., Ned. Kruidk. Arch. 2 ser., 5: 504 (1889).

Conidiomata 150-250 µm diam., aggregated into linear, black stromata, up to 6 mm long, orientated along the length axes of the stems, ripping open epidermis, colouring substrate yellowish (?); ostiole 30 µm outer diam., circular, central, weakly papillate, at first filled with hyaline cells. Peridium not studied. Conidiophores absent. Conidiogene cells 5-6 x 6-7 µm, ampulliform, hyaline, smooth, holoblastic, proliferating percurrently once or twice. Macroconidia 33-70 x 3-4.5 µm, fusiform with a rounded apex and a narrowed, truncate base, 3-8 septa mostly 5, light brown, with a large, 5-8 µm flaring appendage apically colored in kongored, small basal appendage (not always seen), with few small guttules near the septa, conidia often as long cirri out of ostiole.

Microconidia not observed but conidiogene cells lining the ostiolar channel, conidia 3-4 x 2-2,5 µm, ovoid to turbinate, aseptate and hyaline (Nag Raj, 1993).
Culture remained sterile (VR648).

Ecology: most common on basal part of old, still standing stems. Also in litter layer (see remarks).

Studied specimens: VR189, VR648 (+ dried culture), VR786; GVR1276, 23/01/2001, on stem, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43, (no exsiccatum), drawing

Remarks
Collections from this species should be studied in detail, probably we include two taxa in this 'species'. One species occurring in the canopy of *P. australis* stands with slightly less fusiform, shorter conidia (up to 45 μm) while *S. phragmitis sensu stricto* occurs on old basal stem parts or in the litter layer and has generally longer (40-70 μm), clearly fusiform conidia. Both taxa show a clear apical appendage.

***Septoriella* sp(p).**

Conidiomata 180- 250 μm diam., spherical, brown, immersed, solitary; ostiole protruding, central. Peridium textura angularis, thin-walled 5-7 μm wide, layers out of polygonal flattened cells 5-8 layers, with relatively small cells +/- 7 μm diam.; abundant, light brown hyphae at the exterior. Conidiogene cells 3-5 x 3-5 μm, ampulliform, hyaline, conidia ontogeny holoblastic?
Conidia 23-48 x 3,5-5 μm, oblong to fusiform, mostly 3-7 septate, mostly 3-septa, slightly constricted, straight, smooth, light yellow to brow, with a clear apical appendage and sometimes a small basal appendage (visible in kongored), aguttulate or with small guttules near septa.

Ecology: very common species, dominant on leaves and leaf sheaths in the upper canopy during early fungal successional colonization (see figure).

Studied specimens: VR373, VR422, GVR988, 17/10/2000, leaf sheath, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43, (no exsiccatum), drawing; GVR3174, 30/09/2002, ibid., pictures (no exsiccatum)

Remarks
This species is probably a multispecies complex. However, no consequent features could be observed to separate these *Hendersonia*-like fungi with appendages.
The type of *Hendersonia neglecta* belongs to this group (see above). See also *Mycosphaerella lineolata* anamorph (above).

Sphaerellopsis filum (Biv. ex Fr.) B. Sutton, Mycol. Pap. 141: 196 (1977).

Conidiomata 150-200 µm diam., spherical, in short rows, stromatic in rust (*Puccinia*) telia; ostiole circular. Conidiogene cells not observed. Conidia 16-20 x 4-6 µm, ellipsoid-fusiform, hyaline, 1 septate, not constricted, with slime caps at both (!) ends, the apical cap largest and the basal small.
Found parasitic on *Puccinia magnusiana* Körn. (see above)

Studied specimens: VR538

Stagonospora cylindrica Cunnell, Trans. Brit. Mycol. Soc. 40: 451 (1957).

Conidiomata up to 1 mm wide and 500-600 µm high, one collection only 250 µm diam. (see remark), flattened, large, rather thin-walled, interveinal, linearly on stem, immersed, black, unilocular; ostiole central, not papillate, central, circular. Peridium textura angularis, the base of the conidiomatum with thinner wall, near ostiole more textura prismatica, greyish-black. Conidiophores absent. Conidiogene cells 6-12 high x 7-8 µm wide, ampulliform, annelate, holoblastic, proliferating percurrently once or twice, hyaline. Conidia 55-95 x 8-14 µm, cylindrical-fusiform, with truncate base, slightly narrowed, rounded apex, hyaline, 3-septate, hardly constricted at septa when young or mature, constricted when senescent and becoming subhyaline, smooth, no appendages, many small guttules in each cell.

Ecology: on basal parts of old, still standing dead stems.

Studied specimens: VR483, VR602

Remarks
The species was cultured (VR602). No teleomorph was formed. In culture on CMA, the conidiomata are much smaller and have a thicker wall, the conidia are smaller compared to the field collections; but see VR483 which formed only small conidiomata in the field. These observations show that the conidiomatal size is not a reliable taxonomic characteristic in *Stagonospora* (Sacc.) Sacc. and conidial size seems to be correlated with it.

Stagonospora elegans (Berk.) Sacc. & Traverso, Syll. fung. 20: 878 (1911).

Conidiomata up to 1 mm long, 500 µm wide and 500-600 µm high, flattened, large, rather thin-walled, interveinal, linearly on stem, immersed, black, unilocular; ostiole +/- 40 µm inner diam., central, sometimes papillate, central, circular; conidiomata formed in stem tissue still covered by leaf sheath have the ability to form an ostiole throught the leaf sheath which is formed out of a thick-walled parenchymaceous tissue, which could be interpreted as an

'epiconidiomatum' in the leaf sheath but without conidiogenesis. Peridium textura angularis, the base of the conidiomatum with thinner wall, near ostiole more textura prismatica, greyish-black. Conidiophores absent. Conidiogene cells 9-11 high x 7-8 μm wide, dolliform-ampulliform, holoblastic, proliferating percurrently, 0-2 annelations, hyaline. Conidia 60-75 x 9-13.5 μm, fusiform, narrowed at both sides, basaly more truncate, (4)-5-(6)- septate, constricted, no appendages, many small guttules.

Ecology: on basal parts of old standing dead stems but occasionally also higher up the stems or on leaf sheaths.

Studied specimens: VR272; GVR3220, 28/11/2002, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43, (no exsiccatum), pictures

Stagonospora incertae sedis I

Conidiomata 200- 500 μm diam. on leaf sheath, spherical, lenticular on stems, immersed up to almost superficial e.g. when conidiomata on the inner side of stems, brown-black; ostiole central, not or slightly papillate, protruding. Peridium textura angularis, light brown, near ostiole more textura prismatica, thin-walled; cells about 5 μm diam., small. Conidiophores absent. Conidiogene cells 7-15 x 4-7 μm, ampulliform to mostly lageniform, annelledic, holoblastic, hyaline. Conidia 12-23 x 4-6 μm, cylindrical, oblong, mostly irregularly bended, often slightly smaller in the middle, 1-3 (4) septate, slightly constricted, hyaline, no appendages, with small guttules.

Ecology: on stems, in litter layer, in a further stage of decay.

Studied specimens: VR383 (GVR97), VR440, VR578, VR626, VR698

Remarks
Uncertain in *Stagonospora* s.s. (Sutton, 1980) because of the lageniform conidiogene cells.
Our collections show resemblance to *Stagonospora vitensis* Unam. (see Sutton, 1980) but has, compared to Sutton's drawing, more irregularly shaped conidia.
Variability in this taxon could be large VR578 looks similar to *Stagonospora* incertae sedis I but was aberrant because of the generally longer conidia (20-33 x 4-6 μm) which tended to fall apart in individual cells by strong constriction at the septa when they matured. Several conidia in the pycnidial cavity were observed with their conidiogene cell still connected. Collections with these characteristic were initially referred to *Stagonospora* incertae sedis III but here referred to *Stagonospora* incertae sedis I.

***Stagonospora* incertae sedis II**

Conidiomata 300-400 µm diam., flattened, pycnidial, some fruit bodies elongated (up to 500 x 150 µm) along the axes of the substrate, black; ostiole protruding, central, not papillate. Peridium textura angularis-prismatica, thin-walled, dark brown. Conidiophores present at some parts of the conidiomatum, up to 50 µm long (often formed out of old conidiogene cells that are indeterminate and form new conidiogene cells on top of the old cell), branched, septated, hyaline. Conidiogene cells lageniform to cylindrical, terminal or lateral, indeterminate, percurrent proliferation, holoblastic, annellidic, annellations often wide-spaced but sometimes tight. Conidia 70-100 x 3.5-4,5 µm, scolecospore, cylindrical, rounded at ends, multiseptate (6-12 septa observed), not constricted, smooth, no appendages, hyaline, many small guttules.

Studied specimens: VR562

Remarks
This collection looks very similar to *Stagonospora* sp. I, which has similar conidiomata and conidia. However, in collections of *Stagonospora* sp. I we never saw conidiophores. Whether this collections should be placed in synonymy with *Stagonospora* sp. I has to be sorted out as variability of *Stagonospora* sp. I will become clearer in the future. For the time being we exclude this collection from *Stagonospora* s.s. (Sutton, 1980) and from *Stagonospora* sp. I based on the long lageniform to cylindrical conidiogene cells and the presence of conidiophores.

Stagonospora macropycnidia Cunnell, Trans. Brit. Mycol. Soc. 44: 87 (1961).

Conidiomata up to 500 mm long and 500 µm wide, 300 µm high, flattened, large, rather thin-walled, interveinal, linearly on stem, immersed, black, unilocular; ostiole +/- 40 µm inner diam., central, not papillate, central, circular; conidiomata on leaves are much smaller with a light coloured peridium and a darkened ostiole (see pictures). Peridium textura angularis, the base of the conidiomatum with thinner wall, near ostiole more textura prismatica, greyish-black. Conidiogene cells, 11-13 x 5,5-6 µm, ampulliform to lageniform, hyaline, proliferation percurrently, 0-2 annelations. Conidia 45-77 x 4-6 µm, cylindrical, rounded ends, 3 (-6) septate, not constricted becoming constricted when senescent, straight to slightly curved, with small guttules but less than *S. elegans*.

