Université de Montréal

La face cachée de la dune

Communautés fongiques du sol: dynamique, succession et interactions avec la végétation d'un écosystème dunaire côtier aux Îles de la Madeleine, Qc

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Résumé

Les écosystèmes dunaires remplissent plusieurs fonctions écologiques essentielles comme celle de protéger le littoral grâce à leur capacité d'amortissement face aux vents et vagues des tempêtes. Les dunes jouent aussi un rôle dans la filtration de l'eau, la recharge de la nappe phréatique, le maintien de la biodiversité, en plus de présenter un attrait culturel, récréatif et touristique. Les milieux dunaires sont très dynamiques et incluent plusieurs stades de succession végétale, passant de la plage de sable nu à la dune bordière stabilisée par l'ammophile à ligule courte, laquelle permet aussi l'établissement d'autres herbacées, d'arbustes et, éventuellement, d'arbres. Or, la survie de ces végétaux est intimement liée aux microorganismes du sol. Les champignons du sol interagissent intimement avec les racines des plantes, modifient la structure des sols, et contribuent à la décomposition de la matière organique et à la disponibilité des nutriments. Ils sont donc des acteurs clés de l'écologie des sols et contribuent à la stabilisation des dunes. Malgré cela, la diversité et la structure des communautés fongiques, ainsi que les mécanismes influençant leur dynamique écologique, demeurent relativement méconnus.

Le travail présenté dans cette thèse explore la diversité des communautés fongiques à travers le gradient de succession et de conditions édaphiques d'un écosystème dunaire côtier afin d'améliorer la compréhension de la dynamique des sols en milieux dunaires. Une vaste collecte de données sur le terrain a été réalisée sur une plaine de dunes reliques se trouvant aux Îles de la Madeleine, Qc. J'ai échantillonné plus de 80 sites répartis sur l'ensemble de ce système dunaire et caractérisé les champignons du sol grâce au séquençage à haut débit. Dans un premier temps, j'ai dressé un portait d'ensemble des communautés fongiques du sol à travers les différentes zones des dunes. En plus d'une description taxonomique, les modes de vie fongiques ont été prédits afin de mieux comprendre comment les variations au niveau des communautés de champignons du sol peuvent se traduire en changements fonctionnels. J'ai observé un niveau de diversité fongique élevé (plus de 3400 unités taxonomiques opérationnelles au total) et des communautés taxonomiquement et fonctionnellement distinctes à travers un gradient de succession et de conditions édaphiques. Ces résultats ont

aussi indiqué que toutes les zones des dunes, incluant la zone pionière, supportent des communautés fongiques diversifiées.

Ensuite, le lien entre les communautés végétales et fongiques a été étudié à travers l'ensemble de la séquence dunaire. Ces résultats ont montré une augmentation claire de la richesse spécifique végétale, ainsi qu'une augmentation de la diversité des stratégies d'acquisition de nutriments (traits souterrains lié à la nutrition des plantes, soit mycorhizien à arbuscule, ectomycorhizien, mycorhizien éricoide, fixateur d'azote ou non spécialisé). J'ai aussi pu établir une forte corrélation entre les champignons du sol et la végétation, qui semblent tous deux réagir de façon similaire aux conditions physicochimiques du sol. Le pH du sol influençait fortement les communautés végétales et fongiques. Le lien observé entre les communautés végétales et fongiques met l'emphase sur l'importance des interactions biotiques positives au fil de la succession dans les environnements pauvres en nutriments.

Finalement, j'ai comparé les communautés de champignons ectomycorhiziens associées aux principales espèces arborescentes dans les forêts dunaires. J'ai observé une richesse importante, avec un total de 200 unités taxonomiques opérationnelles ectomycorhiziennes, appartenant principalement aux Agaricomycètes. Une analyse de réseaux n'a pas permis de détecter de modules (c'est-à-dire des sous-groupes d'espèces en interaction), ce qui indique un faible niveau de spécificité des associations ectomycorhiziennes. De plus, je n'ai pas observé de différences en termes de richesse ou de structure des communautés entre les quatre espèces hôtes.

En conclusion, j'ai pu observer à travers la succession dunaire des communautés diversifiées et des structures distinctes selon la zone de la dune, tant chez les champignons que chez les plantes. La succession semble toutefois moins marquée au niveau des communautés fongiques, par rapport aux patrons observés chez les plantes. Ces résultats ont alimenté une réflexion sur le potentiel et les perspectives, mais aussi sur les limitations des approches reposant sur le séquençage à haut-débit en écologie microbienne.

Mots-clés : Dunes côtières; Succession écologiques; Microbiome du sol; Champignons du sol; Champignons ectomycorhiziens; Interactions plantes-champignons; Séquençage à haut-débit

Abstract

Coastal dunes provide several key ecosystem services, such as erosion mitigation and protection of the littoral by forming a barrier against wind and wave action. These ecosystems also importantly contribute to water filtering, groundwater replenishment, maintenance of biodiversity, and have a cultural, aesthetic and recreational importance. Dune ecosystems are highly dynamic and characterized by stark ecological successional gradients. The sequence of plant communities along the gradient extends from upper beach to the foredune stabilized by pioneer species such as beach grass, which facilitates the establishment of other herbs, shrubs and eventually, trees. Plant growth and survival can be limited by environmental factors such as wind, salinity, drought and nutrient deficiency, and is therefore strongly linked to the presence of soil microorganisms. Soil fungi in particular are important plant symbionts and major regulators of organic matter decomposition, nutrient cycling and soil structure. Hence, they are key drivers of soil and vegetation dynamics, as well as important contributors to dune stabilisation. Still, the diversity and structure of soil fungal communities, as well as the mechanisms responsible for their ecological dynamic, remain incompletely understood.

In this thesis, I aimed to characterize fungal communities along a successional and edaphic gradient in a coastal dune in order to improve our understanding of soil dynamics in sand dunes ecosystems. I performed a comprehensive sampling of soils and aboveground vegetation at over 80 sites on a relic foredune plain. Soil fungi were characterized using high-throughput sequencing. A general description of soil fungal communities across dune zones was produced and, in addition to a taxonomic description, I assigned putative roles to all fungal genera to determine how variations in fungal community can be translated in functional changes. I recorded high level of fungal diversity (over 3400 operational taxonomic units) and described distinct communities along the successional and edaphic gradient. These results demonstrated the presence of taxonomically and functionally diverse communities across the dune sequence, including in the barren foredunes.

I also investigated the links between plant and fungal communities across the edaphic and successional gradient. These results showed a clear increase in plant species richness, as well as in the diversity of nutrient-acquisition strategies (belowground trait related to plant nutrition: arbuscular mycorrhizal, ectomycorrhizal, ericoid mycorrhizal, nitrogen-fixing or

unspecialized). I also found a very strong correlation between aboveground vegetation and soil fungal communities, which both responded to soil physicochemical properties. Soil pH importantly shaped plant and fungal communities, and could act as an important environmental filter along this relic foredune plain. The coordinated changes in soil microbial and plant communities highlight the importance of aboveground-belowground linkages and of positive biotic interactions during ecological succession in nutrient-poor environments.

Finally, I compared the ectomycorrhizal fungal communities associated with four co-occuring tree species in the forested zone of the relic foredune plain. High ectomycorrhizal fungal richness was observed across the four hosts, with a total of 200 ectomycorrhizal operational taxonomic units, mainly belonging to the Agaricomycetes. Network analysis did not detect modules (i.e. subgroups of interacting species), indicating a low level of specificity in these ectomycorrhizal associations. In addition, there were no differences in ectomycorrhizal diversity or community structure among the four tree species.

To conclude, I was able to describe diverse communities and distinct community structures across the dune sequence, for both plants and fungi. Succession however seemed less pronounced in fungal communities compared to patterns observed in plants. These results fueled a reflection on the potential and perspectives, as well as the limitations, of high-throughput sequencing approaches in the field of microbial ecology.

Keywords : Coastal dunes; Ecological succession; Soil microbiome; Soil fungi; Ectomycorhizal synbiosis; Plant-fungi interactions; High-throughput sequencing

Table des matières

Liste des tableaux

Liste des figures

Liste des abréviations

(L'italique désigne les termes en anglais)

AM	*arbuscular mycorhizas*
AMF	*arbuscular mycorhizal fungi*
BSA	*bovine serum albumin*
CMA	champignons mycorhiziens à arbuscules
CMN	*common mycelial network*
DMSO	*dimethyl sulfoxide*
EM	*ectomycorhizal*
ITS	*internal transcribed tpacer*
MID	*multiplex identifier*
NODF	*nestedness metric based on overlap and decreasing fill*
OTU	unité taxonomique opérationelle / *operational taxonomic unit*
PCR	*polymerase chain reaction*
PERMANOVA	*permutational analysis of variance*
PCoA	*principal coordinates analysis*
PDI	*paired difference index*
qPCR	*quantitative PCR*
RDA	*redundancy analysis*
rRNA	*ribosomal DNA*
tb-RDA	*transformation based RDA*
WNODF	*weighted NODF*

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"We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time."