Studied specimens: VR627; GVR3442, Switzerland, 2/12/2003, coll. M.O. Gessner, Southeastern shore of Lake Neuchâtel, oligotrophic hardwater lake (no exsiccatum, pictures)

***Stagonospora* sp. I**

Conidiomata up to 500 mm long and 300 µm wide, 300 µm high, flattened, large, rather thin-walled, interveinal, linearly on stem, immersed, black, unilocular; ostiole central, weakly papillate. Peridium textura angularis-prismatica, brown. Conidiophores absent or with 1-2 basal cells, hyaline, short. Conidiogene cells 10-20 long x 4-7 wide, lageniform to cylindrical, holoblastic, proliferation percurrent, 1-4 annelations. Conidia 69-112 x 4-4.5 µm, scolecoconidia, slightly fusiform, 6-13 septa, mostly 7-9 septa, not constricted when mature but becoming constricted when senescent, smooth, no appendages, cells full with small guttules.

Studied specimens: VR623, pictures + drawing + culture (dried)

Remarks
Species shows affinities with *Stagonospora macropycnidia* but differs in the conidia which are longer, smaller and more septated.

***Stagonospora* sp. II** [*Stagonospora megaconidia* Van Ryck. nom. prov.]
Etymology: referring to the extraordinary large conidia

Conidiomata up to 1 mm diam. mostly smaller than long, up to 500 µm high, thick to thin-walled, immersed, solitary, unilocular; ostiole circular, central, sometimes papillate. Peridium textura angularis-prismatica dark brown to yellow brown. Conidiophores absent. Conidiogene cells 5-10 x 5-7 µm, ampulliform- doliiform, holoblastic, proliferation percurrently, 0-2 annelations, hyaline. Conidia 100-160 x 11-20 µm, fusiform, apex narrowed, base truncate, straight or slightly curved, 7-12 septate, slightly constricted at septa, hyaline, smooth, no appendages, cells of irregular size, full with small guttules.

Ecology:

Studied specimens: VR187, VR654 (culture dried)

Remarks
No *Stagonospora* sp. with such large conidia was found in literature.
This species is close to *Stagonospora anglica* Cunnell but our collections have larger conidiomata and larger, more septated conidia. Perhaps also close to *Stagonospora macrosperma* Sacc. & Roum. in Sacc. Michelia II: 629 (1882) sub *Hendersonia* (Collection Libert). In leaf of grass. Conidia 85-95 x 12-14 µm, 6-8 septate, not constricted, with guttules, hyaline. Herbarium material should be traced and compared.
Species cultured (now dried herbarium collection, VR654). All cultures from single conidium isolation resulted in the formation of a *Phoma*-like anamorph. No teleomorph, nor the *Stagonospora*-state was observed. A short description of the *Phoma* (microconidial state) of this *Stagonospora* is given. Conidiomata 300-650 µm diam., large and variable in shape; ostiole large

up to 500 μm long, hyaline and more a textura globulosa; peridium textura angularis, black, relatively large cells up to 12 μm diam. Conidiogene cells 6-8 μm diam., hyaline, poly-enteroblastic, formed from the inner cells of the peridium. Conidia 7-10 x 3-4 μm, hyaline, ellipsoid, fusiform, with several small guttules near the ends, smooth, aseptate, no appendages.

***Stagonospora* sp. III**

Conidiomata ± 300 μm diam., immersed, black, aggregated, with dark-brown mycelia, aggregated; ostiole, central; peridium textura angularis, thick-walled cells, with many hyphae at the exterior, cells 8-10 μm diam. Conidiophores absent. Condiogene cells 7-10 x 5-5.5 μm, ampulliform to slightly lageniform, holoblastic, annelledic. Conidia 29-34 x 6-6.5 μm, fusiform, with one side tapered more, hyaline, 3 septate, not constricted, multiguttulate, without appendages but apparently an entire, thin slime coat.

Studied specimens: VR784 (GVR 3239)

Remarks
This species resembles *Neottiosporina australiensis* but not a single conidia had an appendage. Appendages in *Neottiosporina* are persisting and should be visible (see above). It also resembles *Stagonospora vitensis* (Sutton, 1980) however this species has more cylindrical conidia. *S. caricinella* has smaller spores which are fusiform but generally with 2 septa (Sutton, 1980)..

Stagonospora vexata Sacc., Michelia, 2: 112, 1882. Syll. Fung. III: 455 (1884).
Sensu Diedicke (1915)

The species is erroneously indicated as a synonym of *Massarina arundinacea* in the Index of Fungi; the type description is of this *Stagonospora* is quite different from *M. arundinacea*, however in Saccordo (1884) we can read that on the type material *S. vexata* is **associated** with *M. arundinacea.*

Conidiomata up to 500 μm long, 200-300 μm wide and high, flattened, lenticular on stems more spherical on leaf and leaf sheaths, greyish-brown, immersed, often in linear rows, solitary; ostiole central, becoming slightly papillate, with periphyses, conidia often as cirri on ostiole; when cutting the conidiomatum open the conidia appear as a pink mass when fresh. Peridium textura angularis-prismatica, unclear out interwoven hyphae, rather thin-walled (variable). Conidiophores absent or sometimes 1-2 basal cells, hyaline. Conidiogene cells 6-20 long and 5-6 μm wide, ampulliform to cylindrical, holoblastic, hyaline, percurrent proliferation seldom, 1-2 annellations. Macroconidia 34-70 x 4-5.5 μm, cylindrical, fusiform, base slightly truncate, apex attenuated, 5-10 septate (mostly 7), slightly constricted, mostly slightly curved, smooth, hyaline, no appendages, with many guttules each cell (often 2 larger and the remaining small).

Ecology: on standing dead stems at middle height in the canopy.

Studied specimens: VR249, VR366, GVR1022, 20/09/2000, stem, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43, (no exsiccatum), drawing; GVR3165, 30/09/2002, stem, ibid. (no exsiccatum), pictures

Remarks
This species agrees well with the species concept that Diedicke (1915) gives for *Stagonospora vexata* Sacc and also his figures agree well with ours. Saccardo (1884) characterized the conidia as 60-70 x 7 µm, 10-12 septa. Diedicke (1915) considered that the observations of Saccardo might be done on further developed conidia. The latter author observed conidia 65-67 x 5-7 µm, 5-7 septa. Both authors give wider measurements for the conidia than seen by us. However we believe that our collections agree with *Stagonospora vexata* Sacc., which is a common, variable species on reed and the only species in literature that fits pretty well our collections.

Hyphomycetes

Alternaria alternata (Fr.) Keissl., Beih. Bot. Centralbl. 29: 434 (1912).
= *Alternaria tenuis* Nees

Colonies not very dense, black-greyish, individual conidiophores visible under stereomicroscope. Conidiophores 25-60 x 3-3.5 µm, straight or flexuous, sometimes with angular appearance, brown, septated with dark scars (1 to 5), slightly lifted. Conidia 25-54 x 10-16 µm, clavate, muriform, with up to 7 transverse septa, apical dark scar, end cell up to 10 x 3-4 µm, often shorter, lighter coloured, initially smooth, later verruculose, in short chains.

Ecology: during summer and autumn on leaves (blades and sheaths), often isolated in culture but less found on plant parts after direct screening.

Studied specimens: VR376

Arthrinium phaeospermum (Corda) M.B. Ellis, Mycol. Pap. 103: 8-10 (1965).

Colonies variable in size and structure, with more or less basally developed, hyaline stroma, linear on stems splitting the epidermis open, on leafs and leaf sheaths more compact and pulvinate, with black, shinning mass of conidia. Conidiophores with basal conidiophore mother cell on mycelium; mother cell 6-10 x 4-5 µm, ampulliform- lageniform, with periclinal thickening, light brown; Conidiophores up to 30 x 1-1.5 µm, simple, hyaline, septated, smooth.

Conidia 8-11 µm diam., in face view, subglobose, smooth, light brown; side view lenticular, with a hyaline band.
Culture remained sterile (dried, VR571)

Ecology: most often on stems, less on leaves. Stems are often more than one year in a standing dead position before this taxon appears.

Studied specimens: VR563, VR571 (dried culture)

Remarks
A. phaeospermum differs from *Arthrinium* state of *Apiospora montagnei*, another species encounter on *P. australis* by larger conidia.

Arthrinium* state of *Apiospora montagnei Sacc., Nuovo G. Bot. Ital. 7: 306 (1875).
See Hudson (1963) for a description and the discussion on the ana-teleomorph connection.
(no figure)

Has the aspect of *Arthrinium phaeospermum* but smaller conidia. Conidia 4.5-6.5 µm in face view, subglobose, slightly ellipsoid, light brown, smooth; side view lenticular, with lighter zone.

Ecology: less frequently found than *A. phaeospermum*, on stems and on inflorescence.

Studied specimens: VR9

***Arthrobotrys* sp.**

Conidiophores 400-500 x 4-5 µm, solitary, erect, septated, hyaline, with hyaline heads, not branching, enlarged apex denticulate. Conidia 28-35 x 14-15 µm, clavate, 1-septate, submedian, weakly constricted, hyaline.

Ecology: in litter layer, not often found after direct screening of material from the field.

Studied specimens: GVR323, Van Ryckgem (1999) n° 127, 20/02/1999, on leaf sheath, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43, (no exsiccatum), drawing

Bactrodesmium atrum M.B. Ellis, Mycol. Pap. 72: 9 (1959)

Conidiophores up to 60 µm long, 6-7 µm wide, brown, septated, unbranched, monoblastic, solitary or in small groups forming scattered sporodochia seen under stereomicroscope as

black shinning dots. Conidia 35-50 x 17-26 µm, clavate, broadly rounded at apex, tapered to the base, terminal, solitary, mostly 3-septated, not or slightly constricted, totally black.

Ecology: often on stems in litter layer or on basal parts of standing stems in the water or very humid conditions.

Studied specimens: VR13

Remarks
Bactrodesmium is a genus well presented as submerged-aquatic hyphomycetes (Goh & Hyde, 1996) mostly found on wood. I found it not to be uncommon on reed stems.
Several hyphomycetes look similar to this species and could be confused. *Phragmocephala glanduliformis* (Höhn.) S. Hughes has smaller conidia with proliferating conidiogenesis and is not known to form small sporodochia (Ellis, 1971). *Trichocladium achrasporum* (Meyers & Moore) Dixon forms also comparable, although smaller, conidia. Those are formed on the hyaline mycelium without conidiophores (Shearer & Crane, 1971).

Cerebella andropogonis Ces., *in* Rabenhorst, Bot. Ztg, 9: 669 (1851).

Sporodochia up to 0,5 cm diam., brain-like folded, dark-brown to black, sometimes gregarious, dry surface. Conidiophores 15-30 x 3-4 µm, short, brown, septated. Conidia 20-24 x 18-21 µm, globose to subglobose, brown, consisting out 5-8 cells, verrucose, short stipe, stipe-cell lighter colored.

Ecology: not common in the inflorescence of reed growing on sclerotia of *Claviceps purpurea*

Studied specimens: VR362

Remarks
This species is always associated with the sclerotia of *Claviceps*-spp. *C. andropogonis* lives saprotrophical on the honey-dew substance produced by the *Spacelia* stadia (Langdon, 1941). For a more detailed discussion on this species see Pellicier (2000). This species could be confused with an *Epicoccum* sp.

Chrysosporium **sp.**
(no figure)

Conidiophores weakly developed, much like vegetative hyphae, hyaline, 3 µm wide, with few branches. Conidiogene cells cylindrical. Conidia produced in short chains, small (no measurements), aseptate, lemon shaped, with basal scar, verrucose, hyaline, in mass light yellow.

Studied specimens: GVR33, on leaf, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43, (no exsiccatum)

***Cladosporium* sp(p).**
(no figures)

Stromata, small, mainly located in the stomatal cavity of leaves (sheaths and blades), conidiophores in small groups exposed through the stomatal opening. Conidiophores 100-130 x 4-4.5 μm, dark brown, not branched, septated every 10-12 μm, not nodulose. Conidiogene cells integrated on conidiophore with 2-3 enteroblastic scars. Conidia 5-15 x 3-5 μm, very variable in shape and size, hyaline, aseptate or 1 septum, verrucose in most cases, secondary budding of conidia from mother conidia seen.

Ecology: on leaves (blades and sheaths) in the canopy during growing season or soon after senescence.

Studied specimens: VR567

Remarks
All collections were lumped because determination of cladosporia's is very difficult (impossible?) by means of light microscopy. At least 4 different *Cladosporium* species occur together on *P. australis*, not all of them morphologically distinct (Wirsel et al., 2002).

Cochliobolus cf. australiensis (Tsuda & Ueyama) Alcorn, Mycotaxon 16: 373 (1983).

Colonies like brown mats on stem, mycelium brown mostly in substrate. Conidiophores 120-170 x 5.5-6 μm, brown, septated, unbranched, polytetric, with conidia halfway down, with prominent scars. Conidia 24-27 x 7-10 μm, cylindrical- fusiform, 3-septate, septa relatively thick-walled (pseudosepta?), slightly echinulate, hilum inconspicuous slightly darker.

Studied specimens: VR378

Remarks
Conidia are slightly echinulate and the septa are not seen as typical pseudosepta. The rest of the description fits well for *C. australiensis*. In Ellis (1971: 413) this species is described under the synonym *Drechslera australiensis*.

***Corynespora* sp.**

Conidiophores 30-42 x 3-4 μm, dark brown, not branched, 2-4 septate, thick-walled, smooth, monotetric, with terminal conidiogene cell, basally somewhat enlarged, solitary. Conidia 45-60 x 7-9,5 μm, 6-10 pseudoseptate (?), obclavate, brown, slightly lighter at apex, some with slime appendage at apex, hilum dark coloured, smooth, with small guttules in cells.

Ecology: on stems and leaf sheaths submerged in fresh till brackish water.

Studied specimens: Van Ryckegem, (1999) sp. n° 130: 15/11/1999, on stem, Belgium, Scheldt estuary, tidal marsh Burcht, 28/11/2002, IFBL C4.26.31 and 17/02/1999, on stem, Belgium, Scheldt estuary, tidal marsh Burcht, 28/11/2002, IFBL C4.26.31, drawing; GVR1517, 27/04/2001, Luxembourg, Boufferdange Muer (Fining) (Carte géographique n° 20)

***Cylindrocarpon* sp.**

Mycelium white, superficial, with conidiophores in rows on leaf sheath ribs, rather compact, fluffy. Conidiophores, short, 1-2 celled, erect, hyaline, terminating in slender phialides. Condiogene cells 27-30 x 2-3 μm, long cylindrical, tapering to apex, percurrent proliferation enteroblastic, with collaretta, hyaline. Conidia 29- 40 x 3.5-4 μm, cylindrical, slightly tapered to ends, 3-septated, not constricted, smooth, aguttulate.

Studied specimens: VR593

***Dactylaria* sp.** – isolate from green leaf sheaths in CMA

Mycelium hyaline, with spherical cell, some appear to be terminal and other integrated, given some parts of the mycelium a nodulose aspect. Conidiophores absent or short consisting out of 1-2 cells. Condiogene cells separate on mycelium, sympodial, with short denticles, polyblastic. Conidia 9-13 x 2.5-3 μm, hyaline, thin-walled, one septum, with apiculus, with guttules, slightly constricted.

Studied specimens: GVR3440, on leaf blade, in culture, 27/05/2003, The Netherlands, Scheldt estuary, brackish tidal marsh, near the 'Verdronken land van Saeftinghe', IFBL B4.33.43, (no exsiccatum)

Remarks
This isolate from leaf sheaths could not be named to species level, although it shows clear affinities to *Dactylaria dimorphospora*. However, the latter species is characterized by two types of conidia and we only observed one type (see De Hoog, 1985). Our collection showed less

variance in conidial shape and size. Looks superficially similar to *Neoramularia phragmitis* (see Braun, 1993) but conidiogenesis is different.

Deightoniella roumeguerei (Cavara) Constant., Proc. Kon. Ned. Akad. Wetensch. C 86(2): 137 (1983).

Colonies 4-6 x 1-2 mm, elliptical, brown, near the main axis leaf, between the nerves, not compact, under binocular stereomicroscope individual conidiophores visible. Conidiophores 15-17 x 15 µm, only basal cell, subglobose, thick-walled (1-3 µm), smooth, brown. Conidiogene cell 20-30 x 9-11 µm, light brown, thick-walled, lighter apex, pore apical. Conidia 25-30 x 17-20 µm, ovoid, ellipsoid, rounded, hyaline- light yellow, no septa or when mature one septum, supramedian, slightly constricted, with basal hilum darker.

Ecology: parasite on leaf blades

Studied specimens: VR132, VR537

Remarks
Deightoniella arundinacea (Corda) S. Hughes also occurs on *P. australis*. This species is distinct from *D. roumeguerei* by effuse less defined disease symptoms more with a greyish appearance, longer conidiophores and obclavate conidia which are at first submedian (Constantinescu, 1983; Bán et al., 1996). Although *D. arundinacea* would be common on *P. australis* it appears to be rare in Belgium as all my collections fit well for *D. roumeguerei*.
D. roumeguerei was nearly exclusively found on leaf blades.

Dictyochaeta simplex (S. Hughes & W.B. Kendr.) Hol.- Jech., Folia Geobot. Phytotax. 19: 434 (1984).

Colonies forming greyish patches on stems, not compact, no setae. Conidiophores 80-90 x 4-4,5 (wide at base) µm, brown, subhyaline apically, grouped together with 4-5 conidiophores on basal, small stroma, multiseptate, on top with hyaline, slimy heads of conidia. Conidiogene cells first terminal but later appearing sympodial as new growing points formed just below apex, polyphialidic, with clear colarette; colarettes funnel-shaped, 2µm high, 4 µm wide. Conidia 11-13 x 3-4 µm, slightly curved, rounded ends, aseptate, hyaline, with several guttules; setulae 4-9 µm long, terminal, straight or slightly curved, implanted at concave side.

In culture: Colonies hyaline, smooth, shinning not a lot of air mycelium is formed. Conidiophores much longer up to 250 µm x 4,5 µm, sometimes percurrently forming new hyphae or conidiophore out of conidiogene cell, sometimes geniculate appearance. Conidia 14-23 x 3-4 µm

Ecology: on reed parts submerged in freshwater

Studied specimens: VR636

Remarks
Originally determined as *Codinaea simplex* S. Hughes & W.B. Kendr. but recombined by Holubová-Jechová (1984).

Dictyosporium oblongum (Fuckel) S. Hughes, Canad. J. Bot. 36: 762 (1958).

Colonies effuse, sparse mycelium on substrate, black-brown, mycelium hyaline to light brown. Conidiophores/conidiogene cell 'absent', conidia formed on mycelium. Conidia 30-42 x 15-35, several rows (3-8) of cells of same or different length, oblong to irregular shape, not flattened in one plane, thick-walled, constricted at septa, cells about 6 µm wide, brown.

Ecology: *D. oblongum* shows a clear preference for submerged or very moist conditions.

Studied specimens: VR52

Remarks
This species was also found on *P. australis* by Taligoola (1969).

Drechslera biseptata (Sacc. & Roum.) M.J. Richardson & E.M. Fraser, Trans. Brit. Mycol. Soc. 51: 148 (1968).

Colonies effuse, scattered. Conidiophores 140-160 x 6-8 µm, brown, paler to the apex, solitary, thick-walled, tapered, straight, unbranched, round scars apically. Conidia 19-30 x 10-14 µm, ovoid to clavate, light brown to brown, 2-3 (mostly 3) pseudosepate, smooth.

Ecology: common in non-tidal reed wetlands in the litter layer on stems and leaf sheaths.

Studied specimens: VR748, Van Ryckegem (1999) n° 134, 14/10/1999, stem, Belgium, Boerekreek (Sint-Laureins, Sint-Jan-in-Eremo), IFBL C2.18.11, drawing.