— *T.S. Eliot*

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Chapitre 1 | Introduction générale

1.1 Les microorganismes du sol

1.1.1 Abondance, diversité et fonctions

Le sol, ou la pédosphère, est à la fois le support de la vie terrestre et son produit. Les sols sont le résultat de la transformation et de la dégradation de la roche-mère, et de l'intégration de matière organique par des processus biologiques. Ils constituent l'interface entre l'atmosphère, la lithosphère, l'hydrosphère et la biosphère. Composés d'une phase solide comprenant minéraux et matière organique, de pores remplis d'eau et d'air et d'une myriade d'organismes vivants, les sols sont des systèmes complexes et dynamiques. Ce sont probablement parmi les systèmes qui demeurent encore aujourd'hui les plus méconnus, tant au niveau de leur fonctionnement que de la diversité et du rôle des organismes qu'ils abritent (Gobat *et al.*, 2004). L'ampleur de la diversité et de l'abondance des microorganismes (bactéries, virus, champignons, algues, etc.) évoluant dans les sols est d'ailleurs souvent surprenante. Par exemple, un seul gramme de sol pourrait contenir jusqu'à 10^{10}-10^{11} bactéries, 6000-50000 espèces bactériennes et jusqu'à 200 m d'hyphe fongique (van der Heijden *et al.*, 2008).

Les microorganismes, ainsi surnommés la « majorité invisible[1] », remplissent plusieurs fonctions essentielles dans les sols. Notamment, ils contribuent de façon importante à la nutrition des plantes par la décomposition de la matière organique et la dégradation de la roche qui permettent l'apport de nutriments essentiels (Uroz *et al.*, 2009 ; Courty *et al.*, 2010). Bien que la contribution des microorganismes aux cycles biogéochimiques soit largement reconnue, l'impact des microorganismes du sol sur le fonctionnement et les propriétés des écosystèmes ainsi que sur la productivité et la diversité des plantes demeure mal compris (van der Heijden *et al.*, 2008).

[1] Expression introduite par Whitman *et al.* (1998), qui estimaient la masse totale des microorganismes à plus du tiers de la biomasse de la terre.

1.1.2 Les Eumycètes

Le règne des champignons, les Eumycètes, constitue un des groupes du vivant les plus diversifiés. Le nombre d'espèces décrites à ce jour est d'environ 100,000 (Tedersoo *et al.*, 2014), mais le nombre total d'espèces estimé varie entre 0,8 à 5,1 millions (Hawksworth, 2001; Blackwell, 2011). Il resterait donc plus de 90 % de la diversité fongique à découvrir.

Les champignons sont présents en abondance dans les sols et, en tant que principaux régulateurs de la décomposition, de la séquestration de carbone (Clemmensen *et al.*, 2013) et de la respiration, leur importance y est incontestable. Ils contribuent aux cycles biogéochimiques et régissent la disponibilité de plusieurs nutriments. En plus de décomposer la matière organique à l'aide de multiples enzymes (Baldrian *et al.*, 2011), certains champignons ont la capacité de dégrader la roche (Landeweert *et al.*, 2001). La sécrétion d'acides organiques et de composés phénoliques permet de solubiliser la roche et de libérer des nutriments comme le phosphore qui sont essentiels à la nutrition des plantes.

Boîte 1.1 – Considérations méthodologiques

Les Eumycètes étant principalement microscopiques, des contraintes méthodologiques ont longtemps limité l'étude des champignons du sol à l'identification des taxons isolés et cultivés en laboratoire ou à l'examen morphologique des spores recueillies par tamisage des échantillons de sol. Or, la majorité des champignons du sol ne peuvent être cultivés (Hawksworth *et al*., 2001) et plusieurs études ont démontré que l'inspection des spores représente souvent assez mal la structure des populations (Horton and Bruns, 2001; Kowalchuk *et al*., 2002; Tedersoo *et al*., 2010). Ainsi, de telles études ne peuvent que dresser un portrait incomplet des populations fongiques du sol.

Les avancées technologiques importantes des dernières années, notamment le développement des outils moléculaires comme le séquençage à haut débit pour l'analyse des communautés microbiennes, fournissent des opportunités de décrire la diversité fongique des sols comme jamais auparavant (Tedersoo *et al*., 2014), et ce, à des coûts de plus en plus bas. En effet, ces méthodes ont considérablement accéléré la découverte et la description d'espèces microbiennes dans les dernières années (Sota *et al.*, 2014). Le séquençage d'amplicons à partir d'ADN environnemental (i.e. métagénomique ciblée) en utilisant des marqueurs ciblant des régions variables – comme l'ITS (internal transcribed spacer) qui est largement utilisé chez les champignons (Nilsson *et al.*, 2012; Lindahl *et al.*, 2013) – a rendu possible le profilage taxonomique des communautés microbiennes dans des environnements diversifiés, incluant les sols (ex : O'Brien *et al*., 2005; Buée *et al*., 2009; Tedersoo *et al*., 2014).

1.1.3 Modes de vie fongiques

Les Eumycètes sont un groupe très vaste et diversifié parmi lequel se sont développés différents modes de vie associés à des stratégies nutritionnelles distinctes. La majorité des champignons sont dits saprotrophes : ils produisent et sécrètent des enzymes extracellulaires qui dégradent la matière organique inerte afin d'en extraire des éléments comme le carbone et l'azote (Moore *et al.*, 2011) (Figure 1.1A). Les champignons saprotrophes décomposent efficacement la lignine, la cellulose et la chitine, soit les biopolymères les plus abondants dans les biomes terrestres et principales sources de carbone dans les sols forestiers (Baldrian *et al.*, 2011). Les saprotrophes produisent différentes enzymes qui leur confèrent des capacités métaboliques distinctes. Par exemple, les « white-rot fungi » comprennent environ 2000 espèces de champignons (principalement des Basidiomycètes) qui métabolisent la lignine, alors que les « brown-rot fungi » sont spécialisés dans la dégradation de la cellulose et de l'hémicellulose (Moore *et al.*, 2001). Les champignons saprotrophes sont d'une grande importance dans les sols à cause de leur contribution à la décomposition de la litière des débris végétaux et animaux, et de leur participation directe aux cycles de nutriments comme l'azote et le carbone (Baldrian *et al.,* 2011).

D'autres sont parasites, c'est-à-dire qu'ils tirent parti de la matière organique vivante. Souvent pathogènes, ces champignons peuvent causer des maladies et entraînent parfois la mort de leur hôte qui peut être végétal, animal ou même fongique. Certains ont des stratégies qui se rapprochent de la prédation, comme certains champignons nématophages qui forment des structures mycéliennes spécialisées, des anneaux constricteurs, leur permettant de capturer, puis de tuer et digérer des nématodes (Barron, 1977; Liu *et al.*, 2009) (Figure 1.1B).

Enfin, certains champignons vivent en symbiose avec un autre organisme vivant. Par exemple, l'association des champignons mycorhiziens avec les végétaux implique un échange nutritionnel entre le champignon et son hôte. Le champignon est approvisionné en sucres photosynthétiques par la plante; en contrepartie, le réseau de mycélium associé au système racinaire de la plante continue de se développer dans le sol, digérant et absorbant des nutriments ainsi que de l'eau qu'il partage avec son hôte (Smith & Read, 2008). Il existe plusieurs types d'associations mycorhiziennes qui impliquent différents champignons et hôtes.

Les mycorhizes à arbuscules qu'on retrouve chez environ 80% des espèces de plantes vasculaires sont de loin les plus répandues (Wang & Qiu, 2006; Brundrett, 2009). Cette symbiose est le résultat de la colonisation des racines par des hyphes de champignons appartenant à la classe des Gloméromycètes. Ceux-ci s'insèrent dans les cellules du cortex racinaire, entre la paroi et la membrane cytoplasmique, où ils forment des structures caractéristiques appelées arbuscules (Figure 1.1C). Les hyphes fongiques explorent le sol au-delà de la rhizosphère et mobilisent des nutriments, principalement du phosphore, qui seront transportés vers les arbuscules où ont lieu les échanges. Les champignons mycorhiziens à arbuscules (CMA) s'associent à une grande diversité d'hôtes appartenant aux angiospermes, gymnospermes et ptéridophytes, et ce, depuis plus de 500 millions d'années, soit l'origine des plantes terrestres (Simon *et al.*, 1993; Redecker *et al.*, 2000; Corradi & Bonfante, 2012). D'autre part, les arbres et arbustes des forêts boréales et tempérées sont caractérisés par l'association de leurs racines à certains champignons du sol (un large spectre de basidiomycètes et ascomycètes) qui forme un autre type de symbiose, les ectomycorhizes. Cette association qui implique environ 3% des spermatophytes (plantes à graines) incluant la majorité des arbres forestiers, est reconnue pour améliorer les capacités d'acquisition de l'azote, mais aussi du phosphore (Read & Perez-Moreno, 2003). Les hyphes des champignons ectomycorhiziens recouvrent les racines fines de l'hôte et pénètrent entre les cellules de l'épiderme, et même du cortex chez les gymnospermes, pour former le réseau de Hartig, site des échanges bidirectionnels entre les deux partenaires (Figure 1.1D). Tout comme dans le cas des champignons mycorhiziens à arbuscules, les hyphes des champignons ectomycorhiziens se propagent hors de la racine. Le réseau mycélien qui se forme dans les sols sécrète différentes enzymes afin de dégrader la matière organique et d'absorber des nutriments (Smith & Read, 2008). Une des particularités des champignons ectomycorhiziens est leur capacité à former de vastes réseaux appelés « common mycorrhizal networks » connectant des arbres, de la même espèce ou pas, et permettant un transfert efficace de l'eau, des nutriments et du carbone entre les individus (Simard *et al.*, 2012). Les symbioses mycorhiziennes à arbuscules, ainsi que les symbioses ectomycorhiziennes, sont aussi reconnues pour l'amélioration de la résistance aux pathogènes, de l'absorption de l'eau et de la résistance aux stress abiotiques chez l'hôte (Ruiz-Lozano *et al.*, 1995; Pozo & Azcón-Aguilar, 2007; Evelin *et al.*, 2009; Wehner *et al.*, 2010). Enfin, les mycorhizes éricoïdes impliquent certains

ascomycètes du sol associés à des plantes de la famille des éricacées, alors que les mycorhizes les orchidoïdes sont essentiels à la germination des graines des orchidées (Smith & Read, 2008).

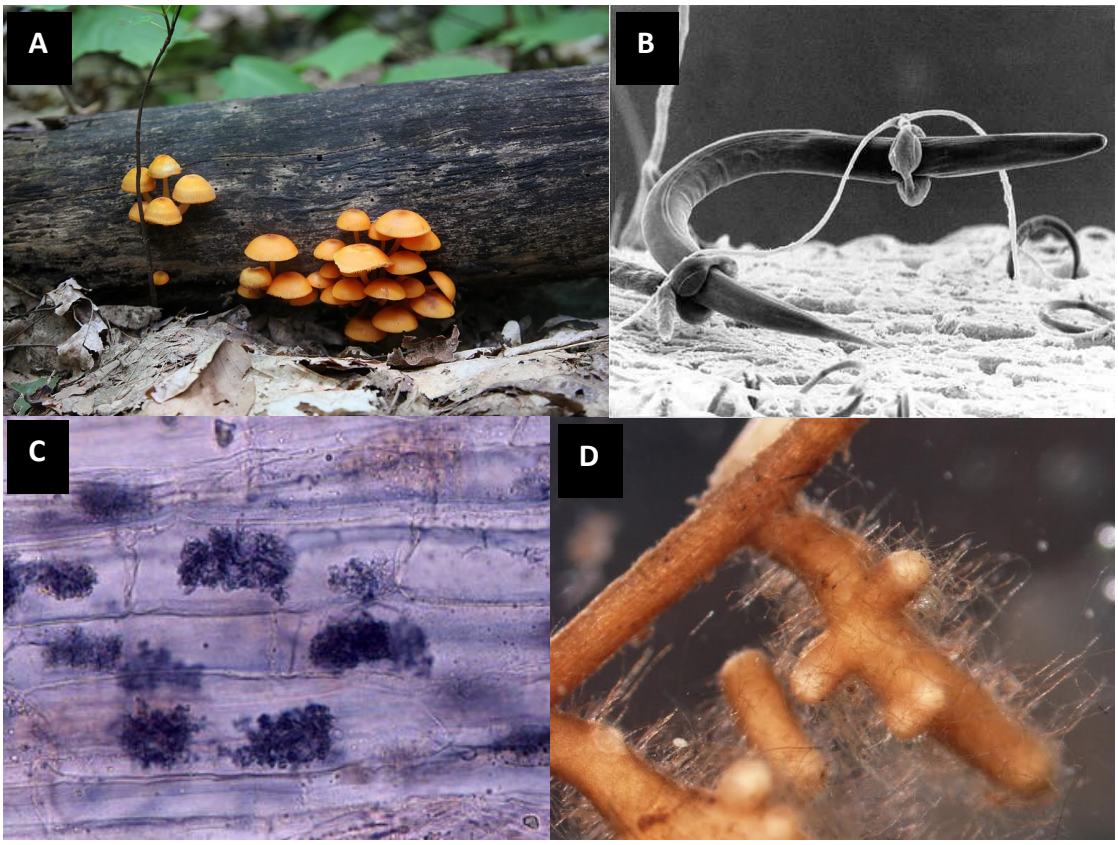

Figure 1.1. Différents modes de vie fongiques. Les champignons du genre *Mycena* (A) sont saprotrophes et se développent sur la matière organique en décomposition comme sur des souches. *Drechslerella anchonia* (B) est un nématophage; on voit sur l'image un nématode pris au piège dans ses anneaux constricteurs. Les champignons mycorhiziens à arbuscules (C) produisent des arbuscules et des hyphes intercellulaires dans les racines d'environ 80% des plantes vasculaires. Manchon mycellien formé par un champignon ectomycorhizien (D) sur les racines fines d'un Hêtre à grandes feuilles.

Source des images: (A) Mohamed Hijri (B) Georges L. Barron, https://atrium.lib.uoguelph.ca/ (C) Mohamed Hijri. (D) Etienne Laliberté. Toutes les images ont été reproduites avec la permission des auteurs.

1.2 Interactions entre le sol et les plantes

Plusieurs des modes de vie et fonctions décrits ci-haut sont liés directement ou indirectement à l'établissement, à la croissance, à la survie des plantes, à leur diversité et à la structure de leurs communautés (van der Heijden *et al.*, 2008). Les microorganismes du sol et

les plantes interagissent et s'influencent mutuellement de plusieurs façons, bien que plusieurs des mécanismes spécifiques demeurent incompris. Les communautés végétales et fongiques sont liées par divers mécanismes complexes, incluant des effets positifs ou négatifs, et directs ou indirects, qui ont été revus par quelques auteurs dans les dernières années (Wardle *et al.*, 2004 ; van der Heijden *et al.*, 2008; Bever *et al.,* 2010).

1.2.1 Effet des champignons du sol sur les plantes

Les modes de vie et fonctions des champignons ont été décrits ci-haut et ne seront qu'exposés rapidement dans le contexte des interactions plante-sol, principalement à l'échelle des communautés. Les champignons parasites et pathogènes ont un effet négatif sur la croissance des plantes hôtes et altèrent la composition et la diversité des communautés végétales (Hansen & Stone, 2005). D'autre part, les champignons mycorhiziens favorisent la survie et la croissance des plantes auxquelles ils sont associés. Ces deux types d'interactions, respectivement négative et positive, agissent de façon directe et ont un impact différentiel selon l'identité de l'hôte et du partenaire fongique (Bever *et al.*, 1997 ; van der Putten, 2003). Ainsi, les interactions parasitiques et symbiotiques ont un impact important sur la composition des communautés végétales. Les champignons du sol ont aussi plusieurs effets indirects, notamment en ce qui a trait à la nutrition des plantes par la décomposition de la matière organique, l'ajout de biomasse à la chaîne alimentaire des détritivores, la solubilisation des minéraux et la dégradation de la roche (Read & Perez-Moreno, 2003; Smith & Read, 2008; Baldrian *et al.,* 2011).

1.2.2 Effet de la végétation sur les champignons du sol

De nombreuses études se sont intéressées à l'effet de la végétation sur les communautés microbiennes du sol (ex : Kourtev *et al.*, 2003 ; Aponte *et al.*, 2010 ; Bever *et al.*, 2013 ; Prescott & Grayston, 2013 ; Roy *et al.*, 2013 ; Barberán *et al.*, 2015). Un des principaux effets de la végétation sur le sol est lié à la production de litière et de matière organique (Aponte *et al.*, 2010 ; Prescott & Grayston, 2013). En effet, la quantité et la qualité des débris végétaux varient grandement d'une espèce à l'autre. La composition de la communauté végétale aura donc une influence sur les conditions du sol (notamment la quantité et le type de carbone organique ajouté au sol par la litière, sur le pH ou sur la

disponibilité des nutriments) et sur la composition des communautés de champignons, dont plusieurs sont saprotrophes (van der Heijden *et al.*, 2008 ; Prescott & Grayston, 2013). D'autres effets connus de la végétation sur les conditions du sol sont liés aux exsudats produits par les racines des plantes qui peuvent aussi modifier le pH et la solubilisation des nutriments, en plus de faire de la rhizosphère un environnement riche en carbone (Grayston *et al.*, 1997 ; Bais *et al.*, 2006). Les exsudats racinaires influencent également les interactions entre plantes et champignons du sol par la production de molécules de signalisation qui peuvent attirer ou repousser les microorganismes (Lugtenberg *et al.*, 2002). Les groupes d'organismes intimement liés aux racines, comme les champignons mycorhiziens, sont influencés par la composition de la végétation. Par exemple, l'identité de l'hôte a été identifiée comme le meilleur prédicteur de la composition des communautés de champignons ectomycorhiziens dans plusieurs études (Ishida *et al.*, 2007 ; Morris *et al.*, 2009 ; Murata *et al.*, 2013 ; Tedersoo *et al.*, 2013). Toutefois, ce phénomène nommé l'effet de l'hôte (« host effect ») demeure partiellement incompris et il n'existe pas de consensus dans la littérature quant à l'étendue de son importance. De plus, les interactions entre les plantes et le sol ainsi que leurs effets sur les communautés végétales et fongiques sont largement dépendants du contexte et peuvent être influencés par des acteurs appartenant à d'autres niveaux trophiques comme les herbivores, ce qui rend difficile la généralisation des mécanismes et la prédiction des réponses (Bever *et al.*, 2010). De plus, l'assemblage des communautés végétales et microbiennes peut se produire de façon indépendante en réponse à des conditions abiotiques du sol semblables (Hines *et al.*, 2006), ou à des échelles temporelles et spatiales différentes (Bardgett *et al.*, 2005).