Epicoccum purpurascens Ehrenb. & Schltdl. Synop. Pl. Crypt.: 136 (1824).

Sporodochia up to 1 mm, rust-brown, ellipsoid to round. Conidiophores 10-20 x 4-6 µm, hyaline to light yellow, on small stroma. Conidia 12-23 µm diam., brown, terminal, solitary on conidiophore, first aseptate but later muriform, verrucose, basal cell lighter and not verrucose.

Ecology: in the canopy, on leaves.

Studied specimens: VR48

***Fusarium* sp. I**

Sporodochia hypersaprotrophical (or parasitic) as white flags on old perithecia of *Massarina arundinacea* or *Lophiostoma* spp. without clearly developed stroma on or in perithecia. Conidiophores short 15-40 μm long, hyaline, septated, branched. Conidiogene cells difficult to observe, small, phialidic, cylindrical. Conidia 90-200 x 5-7 μm, fusiform, curved, 8-11 septate, not constricted, aguttulate.

Studied specimens: VR148

***Fusarium* sp II**

Sporodochia well developed, hypersaprotrophical (or parasitic) on perithecia of *Massarina arundinacea*, yellowish. Conidiophores hyaline, septated rather well developed, up to 90 μm x 3 μm, branched. Conidiogene cells +/- 15 x 4 μm, lageniform, phialidic, hyaline, lateral and terminal. Conidia 36-60 x 2.5-3.5 μm, curved or straight with only the ends curved, 3-5 septated, no guttules.

Studied specimens: VR500

***Fusarium* sp. III**

Spordochia effuse, with macroconidia formed scattered on mycelium, white fluffy. Conidiophores erect, branched, hyaline, up to 250 μm long. Conidiogene cells variable (several types?) 10-25 x 3 μm, lateral, terminal, phialidic, cylindrical to lageniform, percurrent, annellidic, some appear polyblastic. Conidia very variable probably micro-and macroconidia or they could represent a continuum of small to large conidia; macroconidia 22-40 x 4-5 μm, curved, fusiform, typically becoming 3-septate (but occasionally 4-5 septa), hyaline, aguttulate; microconidia 7-16 x 3.5-4.5 μm, fusiform, not curved, 0-1 septum, hyaline, aguttulate.

Ecology: in the litter layer of non-tidal reed wetlands on leaf sheaths and stems.

Studied specimens: VR576, VR810, VR824

Remarks

Determination of *Fusarium* from the field without culturing is impossible. Even after we cultured the species we could not get a proper name. In culture we did not observe clamydospores, agar was pinkish red coloured in the first culture but pigmentation was not observed in further transfers; fast growing and no *Gibberella* state was found. It could perhaps fit for *Fusarium graminearum* (Booth 1971: 179) the anamorph of a *Gibberella zeae* which was found several times on *P. australis* in the field. However this species would have no microconidia (Booth, 1971). Our collections could also agree with *Fusarium phragmitis* Matsush. [invalid name: Art. 37.1], but conidia of this taxon seem generally larger. We should re-examine our material.
This *Fusarium* sp. is relatively frequently encountered in the field.

Helicomyces tenuis Speg. , Anales Mus. Nac. Hist. Nat. Buenos Aires 20: 423 (1910).
Emended, R.D. Goos. See Mycologia 77: 614-615 (1985).

Conidiophores not observed. Conidia 18-22 μm diam., 1-1,5 μm breed, geseptated, hyaline.

Ecology: on submerged reed.

Studied specimens: GVR1518 (slide, no exsiccatum), 27/04/2001, Luxembourg, Boufferdange Muer (Fining) (Carte géographique n° 20)

Helicosporium phragmitis Höhn., Ann. Mycol. 3: 338 (1905).
= *Helicosporium* state of *Tubeufia helicomyces* Höhn. Sber, Akad. Wiss. Wien 118:1477 (1909).
The connection between anamorph and teleomorph was demonstrated by Webster (1951).

Colonies appear like sporodochial aggregates up to 5 mm diam., pinkish coloured. Conidiophores long up to 350 μm, erect, branched or not, upper part sterile (visible under dissecting microscope), brown, upper part lighter coloured. Conidiogene cells integrated, poly- or monoblastic; denticles cylindrical often narrowed. Conidia 20-25 μm diam., cells 2-3 μm wide, hyaline, helicoids, 3 dimensional, guttulated, multiseptated (when alive septation can not be observed), when guttulation disturbed, 6-10 septa were seen, coiled 2-4 times.

Ecology: on submerged reed

Studied specimens: GVR1860, 2/7/2003, Switzerland, Southeastern shore of Lake Neuchâtel, oligotrophic hardwater lake (46°54'N, 6°54'E).

Remarks
This collection fits well with the description provided by Ellis (1971), with the exception of the 3 dimensional conidia and not in one plane as figure by Ellis.

Hymenella arundinis Fr. Syst.Myc. II: 234 (1822).

Proposed synonyms:
= *Hymenula arundinis* Fr. Elenchus Fungorum II: 37 (1828)
= *Hymenella arundinis* (Fr.) Vestergr. (1899) (in Saccardo, 1902 (part XVI): 1105); Superfluous recombination
= *Dacrymyces phragmitis* Westend. (Kickx 1867, Fl. Flandres, II: 115; Westendorp Not. VII: 13 date?).
= *Dendrodochium microsorum* var. *phragmitis* Fautrey, Rev. Myc. Oct. 1891.
in Saccardo's Syll. Fung. X: 708 (1892).

Sporodochia up to 500 μm diam., irregularly shaped from discoid, cylindrical to pyramidal, often with basal receptaculum, ochre-rust brown, textura prismatica, sessile or short stipe, on receptaculum conidiophores develop with abundant conidia on top of sporodochia, dark yellow when dry, cream-coloured when moist. Conidiophores 40-80 μm x 1μm, branched, septated, hyaline, branches variable in length, tight together. Conidiogene cells sometimes in ventricles (*Verticillium*-like), phialidic, with small periclinal thickening at the tip, long cylindrical only slightly tapering to the apex, terminal or lateral, conidia mostly attached with some angle to the conidiogene cell. Conidia 3-4.5 x 0.8-1.2 μm, cylindrical, straight or slightly asymmetric, smooth, with terminal guttules (1 or more).

Ecology: on stems and leaf sheaths in the litter layer, common in non-tidal reed wetlands, not submerged.

Studied specimens: VR218, VR815

Remarks
Our collections seem to be conspecific with *Dendrodochium microsorum* var. *phragmitis* Fautrey described in Saccardo (1892): "Sporodochiis flavescentibus, superficialibus, discoideis, sparsis vel confluentibus; sporophoris furcatis, longissimis, 80-90 μ. long.; conidiis acro-pleurogenis, cylindraceis, rectis vel subcurvis, 4,5 ~1".
Why this taxon received the rank of variety is unclear to me, perhaps it should be synonymized with *D. microsorum* Sacc. In any case, *Dendrodochium* is placed in synonymy with *Clonostachys* Corda (Schroers et al., 1999). Our taxon differs from the genus concept described by Schroers et al. (1999) for *Clonostachys* by the peculiar receptaculum, which is generally well developed.
Our taxon is congeneric with the type species of *Hymenella* Fr. Syst. mycol II: 233 (1822) (to check!), described by Tulloch (1972). Fries published the genus later incorrectly as *Hymenula* (Fries, 1828 Elenchus Fungorum II: 37). Several names with descriptions all very similar to ours are available to name our taxon. All these taxa are probably the same species (see synonyms). Another name to check is

Hymenella rubella [Fr.?] unpublished name by Fries? Published as *Hymenula rubella* Fr. in Elenchus Fungorum II (1828) (see also Saccardo, 1886 (Syll. Fung. part IV): 670). This species was also recorded from *Phragmites* and could also be a synonym but conidia are mentioned to be slightly larger than our observations.

Hyphomycete sp. I

Mycelium 3 µm wide, hyaline, with clamp connections (? perhaps other species interwoven). Conidiophore up to 200 x 4 µm, brown, apically paler, foot-cell enlarged, unbranched, nodulose. Conidiogenesis enteroblastic, tetric with scars on nodules, sympodial. Conidia 8-12 x 3-4 µm, hyaline, 1-septate, aguttulate, apiculate.

Studied specimens: VR71

Hyphomycete sp. II

Colonies up to 500 µm, spherical, shinning black, superficial mycelium, the appearance of a smut fungus. Conidiophores brown, branched, thallo-conidia. Conidia 5-8 x 3-5 µm, in chains, thick-walled, smooth, thallic.

Studied specimens: VR91, VR347

Hyphomycete sp. III

Colonies up to 1 mm long between the veins of the leaf sheaths, pink, immersed under epidermis, sometimes epidermis seems to dissolve showing the colonies naked becoming even slightly superficial, sometimes forming closed conidiomata within the hyphal mass. Mycelium hyaline, flexible, rather thick-walled, forming sympodial conidiogene cells. Conidiogene cells phialidic, lageniform, 5-7 x 2-3 µm, with long bottle-neck, swollen base, formed on hyphae at regular intervals. Conidia 5-8 x 1.5-2.5 µm, cylindrical, slightly tapered to the ends, hyaline, biguttulate or several small guttules.
Within the pink mycelium, initially tight clusters of hyphal mass forms *Phoma*-like conidiomata, about 100 µm diam., sometimes present. Swollen phialidic conidiogene cells form the inner layer of the conidioom, producing the same conidia. This fungus seems to produce a Coelomycetous and a Hyphomycetous stage that form identical conidia. (see also *Pseudoseptoria donacis*)

Ecology: immersed patches in leaf sheaths in the canopy

Studied specimens: VR370, GVR2526, leaf sheath, 31/01/2002, The Netherlands, Het verdronken land van Saeftinghe, near Belgium border, IFBL, B4.33.43, drawing, no exsiccatum

Remarks
The hyphomycete-stadium shows some resemblance to *Tolypocladium* with its swollen phialids (Gams, 1971) but differs in many ways to any of the species belonging to this genus.