1.2.3 Interactions entre groupes fongiques

Il existe aussi des effets antagonistes connus entre différents modes de vie fongiques. Par exemple, l'interaction des plantes avec des CMA est reconnue pour améliorer la protection contre les pathogènes (Wehner *et al.*, 2010). Les champignons ectomycorhiziens joueraient un rôle semblable en formant une barrière physique autour des racines (Marx, 1972) et en produisant des antibiotiques (Duchesne *et al.*, 1988). Ainsi, les champignons mycorhiziens (EM et AM) pourraient avoir un effet négatif sur l'abondance de champignons pathogènes dans la rhizosphère des plantes avec lesquelles ils interagissent. Aussi, plusieurs auteurs ont observé une corrélation négative entre les CMA et les champignons ectomycorhiziens qui

pourrait être due à de la compétition (ex : Lapeyrie & Chilvers, 1985; Lodge & Wentworth, 1990).

Les interactions, multiples et complexes, entre la végétation et les microorganismes du sol prennent place dans tous les écosystèmes terrestres. Les effets de la végétation sur les microorganismes du sol (et vice-versa) sont difficiles à prédire et dépendent largement du contexte et des caractéristiques de l'environnement (Wardle *et al.*, 2004). Ces interactions sont en effet dynamiques et varient avec le développement des écosystèmes (Kardol *et al.*, 2013). L'importance relative des différents acteurs ainsi que la nature des interactions sont influencés par les changements dans les conditions environnementales, tel qu'observés dans des écosystèmes en développement comme les milieux dunaires.

1.3 Écosystèmes en développement : les dunes côtières

Les écosystèmes dunaires sont depuis longtemps des terrains d'étude de prédilection pour les écologistes intéressés par les dynamiques végétales et la succession primaire (ex : Cowles, 1899 ; Olson, 1958 ; Lichter, 1998 ; Laliberté *et al.*, 2012). Les milieux dunaires présentent un gradient complexe de contraintes environnementales qui ont un impact différentiel sur la survie, la croissance et la reproduction des différentes espèces végétales, créant ainsi une séquence marquée de communautés végétales et types de sol. Les jeunes dunes situées à proximité du littoral sont caractérisées par de forts vents, des hauts taux d'ensablement et d'érosion, et des sols très pauvres en nutriments et en eau (Cowles, 1899 ; Lichter, 1998). Le peu de matière organique qui s'y trouve y est déposé essentiellement par le vent et les vagues, sous forme de débris marins (Maun *et al.*, 2009). Les conditions environnementales varient selon l'âge de la dune et la distance avec la mer. En s'éloignant du littoral, on observe typiquement une réduction du vent, de l'ensablement, de l'érosion et de l'embrun salin grâce à la présence de la dune bordière qui forme un écran protecteur (Lichter, 1998). Les jeunes dunes sont colonisées par une végétation pionnière, souvent quasi mono-spécifique, qui permet la stabilisation des premières lignes de dune (dunes bordières). Au fil du développement de l'écosystème, la végétation évolue vers des communautés plus complexes typiquement caractérisées par une augmentation de la diversité végétale et la

présence accrue d'espèces ligneuses (Laliberté *et al.*, 2012) (Figure 1.2). En absence de perturbations anthropiques, ces changements s'accompagnent d'une accumulation de litière et de matière organique dans les sols ainsi que d'une augmentation des concentrations de carbone et d'azote, de la formation graduelle de sols stratifiés et d'une meilleure capacité de rétention d'eau (Gooding, 1947 ; Olson, 1958; Lichter, 1998). On observe aussi généralement une acidification des sols causée par la production d'acides organiques par la végétation et la décomposition de la matière organique (Olson, 1958 ; Lichter, 1998), et un déclin du phosphore disponible dans le cas où l'écosystème atteint une phase de rétrogression (Vitousek & Farrington, 1997 ; Lichter, 1998 ; Laliberté *et al.*, 2012).

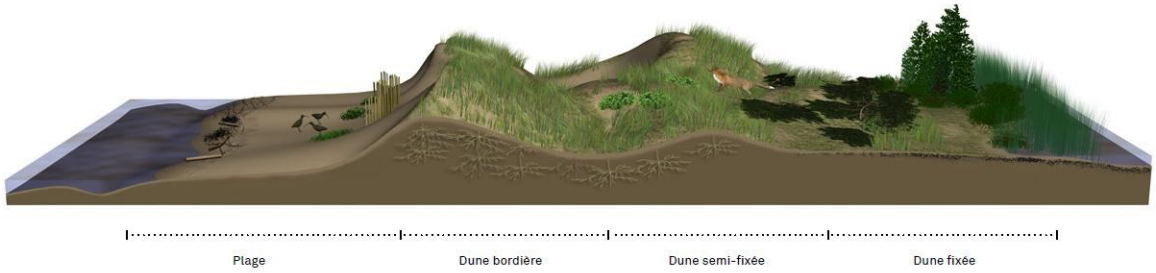

Figure 1.2. Profil typique de succession végétale dans un écosystème dunaire (source : Attention Fragîles).

1.3.1 Étude de la succession écologique

La succession écologique, telle que décrite rapidement ci-haut pour les milieux dunaires, se définit comme un changement directionnel dans la composition et la structure d'une communauté. C'est le processus naturel de développement d'un écosystème qui consiste en la recolonisation d'un milieu suite à une perturbation (ex : feu, inondations, coupes forestières) ou d'un milieu vierge dans le cas de la succession primaire. La succession écologique décrit l'ensemble des stades (« séries ») que traverse une communauté et implique une multitude de processus et mécanismes – par exemple des interactions inter-spécifiques comme la compétition, l'inhibition ou la facilitation (ex : Connell & Slatyer 1977) – qui définiront la direction des changements. La succession est l'un des plus vieux concepts en écologie et demeure une source de débats et de divergences au sein de la communauté

scientifique. Certains voient la succession écologique comme un phénomène ordonné, linéaire et prédictible qui est le résultat de propriétés émergentes des communautés et liée aux caractéristiques de chacune des espèces (Gurevitch et al. 2002). Le climax est la communauté théorique qui correspond à la finalité de la succession dans une vision déterministe. À l'autre extrême, certains écologistes définissent la succession comme une série d'évènements imprévisibles qui résultent des interactions entre les individus et l'environnement (Gurevitch et al. 2002).

Comme la succession végétale est généralement un phénomène qui s'effectue sur une échelle de temps relativement longue, il faudrait des décennies pour observer une succession complète. Une des façons traditionnelles de contourner ce problème est l'observation d'une chronoséquence, soit une séquence de sites d'âges différents partageant les mêmes caractéristiques (abiotiques, climatiques, géographiques, écologiques). Cette approche repose sur la substitution espace-temps, c'est-à-dire qu'une progression dans l'espace devrait correspondre à une variation de l'âge des sites. Cela permet d'étudier des phénomènes sur une période de temps beaucoup plus longue que l'on pourrait le faire par observation directe.

Cowles (1899) a été l'un des premiers écologistes à utiliser cette approche, dans un système de dune au Lac Michigan. Le recul du Lac suite à la dernière glaciation a entraîné la formation d'une séquence de crêtes dunaires. Cowles (1899) a décrit la végétation évoluant sur cette série de dunes d'âges différents et définit une séquence simplifiée de végétation. Par la suite, plusieurs écologistes ont eu recourt à l'utilisation de chronoséquences pour étudier la succession. Dachnowski (1912), par exemple, s'est interessé à la succession des tourbières et Oosting (1942) aux champs abandonnés. Des plaines glacières, où le recul des glaciers fait place à des sols d'âge croissant, forment aussi des chronoséquences. Celle de Glacier Bay en Alaska a d'ailleurs été amplement étudiée (ex : Cooper, 1923; Crocker & Major, 1955). Les îles volcaniques d'Hawaii (ex : Crews *et al.*, 1995) et la chronoséquence dunaire de Jurien Bay en Australie (ex : Laliberté *et al*., 2012) sont d'autres exemples de chronoséquences bien connues des écologistes. Cette approche demeure encore très répandue à ce jour pour l'étude de la succession écologique (Johnson & Miyanishi, 2008).

L'étude de la succession écologique à l'aide d'une chronoséquence comprend des limitations qui ont été sévèrement critiquées par certains écologistes (ex : Johnson & Miyanishi, 2008). Notamment, la supposition fondamentale à cette approche – c'est-à-dire que

chacun des sites de la séquence ne diffèrent qu'au niveau de l'âge et que les sites plus jeunes se développent de la même façon que les sites plus vieux l'ont faits – est souvent peu, voire pas supportée dans les études s'intéressant à des chronoséquences (Johnson & Miyanishi, 2008). Une utilisation inappropriée des chronoséquences peut amener à des conclusions fausses sur les dynamiques temporelles et les processus impliqués dans la succession. Par exemple, Cowles (1899) décrivait une succession basée sur la facilitation, plus précisément sur la modification des conditions édaphiques et de lumière par les plantes qui entraînerait un changement directionnel progressif de dunes à Ammophile vers des forêts mésiques. Or, plusieurs études ont par la suite fournit des évidences contre l'hypothèse de la facilitation (revues dans Johnson & Miyanishi, 2008). De plus, Olson (1958) a aussi étudié la succession végétale des dunes du Lac Michigan. Il a pu déterminer l'âge des dunes et a constaté que des sites du même âge supportaient des végétations très différentes. Les communautés végétales établies à un endroit donné semblent donc dépendre beaucoup plus de la localisation topographique et de l'historique de perturbation que d'interactions de facilitation. Malgré ces critiques, les chronoséquences demeurent pertinentes pour clarifier les dynamiques écologiques temporelles de la végétation. En effet, il n'existe souvent pas d'alternatives pour étudier le développement des écosystèmes à long terme. De plus, en interprétant les observations avec prudence et en formulant les conclusions avec conscience des limitations de l'approche, l'étude des chronoséquences peut sans aucun doute permettre d'améliorer la compréhension de la dynamique des écosystèmes (Walker *et al.*, 2010).

1.3.2 Interactions plantes-microorganismes dans les sols en développement

Il existe une littérature abondante sur l'évolution de la végétation au fil du développement des écosystèmes, mais relativement peu sur les communautés microbiennes du sol dans ce contexte (Dickie *et al.*, 2013). Le peu de connaissances sur la succession microbienne, bien que largement lié à des contraintes méthodologiques (voir boîte 1.1), est contradictoire avec le fait que les microorganismes du sol soient de plus en plus reconnus comme des moteurs du développement du sol et des communautés végétales.

Quelques chronoséquences ont été étudiées particulièrement extensivement – notamment Glacier bay, Jurien bay et une série d'îles à Hawaii (voir Wardle *et al.*, 2008 et

Laliberté *et al.*, 2013) – et ont permis l'acquisition d'une meilleure compréhension des liens entre la pédogénèse et les dynamiques de la végétation. De façon générale, on a observé une augmentation de la diversité végétale liée au développement de l'écosystème (Wardle *et al.*, 2008; Laliberté *et al.*, 2013; Zemunik *et al.*, 2015). À Jurien Bay, une chronoséquence dunaire australienne vieille de 2 millions d'années et identifiée comme un « hot-spot » de biodiversité, on a observé une augmentation significative de la diversité végétale avec l'âge des dunes (Laliberté *et al.*, 2013; Zemunik *et al.*, 2015). Des patrons semblables ont été constatés dans d'autres chronoséquences : Wardle *et al.* (2008) a étudié la diversité des arbres et plantes vasculaires à 6 sites différents et observé une diversité croissante (sauf pour 1 site) ainsi qu'une augmentation des plantes ayant une stature réduite. Dans la chronoséquence de Jurien Bay, on s'est aussi intéressé aux traits liés à l'acquisition ou à l'allocation et au recyclage des nutriments à travers les phases de progression et de rétrogression de l'écosystème (ex : Hayes *et al.*, 2014; Zemunik *et al.*, 2015). En effet, les plantes ont recours à différents mécanismes pour acquérir des nutriments, dont plusieurs sont liés à des microorganismes du sol. Lambers *et al.* (2008) ont proposé un modèle décrivant les changements des stratégies d'acquisition de nutriments avec l'âge des sols (Figure 1.3). Selon ce modèle, les plantes formant des symbioses avec des CMA seraient dominantes dans les sols jeunes, suivies des plantes ectomycorhiziennes, puis éricoïdes. Finalement, les sols les plus matures seraient principalement colonisés par des plantes adoptant des stratégies non-mycorhiziennes, notamment les racines proteoïdes que l'on retrouve chez des plantes de la famille des Proteaceae, Betulaceae, Leguminoseae, Myricaceae et autres. La diversité des stratégies d'acquisition de nutriments a été proposée comme mécanisme favorisant la diversité végétale au niveau taxonomique (Zemunik *et al.*, 2015; Lambers *et al.*, 2008). En effet, la combinaison de stratégies, ainsi que l'accès à des ressources nutritionnelles distinctes, pourraient réduire la compétition interspécifique et favoriser la diversité locale (Turner, 2008; Laliberté *et al.*, 2013). Il existerait même une complémentarité entre certaines stratégies, ce qui favoriserait la croissance d'espèces voisines utilisant des stratégies différentes et pourrait augmenter la diversité et la productivité globale du système (Teste *et al.*, 2014).

1.3.3 Importance des champignons du sol dans les milieux dunaires

Les fonctions remplies par les champignons peuvent prendre une importance particulière dans des milieux où les conditions abiotiques et les propriétés du sol limitent la croissance, voire menacent la survie des plantes (Maun, 2009). Dans les dunes côtières, des systèmes pauvres en eau et en nutriments (Lichter, 1998), on s'attend à ce que les traits des plantes ainsi que les symbiotes fongiques liés à l'acquisition de nutriments influencent significativement l'assemblage des communautés végétales (Lambers *et al.*, 2008). En effet, plusieurs études ont démontré l'importance des microorganismes du sol, et des champignons mycorhiziens plus particulièrement, dans les écosystèmes dunaires.

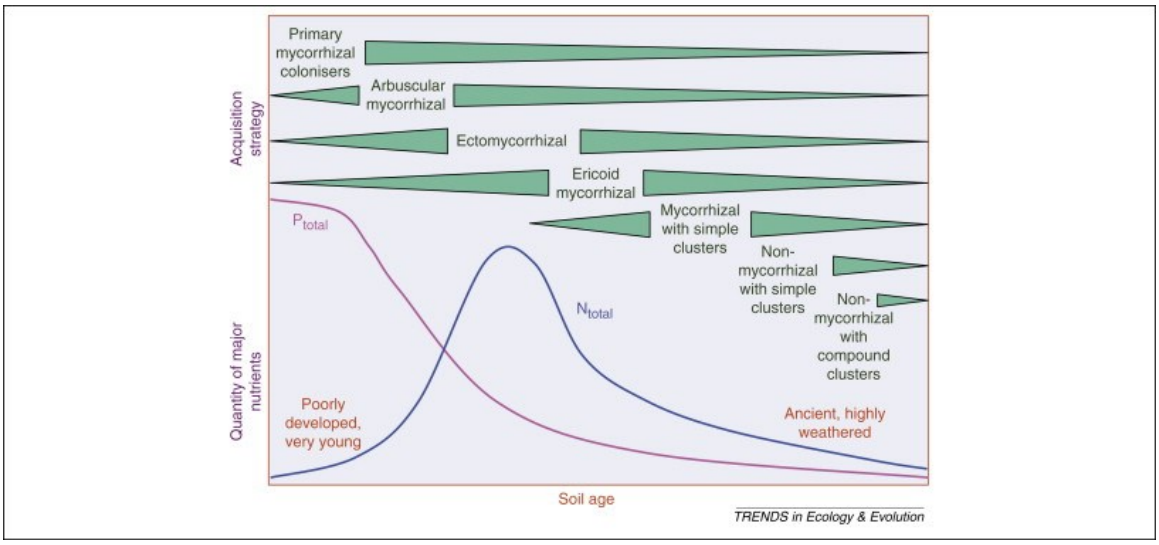

Figure 1.3. Changements dans la concentration de phosphore total (mauve) et d'azote (bleu), ainsi que dans l'abondance des différentes stratégies d'acquisition de nutriments en fonction de l'âge des sols. Tiré de Lambers *et al.* (2008).

Les premières études sur le sujet (Webley *et al.*, 1952 ; Brown, 1958) suggéraient déjà un rôle clé des microbes du sol pour la maturation de l'écosystème. Notamment, Webley *et al.* (1952) a observé une augmentation au niveau de l'abondance des bactéries et des champignons cultivables dès le début de la colonisation du sable par la végétation ainsi qu'un changement dans la structure des communautés microbiennes liés aux changements de végétation, suggérant une contribution importante de l'activité des microorganismes du sol à l'avancement de la succession végétale et à la maturation de l'habitat. Juliet Frankland

(Brown, 1958 ; Frankland, 1998) qui s'est intéressée à la succession fongique, a observé dans une chronoséquence dunaire en Angleterre des communautés de macro-champignons distinctes en fonction du stade de succession. La plus grande diversité fut observée dans les dunes semi-fixées, où les plantes pionnières et les communautés végétales typiques des stades successionnels plus tardifs se chevauchent.

De nombreuses études se sont consacrées spécifiquement à l'étude des CMA en utilisant principalement des méthodes traditionnelles (c'est-à-dire l'examen morphologique des spores et/ou la quantification de la colonisation des racines) et ont montré une présence importante de CMA en milieux dunaires (ex : Nicolson, 1959; Koske & Halvorson, 1981; Koske & Polson, 1984; Gemma *et al.*, 1989; Corkidi & Rincon, 1997; Koske, 1997; Błaszkowski & Czerniawska, 2011; Stürmer *et al.*, 2013). Plus précisément, un lien entre la performance des plants d'ammophile (*Ammophila breviligulata* Fern., la plante pionnière des dunes par excellence en Amérique du nord) et une activité élevée des CMA dans la rhizosphère a été établi dans plusieurs études indépendantes (Nicolson, 1959; Nicolson, 1960; Koske and Halvorson 1981; Koske and Polson, 1984; Koske 1997). De plus, l'inoculation avec des CMA sur le terrain a amélioré significativement les taux de survie et la croissance des plantules d'ammophile (Gemma *et al.,* 1989). Ces résultats suggèrent donc un rôle clé des CMA pour l'établissement, la survie et la croissance des plantes pionnières en milieux dunaires, particulièrement de l'ammophile, contribuant ainsi à la stabilisation des dunes. Kowalchuk *et al.* (2002) ont atteint des conclusions semblables, soit une relation significative entre la vigueur des peuplements d'ammophile et l'abondance et la diversité des CMA, en ayant recours à une méthode moléculaire (DGGE).