Hyphomycete sp. IV

Sporodochia ± 300 µm, hyaline-pinkish, slimy, parasitic or hypersaprotrophical on *Arthrinium phaeospermum*. Conidiophores up to 250 µm long, branched, branches just below septa. Conidiogene cells integrated, same appearance as conidiophore, long cylindrical, holoblastic (?), terminal or lateral. Conidia 42-55 x 4.5-6 µm, cylindrical, with truncate base, rounded apically, 3-septate, slightly or more clearly constricted at septa, smooth, no appendages, filled with very small guttules.

Ecology: in a standing position or in the litter layer parasitic on *Arthrinium phaeospermum.*

Studied specimens: VR599, VR769

Remarks
Perhaps a *Cylindrocarpon* sp?

Hyphomycete sp. V

Colonies forming small, whitish to pinkish-orange spots beneath the epidermis out of stroma-like basal part, consisting out tightly interwoven hyphae. Conidiophores not observed ?absent. Conidia 17-30 x 11-13 µm, broadly cylindrical, ovoid, irregular, smooth or slightly echinulate, aseptate, catenulate, compact, formed thallic?, (sub)hyaline.

Studied specimens: GVR3104 (slide), The Netherlands, Het verdronken land van Saeftinghe, near Belgium border, IFBL, B4.33.43 (no exsiccatum) (pictures)

Remarks
The interpretation of this fungus is difficult. Perhaps this is an immature stage, or a stroma-like structure. No appropriate genus is available for this collection.

Hyphomycete sp. VI

This taxon was not properly studied so far. Diagnostic description: tremella-like hyphomycete, perhaps a conidial form of it, found in the upper canopy growing hypersaprotrophical or parasitic mainly on *Stagonospora vexata* (see above) and once it was associated with *Hendersonia*

culmiseda (see above). No drawing, no pictures, difficult to make a good slide. Herbarium collections available.

***Monacrosporium* sp.**

Conidiophores 320-400 x 3-4 μm, hyaline, unbranched, septated, with one apical conidium. Conidia 62-70 x 21-22 μm, fusiform, 3-septate, with central cell much larger than other cells, 2 septa under the middle, 1 septa above the middle, hyaline, smooth.

Studied specimens: VR235

***Myrothecium cinctum* (Corda) Sacc.** Syll. Fung. (Abellini) 4: 751 (1886).

Sporodochia up to 1 mm diam., black, shinning when moisted, sessile or short, white stalk, margin with white hyphae, when senescent only black conidial mass is left on substrate. Conidiophores hyaline, branching, septated. Conidiogene cells 10-18 x 1.5-2 μm, phialidic, cylindrical, lageniform, hyaline. Conidia 10-12 x 3-3.5 μm, cylindrical-fusiform, aseptate, olive-green, striate (or not?), often with four guttules.

Ecology: in non-tidal wetlands, in litter layer on leaf sheaths and stems, not submerged

Studied specimens: VR183

Remarks
Compare this taxon with *Myrothecium atroviride* (Tulloch, 1972; Ellis, 1976), which has also marginal hyphae but conidia without striae and also sessile sporodochia.
If striation of conidia is a good characteristic to distinguish between those two species we possibly lumped *M. atroviride* within our collections of *M. cinctum*. Otherwise those two taxa could represent one and the same species.

Myrothecium masonii M.C.Tulloch, Mycol. Pap. 130: 21-23 (1972).

Synnemata up to 1,5 mm x 50-25 μm, brownish-grey, formed out of helicoidally winded hyphae, septated, without clamp connections; marginal hyphae with capitate endcells, 4 μm wide, verrucose, giving a powdery aspect at the upper part of the synnematum; head black, shinning, with sometimes a new secondary synnematum formed. Conidiogene cells 7-18 μm long, phialidic, enteroblastic and penicillium-like branched, hyaline. Conidia 6-7 x 2-3 μm, light olive-green (dark in mass), aseptate, ellipsoid, subtle striate, aguttulate or two small guttules.

Ecology: in the litter layer on leaves (blades and sheaths)

Studied specimens: VR169

Remarks
This species is shows similarity to *Stilbella clavulata* (see Seifert, 1985) with capitate marginal cells, synnemata and phialides within the size range. *M. masonii* seems to differ in conidial shape and colour. The feature that the conidia are also slightly striated was not observed by Ellis (1976) & Tulloch (1972).
M. masonii is really an outsider in the genus, showing large synnemata and capitated marginal cells.

***Penicillium* sp.**

Conidiophores up to 1 mm long, subhyaline, penicillate, septated, thin-walled, apically branched 3-dim., phialidic conidiogene cells. Conidia 3-4 x 2-3 µm, subglobose, hyaline, with 2 small guttules, mass forming on top of conidiophore, no slime droplet was observed.

Studied specimens: VR69

Periconia cookei E.W. Mason & M.B. Ellis, Mycol. Pap. 56: 72 (1953).

Conidiophores 300-340 high x 21-25 (at basis) µm, causing on substrate pale discoloration, unbranched, 3-septated; apex enlarged, columella-like, (27-30 µm wide), no apical cell, with scars, polyblastic. Conidia 14-20 µm, globose, verrucose, dark brown, at initial stage catenate, aguttulate, 1-2 germ tubes sometimes present.

Studied specimens: VR590

Remarks
This collection is somewhat atypical in the substrate discoloration, which is normally black for *Periconia* species. Also the conidia are rather large for *P. cookei* and fit more for *Periconia byssoides* Pers. ex Mérat. However, *P. byssoides* is characterized by a subhyaline apical cell, which is absent in our collections.

Periconia digitata (Cooke) Sacc., Syll. Fung. 4: 274 (1886).

Conidiophores up to 500 µm long and 18 µm at base, just below conidial head 12-14 µm, brown, with multiple branches in loosely compacted heads. Conidia 7- 8.5 µm diam., globose, catenulate, verrucose.

Studied specimens: VR693

Periconia glyceriicola E.W. Mason & M.B. Ellis, Mycol. Pap. 56: 109 (1953).

Conidiophores ± 300 x 7 μm, brown, smooth, septated, without compact heads, with 3-4 whorls of branches on several heights under the apex. Conidia 6.5-9 μm diam., light brown, finely verrucose.

Studied specimens: VR8

Periconia hispidula (Pers.) E.W. Mason & M.B. Ellis, Mycol. Pap. 56: 112 (1953).

Colonies aggregated in ellipsoid, black patches. Conidiophores ± 700 x 10 μm, with conidiogenesis on the lower part, upper part sterile, tapering to 2-3 μm. Conidia 11-14 μm diam., globose, thick-walled, finely echinulate.

Studied specimens: VR7

Periconia igniaria E.W. Mason & M.B. Ellis, Mycol. Pap. 56: 104 (1953).
(no figure)

This species differs from *Periconia minutissima* by the larger conidia (7-10 μm) with prominent spines and the rough walled branches in the conidial head.

Studied specimens: VR345

Periconia minutissima Corda, Icones Fungorum, 1: 19 (1837).

Conidiophores 300-400 x 5-7 μm, brown, branched apically, septated, with spherical, black conidial head. Conidia 4-6.5 diam. spherical, catenulate, light brown, echinulate.

Ecology: The most common species of the genus *Periconia* recorded on dead reed parts in the canopy and in the litter layer.

Studied specimens: VR7

Rhinocladiella **sp.**

Mycelium brown, forming a brown mat on substrate. Conidiophores 110-180 x 2-3 μm, brown, aggregated, unbranched, septated, conidiogenesis from halfway up the conidiophore

with scars, sympodial, enteroblastic conidiogenesis. Conidia 6-7 x 3 μm, oblong to ellipsoid, asepate, hyaline.

Studied specimens: VR191

Stachybotrys atra Corda, Icon. fung. (Prague) 1: 21 (1837).

Colonies effuse, black. Conidiophores up to 150 μm long, dark brown, covered with granules towards the apex, branched, ending in heads with whorls of phialids. Conidiogene cells, 10-12 x 5-6 μm, lighter coloured than conidiophores, enteroblastic. Conidia 9-12 x 5-6 μm, ellipsoid, aseptate, verrucose, dark brown, two large guttules.

Ecology: Never collected from the field, developed on a leaf blade a moisted chambre. Common indoor mold.

Studied specimens: VR411

Stachybotrys bisbyi **(Sriniv.) G.L. Barron**, Mycologia 56: 315 (1964).

Conidiophores 100-200 μm high 3-5 μm wide, hyaline, simple or branched near the base, 3-4 septated, verrucose in the upper parts, top cell slightly swollen with up to 8 phialids in a whorl, conidia in a slimy, pinkish mass on the top. Conidiogene cells 12-15 μm long, phialidic, slightly clavate, with inconspicuous collarettes. Conidia 12.5-14 x 4.5-5.5 μm, hyaline, aseptate, two large guttules and several small, straight or slightly curved, slightly fusiform, basal part more attenuated with inconspicuous apiculus.

Studied specimens: VR816

Remarks
This species is unusual in the genus *Stachybotrys* by its hyaline appearance; it even served as a type species for a new genus *Hyalostachybotrys* Srinivasan (1958) but was synonymized by Barron (1964) in *Stachybotrys* considering pigmentation as an invalid character to create a new genus. The species concept of this species followed by Jong & Davis, (1976) is rather wide with an unusual high variability in the conidial morphology and placing several species in synonym. Our field collection showed a small range in conidial size. *Stachybotrys bisbyi* seems to be closely associated with grasses as most of the collections are all isolates from above ground plant parts of grasses or from the rhizosphere of grasses.

Torula herbarum (Pers.) Link ex S.F. Gray, Nat. Arr. Br. Pl., 1: 557 (1821).

Colonies 1-3 mm, brown to black, irregularly shaped, powdery; mycelium hyaline, septated, no clamp connections, partly superficial. Conidiophores short, 3-5 µm diam. 1-celled, hyaline to light yellow. Conidiogene cell somewhat larger than conidiophore. Conidia 25-27 x 5-6 µm, broad cylindrical, rounded ends, 3-5 septated, constricted, brown, verrucose.