Bien que les dunes de sable aient été largement investiguées au niveau des CMA, très peu d'études se sont intéressées à l'ensemble de la diversité fongique. Or, tel que discuté précédemment, les interactions multiples et complexes opérant entre les plantes et les communautés fongiques, incluant différents modes de vie, sont déterminants de la diversité et de l'assemblage des communautés végétales et de la formation des sols. On prédit même que ces effets seraient plus importants dans des milieux subissant de fortes contraintes abiotiques comme la sécheresse et des sols pauvres en nutriments (Lambers *et al.*, 2008). De plus, les récentes avancées technologiques en matière de séquençage (voir boîte 1.1) offrent maintenant

la possibilité de caractérister les communautés microbiennes du sol d'une façon de plus en plus fiable, détaillée et rapide.

1.4 Objectifs de la thèse

Les plantes et les champignons présentent des interactions multiples et complexes, qui semblent prendre une importance particulière dans les milieux dunaires, où les organismes vivants font face à de fortes contraintes environnementales (sécheresse, salinité, sols pauvres en nutriments). Or, à ma connaissance, la diversité fongique n'avait encore jamais été recenssée de façon holistique, c'est-à-dire en s'intéressant à l'ensemble des modes de vie et des groupes taxonomiques, dans des dunes côtières et encore moins sur l'ensemble d'une plaine dunaire dans les différents stades de dévelopement. Cette thèse a donc pour objectif général l'acquisition d'une meilleure compréhension de l'écologie des sols et des interactions biologiques d'un écosystème dunaire côtier. Je m'intéresse plus précisément à la dynamique écologique des communautés de champignons dans les dunes afin d'observer le phénomène de succession au niveau des interactions fongiques et de décrire le lien entre les communautés de champignons, la végétation et les conditions abiotiques.
Plus précisément, cette thèse s'articule autour de trois objectifs :

1) Décrire la composition des communautés fongiques, leur diversité et leurs fonctions à travers un gradient de succession et de conditions édaphiques dans un écosystème dunaire côtier ;

2) Décrire les changements au niveau de la végétation et des principales stratégies d'acquisition de nutriments à travers le système de dune, et étudier le lien entre les communautés de champignons du sol et les communautés végétales ;

3) Décrire et comparer les communautés de champignons associées aux principales espèces arborescentes des dunes et étudier l'effet de l'hôte sur la diversité et la structure de la communauté microbienne qu'il supporte ;

Chacun de ces objectifs est traité dans un chapitre de cette thèse (chapitres 2, 3 et 4).

1.5 Sommaire de la méthodologie

1.5.1 Terrain d'étude

Les Îles de la Madeleine (47° 23′ N, 61° 52′ W) sont situées au cœur du golfe Saint-Laurent, entre la péninsule gaspésienne, Terre-Neuve, le Cap-Breton en Nouvelle-Écosse, l'Île-du-Prince-Édouard et l'Île d'Anticosti. L'archipel est constitué d'une douzaine d'îles, dont sept sont reliées par des cordons sableux. Le climat y est tempéré à forte influence maritime avec une température annuelle moyenne de 4.5°C et des précipitations annuelles totalisant 987 mm dont le tiers tombe sous forme de neige (Grandtner, 1967; Houle, 2008). Le territoire est continuellement balayé par des vents violents, facteur affectant de façon importante les sols et la végétation. Ces vents, plus intenses durant la saison hivernale, soufflent à une vitesse moyenne de 31 km/h avec des pointes pouvant dépasser les 100 km/h.

Les dunes de sable, qui couvrent environ 30% du territoire et représentent plus de 50% de la longueur de côte, sont d'une importance particulière aux Îles de la Madeleine où elles remplissent plusieurs fonctions importantes (Bernatchez *et al.*, 2008). Par exemple, ce sont les dunes qui relient les Îles entre elles et permettent la communication et le transport au sein de l'archipel. Elles forment aussi un écran protecteur réduisant l'érosion des côtes et prévenant l'ensablement et l'inondation des routes. De plus, les écosystèmes dunaires constituent un habitat essentiel à de nombreuses espèces végétales et animales, par exemple pour la nidification de plusieurs oiseaux migrateurs. Les dunes y abritent d'ailleurs plusieurs espèces menacées comme le pluvier siffleur (*Charadrius meloduset*), l'Aster du St-Laurent (*Symphyotrichum laurentianum*) et le Corème de Conrad (*Corema conradii*) selon la Loi sur les espèces menacées ou vulnérales du gouvernement du Québec[2].

Bordée d'un côté par la mer et de l'autre par la lagune, la série de dunes fossiles communément appelée Les Sillons (Figure 1.4) est une formation dunaire particulièrement intéressante du point de vue de l'histoire géologique et de l'évolution du paysage. Les Sillons couvrent une superficie d'environ 10 km^2 sur 7 km de long et comprennent une soixantaine de

[2] Pour la faune : http://www3.mffp.gouv.qc.ca; pour la flore : http://www.mddelcc.gouv.qc.ca.

crêtes parallèles qui correspondent à d'anciennes dunes bordières. Cette plaine de dunes reliques est la plus grande formation de ce type dans le Canada Atlantique (Giles and King 2001). On retrouve dans Les Sillons, en plus de la séquence de végétation typique des écosystèmes dunaires – c'est à dire plage, dune bordière, dune mobile, lande, puis forêt dunaire – une alternance de crêtes et de creux qui abritent respectivement des landes ou des forêts et des milieux humides. En effet, certains creux ont atteint un niveau inférieur à celui de la nappe phréatique, entraînant la formation de milieux humides comme des tourbières ou des marécages. On peut donc observer dans ce système de dunes une très grande diversité d'habitats. Cet écosystème a d'ailleurs intéressé des botanistes de renom comme Miroslav Grandtner (1967), ainsi que Gisèle Lamoureux (1977) qui a caractérisé la partie littorale de la flore des Sillons.

Figure 1.4. Vue sur la Dune du Sud et Les Sillons

Ce système de dunes s'est développé durant la période postglaciaire holocène. L'Inlandsis laurentien qui recouvrait une bonne partie de l'Amérique du nord, incluant les Îles de la Madeleine, lors de la dernière glaciation de 95 à 18-13 ka (milliers d'années avant notre ère) pouvait avoir jusqu'à 3 ou 4 km d'épaisseur (Dyke & Prest, 1987). La fonte progressive de l'Inlandsis a provoqué des augmentations successives du niveau de la mer, mais aussi une provision importante de sédiments (Giles and King 2001). Ainsi, ce système dunaire fut en progradation malgré l'augmentation du niveau de la mer, et ce grâce à cet apport sédimentaire significatif. Au fil du temps, la formation de nouvelles dunes parallèles à l'avant du système a entraîné la croissance de la plaine de dunes vers le large. Finalement, la plaine de dunes reliques des Sillons, de par son histoire géologique et la diversité des habitats qu'elle supporte, est un système écologiquement très intéressant. De plus, il présente plusieurs avantages pour

l'étude des dynamiques écologiques et de la succession, notamment la présence d'un gradient marqué sur une courte distance géographique, ce qui diminue la variation de facteurs confondants comme le climat.

1.5.2 Échantillonnage sur le terrain

Afin d'étudier les communautés de champignons associées aux différentes zones de la dune, une collecte de données sur le terrain fut réalisée sur les Sillons, aux Îles de la Madeleine, Québec, à l'été 2011. Dix points d'échantillonnage furent répartis aléatoirement dans chacune des zones de végétation suivant un gradient édaphique et successionnel, soit de la plage aux forêts, et aux milieux humides précédant la lagune. Un total de 80 sites, furent visités pour une saisie de données environnementales (type de sol, pente, élévation, etc.) et un recensement de la végétation. Des échantillons de sols et de racines furent également prélevés puis transportés au laboratoire pour des analyses de sol (pH, salinité et pourcentage de matière organique) et pour l'identification des champignons grâce au séquençage à haut débit. Un deuxième échantillonnage s'est concentré sur la zone forestière des dunes, où des échantillons de sols et de racines associés aux quatre principales espèces arborescentes furent prélevés.

1.5.3 Identification des champignons

En premier lieu, l'ADN génomique a été extrait à partir des échantillons de sol et de racines. La région ITS (internal transcribed spacer) située entre la grande et la petite sous-unité ribosomale a ensuite été amplifiés par réactions en chaîne polymérase (PCR) avec un couple d'amorces (ITS1F et ITS4) permettant l'amplification de la région ITS complète. Cette région englobe, en plus du gène intercalaire 5.8S, les deux zones très variables ITS1 et ITS2 et présente donc un degré de variation interspécifique pouvant permettre la différentiation des taxons fongiques. En utilisant des marqueurs ciblant les gènes ribosomaux adjacents (18S et 28S) qui sont bien conservés, il est possible d'amplifier la région ITS pour un large éventail de champignons (Lindahl *et al.*, 2013). Cette région est très fréquemment utilisée pour la caractérisation des communautés de champignons et est donc bien représentée dans les bases de données. Les PCR ont été réalisées avec des amorces fusionnées qui comprennent une séquence de nucléotides unique à chaque échantillon (communément appelée MID) et un adapteurs directionels nécessaires pour le pyroséquençage. Les amplicons ont ensuite été

purifiés, quantifiés, puis regroupés à des concentrations égales. Le volume a ensuite été corrigé par précipitation à l'éthanol afin d'atteindre la concentration requise, soit au moins 15 ng/ul, et enfin envoyé au centre d'innovation de Génome Québec (Montréal, Canada) pour le séquençage avec la plateforme 454 GS-FLX+ de Roche Life Sciences. Les séquences obtenues ont ensuite été traitées avec différents outils bio-informatiques afin de contrôler la qualité des séquences, de les regroupées en *Operational Taxonomic Units* (OTUs) et de leur attribuer une classification taxonomique. L'approche moléculaire ainsi que les analyses bioinformatiques utlilisées seront détaillées dans les chapitres suivants.