Ecology: on all reed parts in the canopy, not abundant on reeds.

Studied specimens: VR198

Trichoderma koningii Oudem., Arch. Néerlandaises des Sc. exacts et nat.: 291 (1902).

Sporodochia superficial compact, brightly green on substrate common on dead stems laying in the litter layer in the field. Conidiophores hyaline, about 3 µm wide. Conidiogene cells 6-15 x 3-3.5 µm, phialids, in divergent whorls, slightly swollen and lageniform, straight. Conidia 3.5-4.5 x 2-3 µm, green, oblong to narrowly ellipsoid, smooth.

Ecology: develops most frequently on stems in the litter layer in moisted conditions but in the field it is found in reed stands without water surface above the sediment.

Studied specimens: GVR1862, 2/07/2002, Switzerland, Southeastern shore of Lake Neuchâtel, oligotrophic hardwater lake (46°54'N, 6°54'E), this collection developed in moisted chamber (pictures) (only slide)

Verticillium **sp.**

Conidiophores 300-350 x 3 µm, hyaline, septated, branched with terminal phialides. Conidiogene cells 33-40 x 3 µm, phialidic, with colarettes, 2-4 grouped, tapered to apex, narrow lageniform, hyaline. Conidia 10-16 x 3 µm, 0-1 septate, cylindrical, with rounded ends, straigth

Studied specimens: VR230, developed in moist chamber

References

ANASTASIOU, C.J. (1967). Two species of *Coprinus* from alkali lakes. Canad. J. Bot. 45: 2213-2222.

ANTONIN, V. & NOORDELOOS, M.E. (1993). A monograph of *Marasmius*, *Collybia* and related genera in Europe. Part 1: *Marasmius, Setulipes*, and *Marasmiellus*. Libri Botanici 8: 1-229.

ANTONIN, V. & NOORDELOOS, M.E. (2004). A monograph of the genera *Hemimycena*, *Delicatula*, *Fayodia*, *Gamundia*, *Myxomphalia*, *Resinomycena*, *Rickenella* and *Xeromphalina* (Tribus Mycenae sensu Singer, *Mycena* excluded) in Europe. IHW-Verlag, Eching, 279 pp.

APINIS, A.E., CHESTERS, C.G.C., TALIGOOLA, H.K. (1972a). Colonisation of *Phragmites communis* leaves by fungi. Nova Hedwigia 23: 113-124.

APTROOT, A. (1998). A world revision of *Massarina* (Ascomycota). Nova Hedwigia 66: 89-162.

BÁN, R., FISCHL, G. & VIRANYI, F. (1996). A spatio-temporal analysis of fungal pathogens on reed in natural habitats. Acta Phytopathol. Entomycol. Hung. 31: 219-227.

BARAL, H.O. (1992). Vital versus herbarium taxonomy: morphological differences between living and dead cells of ascomycetes, and their taxonomic implications. Mycotaxon 44: 333-390.

BARAL, H.O. & KRIEGLSTEINER, G.J. (1985). Bausteine zu einer Askomyzeten-flora der BR Deutschland: In Süddeutschland gefundene Inoperculate Discomyceten. Beih. Z. Mykol. 6: 1-160.

BARGHOORN, E.S. & LINDER, D.H. (1944). Marine Fungi: their taxonomy and biology. Farlowia 1: 395-467.

BARNETT, H.L. & HUNTER, B.B. (1987). Illustrated genera of Imperfect Fungi. APS, St. Paul, Minnesota. pp.

BARR, M.E. (1972). Prelimary studies on the Dothideales in temperate North America. Contrib. Univ. Michigan Herb. 9: 523-638.

BARR, M.E. (1980). On the family Tubeufiaceae (Pleosporales). Mycotaxon 12: 137-167.

BARR, M.E. (1989). The genus *Chaetomastia* (Dacampiaceae) in North America. Mycotaxon 34: 507-515.

BARR, M.E. (1990). Promodus to nonlichinized, pyrenomycetous members of Class Hymenoascomycetes. Mycotaxon 39: 43-184.

BARRON, G.L. (1964). A note on the relationship between *Stachybotrys* and *Hyalostachybotrys*. Mycologia 56: 313-316.

BAS, C. (1971). Over halm-bewonende *Coprinus*-soorten (sect. Herbicolae). Coolia 15: 43-48.

BOEREMA, G.H. (1993). Contributions towards a monograph of *Phoma* (Coelomycetes) - II. Section Peyronellaea. Persoonia 15: 197-221.

BOOTH, C. (1959). Studies of Pyrenomycetes IV. Nectria part I. Mycol. Pap. 73: 1-115, pl. 1-3.

BOOTH, C. (1971). The genus *Fusarium*. Commonwealth mycological institute, Kew. 237 pp.

BORSE, B.D. & HYDE, K.D. (1989). Marine fungi from India. III. *Acrocordiopis patilii* gen. et. sp. nov. from mangrowe wood. Mycotaxon 34: 535-540.

BRAUN, U. (1993). Studies on *Ramularia* and allied genera (VI). Nova Hedwigia 56: 423-454.

BREITENBACH, J. & KRÄNZLIN, F. (1981). Pilze der Schweiz, Band 1 Ascomyceten. Verlag Mycologia, Luzern. 313 pp.

BREITENBACH, J. & KRÄNZLIN, F. (1995). Pilze der Schweiz, Band 4 Bläterpilze 2. Teil. Verlag Mycologia, Luzern. 371 pp.

BUCHANAN, P.K. (1987). A reappraisal of *Ascochytula* and *Ascochytella* (Coelomycetes). Mycol. Pap. 156: 1-83.

CANNON, P. & HAWKSWORTH, D.L. (1982). A re-evaluation of *Melanospora* Corda and similar Pyrenomycetes, with a revision of the British species. Bot. J. Linn. Soc. 84: 115-160.

CASTELLANI, E. & GERMANO, G. (1977). Le Stagonosporae graminicole. Ann. Fac. Sci. Agrar., Univ. Torino 10: 1-135.

CHEN, W., SHEARER, C.A. & CRANE, J.L. (1999). Phylogeny of *Ophioceras* spp. based on morphological and molecular data. Mycologia 91: 84-94.

CHESTERS, C.G.C. & BELL, A. (1970). Studies in the Lophiostomataceae Sacc.. Mycol. Pap. 120: 1-55.

CLÉMENÇON, H. (1990). *Tetragoniomyces uliginosus* (Tremellales) auf *Waitea nuda* (Tulasnellales). Mycol. Helvetica 4: 53-73.

CLEMENTS, F.E. & SHEAR, C.L. (1931). The Genera of fungi. Hafner, New York.

CONSTANTINESCU, O. (1983). *Deightoniella* on *Phragmites*. Proc. K. Ned. Akad. Wet., Ser. C 86: 137-141.

CONWAY, K.E. & BARR, M.E. (1977). Classification of *Ophioceras dolichostomum*. Mycotaxon 5: 376-380.

COOKE, W.B. (1961). The cyphellaceous fungi. Beih. Sydowia 4: 1-144.

CORLETT, M. (1981). A taxonomic survey of some species of *Didymella* and *Didymella*-like species. Canad. J. Bot. 59: 2016-2048.
CORLETT, M. (1991). An annotated list of the published names in *Mycosphaerella* and *Sphaerella*. Mycologia Memoir 18
CORLETT, M. & SMITH, J.D. (1978). *Didymella proximella* and its *Stagonospora* anamorph. Canad. J. Bot. 56: 2818-2824.
CRIVELLI, P.G. (1983). Ueber die heterogene Ascomycetengattung *Pleospora* Rabh.; vorschlag für eine Aufteilung. Abh. Eidgen. Techn. Hochsch., Zürich. 213 pp.
CUMMINS, G.B. (1971). The rust fungi of cereals, grasses and bamboos. Springer-Verlag, Berlin. 570 pp.
DE HOOG, G.S. (1985). Taxonomy of the *Dactylaria* complex, IV-VI. Stud. Mycol. 26: 1-124.
DE MEULDER, H. (1999). *Psathyrella almerensis*, Polderfranjehoed, een nieuwe franjehoed voor ons land. Meded. Konink. Antwerpse Mycol. Kring 1999: 46-48.
DE MEULDER, H. (2002). *Halosarpheia spartinae* (E.B.G. Jones) Shearer & J.L. Crane een pyrenomyceet op Grote lisdodde (Typha latifolia). Meded. Konink. Antwerpse Mycol. Kring 2002: 11-12.
DENMAN, S., CROUS, P.W., TAYLOR, J.E., KANG, J., PASCOE, I., WINGFIELD, M.J. (2000). An overview of the taxonomic history of *Botryosphaeria*, and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. Stud. Mycol. 45: 129-140.
DENNIS, R.W.G. (1949). A revision of the British Hyaloscyphaceae, notes on related European species. Mycol. Pap. 32: 1-97.
DENNIS, R.W.G. (1963). A redisposition of some fungi ascribed to the Hyaloscyphaceae. Kew Bull. 17: 319-379.
DENNIS, R.W.G. (1981). British Ascomycetes. J. Cramer, London. 585 pp.
DIEDICKE, H. (1915). Pilze VII. Sphaeropsidae, Melanconieae. Kryptogamenflora der Mark Brandenburg 9. Borntraeger, Leipzig. 962 pp.
ELLIS, J.P. (1980). The genus *Morenoina* in Britain. Trans. Br. Mycol. Soc. 74: 297-307.
ELLIS, M.B. (1971). Dematiaceous Hyphomycetes. Commonwealth Mycological Institute Kew, Surrey. 608 pp.
ELLIS, M.B. (1976). More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute Kew, Surrey. 507 pp.
ERIKSSON, O. (1967). On graminicolous pyrenomycetes from Fennoscandia. II. Phragmosporous and scolecosporous species. Ark. Bot. II 6: 381-440.
ERIKSSON, O.E. & YUE, J. (1986). *Bertiella* (Sacc.) Sacc. & Sydow a synonym of *Massarina* Sacc.. Mycotaxon 27: 247-253.
FALLAH, P.M. & SHEARER, C.A. (1998). Freshwater Ascomycetes: *Phomatospora* spp. from lakes in Wisconsin. Mycologia 90: 323-329.
FELTGEN, J. (1899). Vorstudien zu einer Pilz-Flora des Grossherzogthums Luxemburg. 1. Theil Ascomycetes. Beffort, J., Luxembourg. 417 pp.
FELTGEN, J. (1901). Vorstudien zu einer Pilz-Flora des Grossherzogthums Luxemburg. 1. Theil Ascomycetes. Nachträge II. Beffort, J., Luxembourg. 243 pp.
FISHER, P.J., ANSON, A.E. & PETRINI, O. (1986). Fungal endophytes in *Ulex europaeus* and *Ulex gallii*. Trans. Brit. Mycol. Soc. 86: 153-156.
FRANCIS, S.M. (1975). *Anthostomella* Sacc. (Part I). Mycol. Pap. 139: 1-97.
GAMS, W. (1971). *Tolypocladium*, eine Hyphomycetengattung mit geschwollen Phialiden. Persoonia 6: 185-191.
GOH, T.K. & HYDE, K.D. (1996). Biodiversity of freshwater fungi. J. Industr. Microbiol. Biotechnol. 17: 328-345.
HELLER, A. & KEIZER, P.-J. (2004). Mykologische Notizen von den niederländisch-deutschen Nordsee-Inseln. Z. Mykol. 70: 3-22.
HILBER, O., WEBSTER, J. & HILBER, R. (1987). *Lasiosphaeria dactylina* sp. nov., a grass inhabiting species. Trans. Brit. Mycol. Soc. 89: 589-593.
HILBER, R. & HILBER, O. (1979). Enige Anmerkungen zu der Gattung *Cercophora* Fuckel (Lasiosphaeriaceae). Z. Mykol. 45: 209-233.
HILBER, R. & HILBER, O. (2002). The genus *Lasiosphaeria* and allied taxa. O. Hilber, Kelheim.
HOLEC, J. (2001). The genus *Pholiota* in central and western Europe. Libri Botanici 20: 1-220.
HOLM, L. & HOLM, K. (1981). Ascomycetes on Nordic Lycopods. Karstenia 21: 57-72.
HOLM, L. & HOLM, K. (1988). Studies in the Lophiostomataceae with emphasis on the Swedish species. Acta universitatis Upsaliensis. Symb. Bot. Upsal. 28(2): 1-50.
HOLUBOVA-JECHOVA, V. (1984). Lignicolous Hyphomycetes from Czechoslovakia 7. *Chalara, Exochalara, Fusichalara* and *Dictyochaeta*. Folia Geobot. Phytotax. 19: 387-438.

HUDSON, H.J. (1963). Pyrenomycetes of Sugar cane and other grasses in Jamaica II. Conidia of *Apiospora montagnei*. Trans. Brit. Mycol. Soc. 46: 19-23.
HUDSON, H.J. & MCKENZIE, E.H.C. (1984). Conidial states of *Apiospora* Sacc.. Trans. Brit. Mycol. Soc. 66: 359-362.
HYDE, K.D. (1992). Tropical Australian freshwater fungi. IV. *Halosarpheia aquaticum* sp. nov. *Garethjonesia lacunosispora* gen. & sp. nov. and *Ophioceras dolichostomum* (Ascomycetes). Austral. Syst. Bot. 5: 407-414.
HYDE, K.D. (1994). The genus *Savoryella* from freshwater habitats, including *S. grandispora* sp. nov.. Mycoscience 35: 59-61.
HYDE, K.D., READ, S.J., JONES, E.B.G. & MOSS, S.T. (1997). Tropical Australian Freshwater Fungi. XII *Rivulicola incrustata* gen. et sp. nov. and notes on *Ceratosphaeria lampadophora*. Nova Hedwigia 64: 185-196.
JACOBSSON, S. (1997). New observations on *Pholiota*. Windahlia 22: 23-28.
JANSEN, P.B. (1969). *Psathyrella typhae* en *Naucoria typhicola*. Coolia 14: 40-45.
JOHNSTON, P.R. (1983). *Stictis* and its anamophs in New Zealand. New Zealand J. Bot. 21: 249-279.
JOHNSTON, P.R. (1985). Anamorphs of the Ostropalean genera *Schizoxylon* and *Acarosporina*. Mycotaxon 24: 349-360.
JONG, S.C. & DAVIS, E.E. (1976). Contribution to the knowledge of *Stachybotrys* and *Memnoniella* in culture. Mycotaxon 3: 409-485.
KHASHNOBISH, A. & SHEARER, C.A. (1996). Phylogenetic relationships in some *Leptosphaeria* and *Phaeosphaeria* species, *Passeriniella obiones* and *Melanomma radicans*. Mycol. Res. 100: 1341-1354.
KIRK, P.M. & SPOONER, B.M. (1984). An account of the fungi of Arran, Gigha and Kintyre. Kew Bull. 38: 503-597.
KIRK, P.M., J.C.DAVID, AND J.A. STALPERS. (2001). Ainsworth and Bisby's Dictionary of the Fungi (including the lichens). Vol. XII.
KITS VAN WAVEREN, E. (1979). Soorten van het geslacht *Panaeolus* III, *P. acuminatus* versus *P. fimicola*. Coolia 22: 76-82.
KITS VAN WAVEREN, E. (1985). The Dutch, French and British species of *Psathyrella*. Persoonia Suppl. 2: 1-300.
KOHLMEYER, J. (1972). A revision of Halosphaeriaceae. Canad. J. Bot. 50: 1951-1963.
KOHLMEYER, J. & KOHLMEYER, E. (1979). Marine Mycology, the Higher Fungi. Academic Press, New York. 690 pp.
KOHLMEYER, J. VOLKMANN-KOHLMEYER, B. & ERIKSSON, O. (1995). Fungi on *Juncus roemerianus*. 2. New dictyosporous ascomycetes. Bot. Mar. 38: 165-174.
KUIJPERS, A.F.A. & APTROOT, A. (2002). A revision of *Mycosphaerella* section Longispora (Ascomycetes). Nova Hedwigia 75: 451-468.
LANGDON, R.F. (1941). The genus *Cerebella* Cesati - its biologic status and use. Mycol. Pap. 61: 613-617.
LE GAL, M. (1939). Florule mycologique des Bois de la Grange et de l'Etoile. Rev. Mycol. 4: 25-63.
LE GAL, M. & MANGENOT, F. (1961). Contribution à l'étude des Mollisioïdées IV (3e série). Rev. Mycol. 26: 263-331.
LEUCHTMANN, A. (1984). Über *Phaeosphaeria* Miyake und andere bitunicate Ascomyceten met mehrfach querseptierten ascosporen. Sydowia 37: 75-194.
LEUCHTMANN, A. (1985). Kulturversuche mit enigen Arten der Gattung *Lophiostoma* Ces. & De Not.. Sydowia 38: 158-170.
LUNDQVIST, N. (1972). Nordic Sordariaceae s. lat.. Acta Universitatis Upsaliensis. Symb. Bot. Upsal. 20: 1-374.
MILLER, A.N. & HUHNDORF, S.M. (2004). A natural classification of *Lasiosphaeria* based on nuclear LSU rDNA sequences. Mycol. Res. 108: 26-34.
MINOURA, K. & MUROI, T. (1978). Some freshwater ascomycetes from Japan. Trans. Mycol. Soc. Japan 19: 129-134.
MÜLLER, E. (1950). Die schweizerishen Arten der Gattungen *Leptosphaeria* und ihrer Verwandten. Sydowia 4: 185-319.
MÜLLER, E. & BOOTH, C. (1972). Generic position of *Sphaeria phaeostroma*. Trans. Brit. Mycol. Soc. 58: 73-77.
MUNK, A. (1956). On *Metasphaeria coccodes* (Karst.) Sacc. and other Fungi probably related to *Massarina* Sacc. (Massarinaceae n. Fam.). Friesia 5: 303-308.
MUNK, A. (1957). Danish Pyrenomycetes, a preliminary flora. Dansk Bot. Ark. 17(1): 1-491.
NAG RAJ, T.R. (1993). Coelomycetous anamorphs with appendage-bearing conidia. Mycologue Publications, Ontario, Canada. 1101 pp.