<u>Chapitre 2</u> | Taxonomie, diversité et modes de vie des communautés fongiques du sol à travers un gradient édaphique et successionnel dans un écosystème dunaire côtier

Pour commencer, nous avons voulu dresser un portrait d'ensemble des communautés de champignons du sol à travers les différentes zones des dunes. En plus d'une description taxonomique dérivée de marqueurs ITS, nous avons tenté de prédire les modes de vie fongiques selon les informations disponibles dans la littérature. Par cette approche, nous souhaitions mieux comprendre comment les variations au niveau des communautés de champignons du sol peuvent se traduire en changements fonctionnels.

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Comprehensive sampling of an isolated dune system demonstrates clear patterns in soil fungal communities across a successional gradient

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2.1 Abstract

Coastal sand dunes are extremely dynamic ecosystems, characterized by stark ecological succession gradients. Dune stabilization is mainly attributed to plant growth, but the establishment and survival of dune-inhabiting vegetation is closely linked to soil microorganisms and to the ecological functions they fulfill. Fungi are particularly important in this context, as some interact intimately with plant roots, while others are critical to soil structure and nutrient availability.

Our study aimed to wholly describe fungal diversity and community composition in a secluded coastal dune ecosystem at eight different stages of succession. We comprehensively sampled a relic foredune plain, which is part of an archipelago in the Gulf of Saint Lawrence (Québec, Canada), by collecting soils from 80 sites and measuring soil characteristics. Soil fungal communities were characterized by pyrosequencing, followed by taxonomic classification and assignment of putative roles. Even though we did not observe clear patterns in diversity, we were able to detect distinct taxonomic and community composition signatures across succession stages, which seemed to translate into variations in fungal life strategies. Our results show that a taxonomically and functionally diverse fungal community exists at each dune succession stage, even in the barren foredunes.

2.2 Keywords

454-sequencing / coastal sand dunes / fungal community / microbial diversity and functions / primary ecological succession / soil ecology

2.3 Introduction

Coastal dune ecosystems act as transition zones between marine and continental environments, and are globally distributed (Martínez & Psuty, 2008). Dune ecosystems are present on most major coastlines, and often reduce coastal erosion and protect littoral zones by providing a barrier against wave and wind action (Maun, 2009). They also contribute to water filtering, groundwater replenishment, maintenance of biodiversity, and are of cultural,

aesthetic, and recreational importance (Everard *et al.*, 2010). These services, however, depend on the integrity of the dune ecosystem, and on the ecological interactions that take place within it.

Both fungi and bacteria perform crucial ecological functions in nearly barren habitats such as sand dunes, including nitrogen fixation, decomposition of organic matter into compounds that are easily assimilated by plants, and rock degradation, which releases essential nutrients such as phosphorus (Landeweert *et al.*, 2001; Van Der Heijden *et al.*, 2008). Fungi have particularly important roles in decomposition and in affecting plant nutrition and diversity (Van Der Heijden *et al.*, 2008). Within the estimated 1.5×10^6 fungal species on Earth (Hawksworth, 2001), there are many known lifestyles that both directly and indirectly impact plant survival. For instance, mycorrhizal fungi are ubiquitous plant partners, and increase plant nutrient absorption while protecting them against pathogen attacks and abiotic stresses (Read, 1992; Smith & Read, 2008). Saprotrophic fungi release a range of enzymes into soils, contributing to organic matter decomposition and nutrient cycling (Baldrian *et al.*, 2011), and converting nutrients into forms that are available to plants. Some fungi are parasites of plants and/or animals, while others are nematophagous, and may alter the impact of pathogenic nematode populations on plant performance. The combined activities of these functional guilds ultimately determine the suitability of soil habitats for ecosystem development.

In coastal dunes, the sequence of plant communities along the spatial gradient extending from upper beaches to fixed dunes is often used to study plant succession. The foredune is colonized by pioneer species with dense root systems, allowing dune stabilization, and facilitating the colonisation of other herbs, shrubs, and eventually, trees. Yet, in these extreme environments, plant survival, growth, and reproduction are greatly limited by environmental factors such as wind, salinity, and drought. As a result, the development of pioneer vegetation is strongly linked to the presence of soil microorganisms, and fungi in particular, since these microbes help to offset the negative impacts of environmental constraints (Koske & Polson, 1984; Maun, 2009).

Îles de la Madeleine is an archipelago in the Gulf of St Lawrence in Québec, Canada, containing different successional stages, which are each represented by characteristic plant communities, over a short spatial gradient (generally < 1 km). The simplicity of island

ecological systems and the compacted succession gradients of dunes make each a model system for ecological studies (Lichter, 1998a; Vitousek, 2002), and this system possesses both, reducing the confounding effects of geography and complex biotic interactions. The functional importance of fungi in coastal dunes is not fully understood, but the positive effect of some fungal groups – mainly mycorrhizal taxa – on plant establishment, survival, and growth, has been clearly demonstrated (e.g. Corkidi & Rincón, 1997; Gemma & Koske, 1997; reviewed in Read, 1989). In this study, we used pyrosequencing of the fungal ITS region to comprehensively describe soil fungal composition and diversity across eight zones of an isolated foredune plain of *ca*. 10 km^2 in the Îles de la Madeleine archipelago. Based on previous research on soil microbial populations in forested ecosystems (Gao *et al.*, 2015) and in successional habitats such as glacier retreats (Jumpponen, 2003; Blaalid *et al.*, 2012; Brown and Jumpponen, 2013), we expected to observe important shifts in fungal community composition and in soil abiotic parameters across successional stages. In addition to shifts in taxonomy, we estimated shifts in functional guilds of fungi across dune zones by assigning our fungal genera to the most probable functional roles based on the literature, and on existing databases. While the functional capacity of soil bacterial communities has been estimated from 16S rRNA gene data (Langille *et al.*, 2013; Barberán *et al.*, 2014), this type of approach has only been applied to fungi in a few recent studies (e.g. Clemmensen *et al.*, 2013; Tedersoo *et al.*, 2014; Clemmensen *et al.*, 2015). We aimed to determine whether functional assignments of fungal taxa would reflect the taxonomic patterns we observed, or even strengthen them.

2.4 Results and discussion

2.4.1 Study site description and soil properties

Our unique study site is an isolated coastal dune system known as *Les Sillons*, and was selected because of the large habitat diversity that it supports, as described by Grandtner (1967). In this system, it is possible to observe a complete ecological succession sequence, from the beach to a relic foredune plain, including forested and wetland habitats (Figure 1.2).

The study site comprised eight different zones belonging to three larger regions: the coastal region encompassing the 1) pioneer zone, 2) front of the foredune, 3) back of the foredune and 4) mobile dune; the forested region including the 5) heathland and 6) forest dune, and the humid region with the 7) inter-ridge swale, and 8) lagoon wetland. The coastal region consists of younger sites located close to the shore, which are characterized by dry sandy soils and a plant community that is largely dominated by *Ammophila breviligulata* Fern., the American beachgrass, as well as a few herbaceous plants such as *Artemisia stellaria* and *Festuca rubra* in areas that are not directly exposed to wind and salt spray. Heathlands are dry habitats, dominated by shrubs such as *Myrica pensylvanica*, *Juniperus communis,* and *Spiraea alba,* and these transition into black spruce, fir, and pine forests. The humid region encompasses the inter-ridge depressions and the wetlands that are located close to the lagoon, and harbour an extremely diverse array of vegetation, including several *Ericaceae*, *Sphagnum*, and *Carex* species. The dominant plant species for each zone are listed in Table S2.1. In terms of environmental variables, we observed variations in elevation, slope and soil type at sampling sites from different zones (Table S2.2). Physiochemistry also shifted substantially across the dune succession gradient, with significant increases in organic carbon, total nitrogen, and bio-available phosphorus at later successional stages, as well as decreasing trends in pH and conductivity (Tables S2.3).

2.4.2 Fungal diversity

After sequence processing and quality filtering, we obtained 233,037 ITS reads that were clustered into a total of 3406 97% OTUs (after excluding singletons). Reads per sample ranged from 449 to 6556, but rarefaction curves were always close to saturation (Supplemental Figure S2.1a-c). In support of this, the mean Good's coverage was 0.98 for all three zones. The sampling effort curve (Supplemental Figure S2.1d) shows that the number of samples treated (77) allowed us to capture over 75% (3406 OTUs) of the estimated total diversity (4492 OTUs).

Although one might expect to find few co-existing fungal species in the nutrient-poor, water-limited, and windy conditions of sand dunes, diverse bacterial and fungal communities have been observed in these environments (Webley *et al,.* 1952) and other arid regions (e.g. Fierer and Jackson, 2006; Fukami *et al.*, 2010; Schmidt *et al.*, 2014; reviewed in Zak, 2005).