NAG RAJ, T.R., MORGAN-JONES, G. & KENDRICK, B. (1972). Genera coelomycetarum. IV. *Pseudorobillarda* gen. nov., a generic segregate of *Robillarda* Sacc.. Canad. J. Bot. 50: 861-867.
NOGRASEK, A. & MATZER, M. (1991). Nicht-pyrenokarpe Ascomyceten auf Gefässplanzen der Polsterseggenrasen I. Arten auf *Dryas octopetala*. Nova Hedwigia 53: 445-475.
OBERWINKLER, F. & BANDONI, R.J. (1981). *Tetragoniomyces* gen. Nov. and Tetragoniomycetaceae fam. nov. (Tremellales). Canad. J. Bot. 59: 1034-1040.
OBERWINKLER, F. & BANDONI, R.J. (1983). *Trimorphomyces*: a new genus in the Tremellaceae. System. Appl. Microbiol. 4: 105-113.
PELLICIER, P. (2000). Une nouvelle espèce de micromycète pour la France: *Cerebella andropogonis* Cesati. Bull. Féd. Myc. Dauphiné-Savoie 156: 42-44.
POON, M.O.K. & HYDE, K.D. (1998a). Biodiversity of intertidal estuarine fungi on *Phragmites* at Mai Po marshes, Hong Kong. Bot. Mar. 41: 141-155.
PUNITHALINGAM, E. (1979). Graminicolous *Ascochyta* species. Mycol. Pap. 142: 1-214.
PUNITHALINGAM, E. (1980). New microfungi from cereals. Nova Hedwigia 32: 585-606.
RAITVIIR, A. (2002). A revision of the genus *Dasyscyphella* (Hyaloscyphaceae, Helotiales). Polish Bot. J. 47: 227-241.
RAITVIIR, A. & SACCONI, S. (1991). Some interesting Hyaloscyphaceae from North Italy. 2. Mycol. Helvetica 4: 161-168.
RAPPAZ, F. (1992). *Phomatospora berkeleyi*, *P. arenaria* and their *Sporothrix* anamorphs. Mycotaxon 45: 323-330.
REHM, H. (1896). Die Pilze Deutschlands, Österreichs und der Schweiz. III. Abteilung: Ascomyceten; Hysteriaceen und Discomyceten. *In* L. RABENHORST'S Kryptogamenflora. I. , Leipzig. 1-1275.
REID, D. A. (1963). Notes on some fungi of Michigan I: 'Cyphellaceae'. Persoonia 3: 97-154.
SACCARDO, P.A. (1884). Sylloge Fungorum III. Friedländer & Sohn, Berlin.
SACCARDO, P.A. (1892). Sylloge Fungorum X. Friedländer & Sohn, Berlin.
SCHEUER, C. (1988). Ascomyceten auf Cyperaceen unf Juncaceen im Ostalpenraum. Biblioth. Mycol. 123: 1-274.
SCHEUER, C. (1995). Lectotypification of *Wettsteinia niesslii* (Dothideales s.l., Ascomycetes). Mycotaxon 54: 173-178.
SCHEUER, C., AND A. CHLEBICKI (1997). Recent collections of micellaneous microfungi from South Poland. Acta Mycol. 32: 147-172.
SCHROERS, H.-J., SAMUELS, G.J., SEIFERT, K.A. & GAMS, W. (1999). Classification of the mycoparasite *Gliocladium roseum* in *Clonostachys* as *C. rosea*, its relationship to *Bionectria ochroleuca*, and notes on other *Gliocladium*-like fungi. Mycologia 91: 365-385.
SCHUMACHER, T. (1990). The genus *Scutellinia* (Pyrenomataceae). Opera Bot. 101: 1-107.
SCHWEERS, A.C.S. (1941). Nieuwe vondsten en beschrijvingen. Meded. Ned. Mycol. Ver. 25: 7-11.
SEIFERT, K.A. (1985). A monograph of *Stilbella* and some allied hyphomycetes. Stud. Mycol. 27: 1-235.
SHEARER, C.A. (1989). *Pseudohalonectria* (Lasiosphaeriaceae), an antagonistic genus from wood in freshwater. Canad. J. Bot. 67: 1944-1955.
SHEARER, C.A. & CRANE, J.L. (1971). Fungi of the Chesapeake Bay and its tributaries. I. Patuxent river. Mycologia 63: 237-260.
SHEARER, C.A., CRANE, J.L. & CHEN, W. (1999). Freshwater Ascomycetes: *Ophioceras* species. Mycologia 91: 145-156.
SHERWOOD, M.A. (1977a). The Ostropalean fungi. Mycotaxon 5: 1-277.
SHERWOOD, M.A. (1977b). The Ostropalean fungi II: *Schizoxylon*, with notes on *Stictis, Acarosporina, Coccopeziza*, and *Carestiella*. Mycotaxon 6: 215-260.
SHOEMAKER, R.A. & BABCOOK, C.E. (1989). *Phaeosphaeria*. Canad. J. Bot. 67: 1500-1599.
SIVANESAN, A. (1984). The bitunicate ascomycetes and their anamorphs. Cramer, Vaduz. 701 pp.
SPRAGUE, R. (1950). Diseases of cereals and grasses in North America. The Royal Press Company.
SRINIVASAN, K.V. (1958). Fungi of the rhizosphere of sugarcane and allied plants. I.. J. Indian Bot. Soc. 37: 334-342.
STÄGER, R. (1905). Weitere Beiträge zur Biologie des Mutterkorns. Centr. Bacteriol. Parasitenk., Abt II 14: 25-32.
SUTTON, B.C. (1977). Coelomycetes VI. Nomenclature of generic names proposed for Coelomycetes. Mycol. Pap. 141: 1-253.
SUTTON, B.C. (1980). The Coelomycetes. Commonwealth Mycological Institute. 696 pp.
SUTTON, B.C. & PIROZYNSKI, K.A. (1963). Notes on British microfungi. Trans. Brit. Mycol. Soc. 46: 505-522.
SUTTON, B.C., PIROZYNSKI, K.A. & DEIGHTON, F.C. (1972). *Microdochium* Syd.. Canad. J. Bot. 50: 1899-1907.

TALIGOOLA, H.K. (1969). Stages in the colonization of *Phragmites communis* Trin. by microfungi. unpublished doctoral thesis, Nottingham. 153 pp.
TALIGOOLA, H.K., APINIS, A.E. & CHESTERS, C.G.C. (1972). Microfungi colonizing collapsed aerial parts of *Phragmites communis* Trin. in water. Nova Hedwigia 23: 465-472.
TSUDA, M. & UEYAMA, A. (1977). Descriptions and specimens of genus *Ceratosphaeria*. Trans. Mycol. Soc. Japan 18: 413-428.
TULLOCH, M. (1972). The genus *Myrothecium* Tode ex Fr.. Mycol. Pap. 130: 1-42 + 2 plates.
ULJÉ, C.B. & NOORDELOOS, M.E. (1997). Studies in *Coprinus* IV - *Coprinus* section *Coprinus*, subdivision and revision of subsection Alachuani. Persoonia 16: 265-333.
VAN BRUMMELEN, J. (1967). A world-monograph of the genera *Ascobolus* and *Saccobolus* (Ascomycetes, Pezizales). Persoonia suppl. Vol. 1: 1-260.
VAN DE PUT, K., VAN RYCKEGEM, G. & ANTONISSEN, I. (2000). Eerste vondsten van *Tetragoniomyces uliginosus* (P. Karst.) Oberw. & Bandoni in Vlaanderen. Sterbeeckia 19: 23-26.
VAN RYCKEGEM, G. (1999). Inventarisatie en ecologische voorkeur van fungi op *Phragmites australis* in Vlaanderen. Master thesis. Ghent University – UGent (in Dutch) 162 pp. + 48 plates.
VAN RYCKEGEM, G. & APTROOT, A. (2001). A new *Massarina* and a new *Wettsteinina* (Ascomycota) from freshwater and tidal reeds. Nova Hedwigia 73: 161-166.
VAN RYCKEGEM, G. & DAM, N. (1999). Nieuw voor Nederland en België: *Maireina monacha* (Speg.) W.B. Cooke. Meded. Antwerpse Mycol. Kring 1999: 11-14.
VAN RYCKEGEM, G. & VERBEKEN, A. (2000). *Rosellinia musispora* sp. nov., een nieuw tepelkogeltje op riet (*Phragmites australis*). Sterbeeckia 19: 3-5.
VAN RYCKEGEM, G., VAN DE PUT, K. & ROBERTS, P. (2002). *Tremella spicifera* sp. nov., a parasite of *Massarina arundinacea*. Mycotaxon 81: 185-189.
VERKLEY, G.J.M. & PRIEST, M.J. (2000). *Septoria* and similar coelomycetous anamorphs of *Mycosphaerella*. Stud. Mycol. 45: 123-128.
VOLDERS, J. (2002). Het genus *Tubaria* in Vlaanderen en het Brussels gewest. Sterbeeckia 21/22: 3-28.
VOLKMANN-KOHLMEYER B. & KOHLMEYER, J. (1996). How to prepare truly permanent microscope slides. Mycologist 10: 107-109.
VON ARX, J.A. (1975). On *Thielavia* and some similar genera of Ascomycetes. Stud. Mycol. 8: 1-29.
VON ARX, J.A. (1977). On *Yukonia caricis* and some other Ascomycetes. Rev. Mycol. 41: 265-270.
VON ARX, J.A. (1981). The genera of fungi sporulating in pure culture. Vaduz: J. Cramer, Stuttgart.
WAKEFIELD, E.M. (1939). Nomina generica conservanda. Contributions from the nomenclature committee of the British Mycological Society. II. Trans. Brit. Mycol. Soc. 23: 281-292.
WALKER, J. (1972). Type studies on *Gaeumannomyces graminis* and related fungi. Trans. Brit. Mycol. Soc. 58: 427-457.
WATLING, R. (1982). British Fungus Flora. Agarics and Boleti. 3. Bolbitiaceae: *Agrocybe*, *Bolbitius* & *Conocybe*. Royal Botanic Garden, Edinburgh. 139 pp.
WEBSTER, J. (1955). Gramenicoulous Pyrenomycetes V. Conidial states of *Leptosphaeria michotii*, *L. microscopica*, *Pleospora vagans* and the perfect state of *Dinemasporium graminum*. Trans. Brit. Mycol. Soc. 38: 347-365.
WEBSTER, J. & HUDSON, H. (1957). Graminicolous pyrenomycetes VI. Conidia of *Ophiobolus herpotrichus*, *Leptosphaeria luctuosa*, *L. fuckelii, L. pontiformis* and *L. eustomoides*. Trans. Brit. Mycol. Soc. 40: 509-522.
WEEDA, E.J., WESTRA, R., WESTRA, CH. & WESTRA, T. (EDS.) (1994). Nederlandse oecologische flora, wilde planten en hun relaties. Deel 5. CIP-gegevens Koninklijke Bibliotheek, Den Haag. 400 pp.
WHEELER, B.E.J. (1968). Fungal parasites of plants. *In* AINSWORTH, G.C. & SUSSMAN, A.S. (EDS.) The Fungi, Vol. 3. Academic Press, New York. 179-211.
WIRSEL, S.G.R., LEIBINGER, W., ERNST, M. & MENDGEN, K. (2001). Genetic diversity of fungi closely associated with common reed. New Phytol. 149: 589-598.
WIRSEL, S.G.R., RUNGE-FROBÖSE, C., AHRÉN, D.G., KEMEN, E., OLIVER, R. P. & MENDGEN, K.W. (2002). Four or more species of *Cladosporium* sympatrically colonize *Phragmites australis*. Fungal Genet. Biol. 35: 99-113.

Index to species

E

F

G

H

K

L

M

N

O

P

R

S

T

U

V

W