We expected to see an increase in soil fungal richness with habitat development, soil complexity, and diversification of vegetation (Van Der Heijden *et al.*, 2008; Blaalid *et al.*,

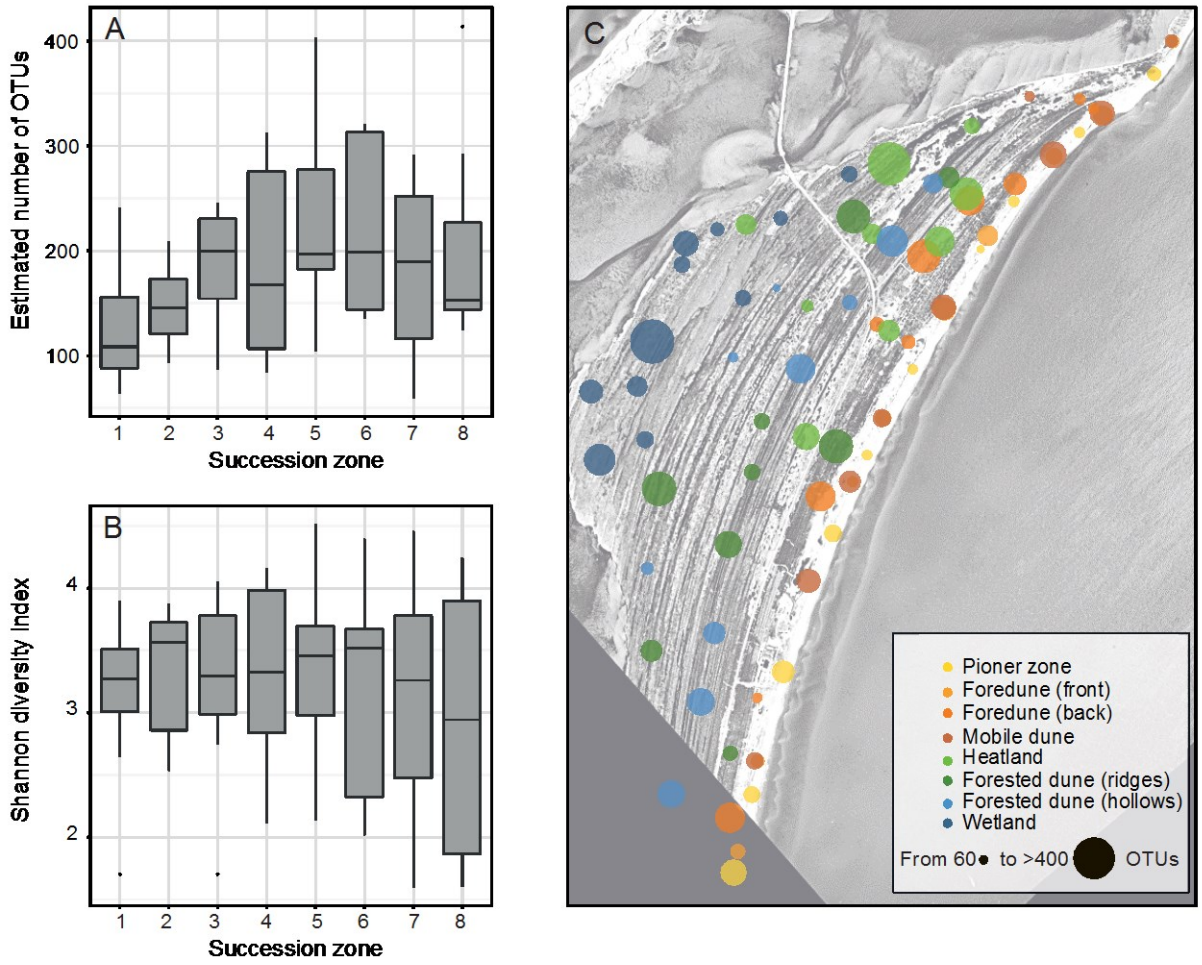

Figure 2.1. Soil fungal diversity across succession stages. Diversity measures are based on rarefaction analysis with the Chao richness estimator (a and c) and on the Shannon diversity index (b). The size of the circles on the map (c) is proportional to the number of estimated OTUs at each sampling point.

2012; Zumsteg *et al.*, 2012). Brown (1958) investigated fungal diversity in coastal dunes of the UK by cultivation and isolation of fungi from sand samples, and found the greatest fungal diversity in the semi-fixed dune, where pioneer and late successional plants overlapped (reviewed in Frankland 1998). In this study, richness ranged from 54 (in the pioneer zone) to 329 (in the lagoon wetland), with an average of 150 OTUs per sample, and ANOVA did not

reveal significant differences in either the variance or means of the Chao richness estimator or the Shannon diversity index across the succession gradient. There was no clear pattern in OTU richness, but the highest mean number of OTUs was recorded in the heathland and forested dune zones (Figure 2.1).

2.4.3 Fungal community structure and links to environmental variables

Redundancy analysis indicated that fungal community composition varied across succession zones (Figure 2.2a), with the objects (sites) clearly segregating into three groups that were fairly consistent within the three regions. The cluster located at the right of the graph consisted mainly of the younger sites of the coastal region. The sites located at the left of the ordination correspond to sites from the more advanced succession zones and the wetland lagoon, the upper part primarily including the drier sites of the forested region, and the lower portion the humid region. In fact, PERMANOVA revealed a significant grouping of sites with respect to the three regions as well as to the eight succession zones (both *P*-values= 0.0001, R^2 of 0.083 and 0.079, respectively). The pairwise comparisons indicated significant groupings for all pairs of regions and succession zones at a significance level of $P \leq 0.05$ (adjusted *P*-values), with the exception of the back foredune, which was not significantly different from the front foredune or the mobile dune. The canonical axes explained 9% and 5% of the response data variance, for a total R^2 of 0.356, and of 0.141 when adjusted for the number of observations and degrees of freedom. The F-statistic ($F_{7,69} = 1.654$) was significant ($P= 0.001$). Despite the clear groupings, compositional shifts were not easily explained by environmental descriptors alone. Of all explanatory variables included in the model, only total nitrogen (F= 1.388, $P= 0.024$) and water content ($F_{7,69} = 1.34$, $P= 0.041$) significantly influenced fungal composition, and total phosphorus ($F_{7,69} = 1.22$, $P= 0.082$) had a nearly significant influence (at the 0.05 level). This suggests that soil properties alone did not have an overwhelming role in shaping soil fungal communities. Unlike bacteria, which appear less limited by dispersal and are shaped heavily by soil parameters, fungi may be somewhat disconnected from the abiotic environment (Schmidt, *et al.*, 2014). For example, correlations to soil pH, as has so often been shown for bacteria (Fierer and Jackson, 2006), are less clear for fungi, possibly because they have wider pH optima (Rousk *et al.*, 2010). Although changes in the community structure of some fungal groups (e.g. EM fungi) have been attributed to shifts in pH, soil

moisture, and nitrogen availability (Erland & Taylor, 2003), the factors determining soil fungal communities remain poorly understood, especially in natural and undisturbed environments (Van Der Heijden & Sanders, 2002). Our results could suggest, as previously supported in microbial studies of glacier retreats (Jumpponen, 2003; Blaalid *et al.*, 2012; Brown & Jumpponen, 2013; Schmidt *et al.*, 2014), that fungal communities assemble in a less deterministic way than bacteria, and that they might be more sensitive to stochastic processes such as history and dispersal limitation.

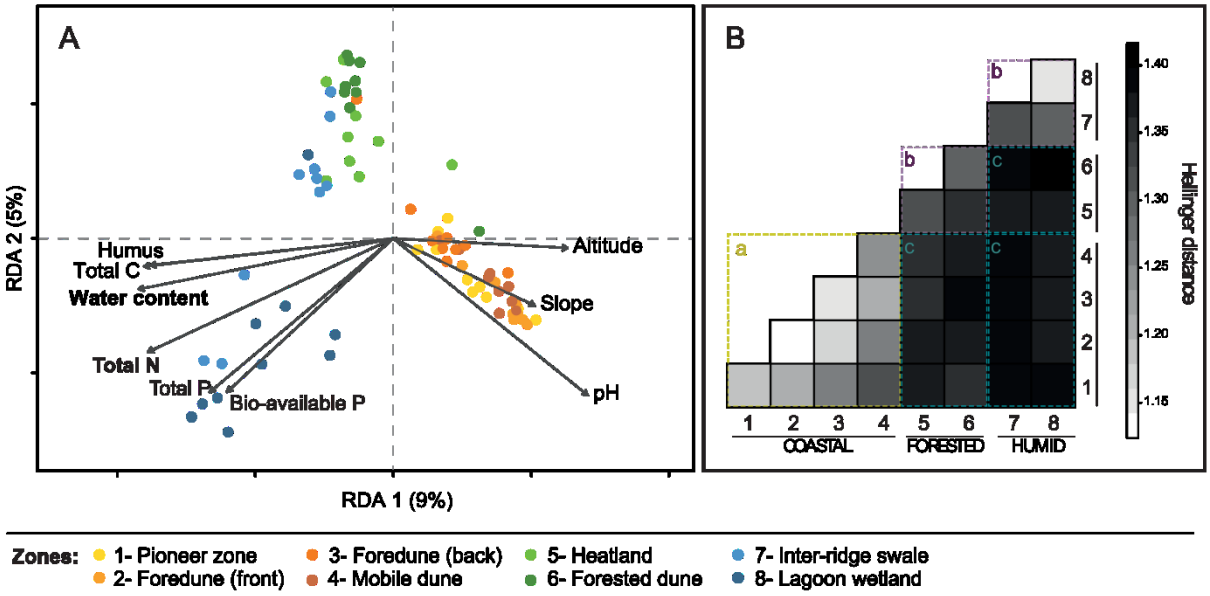

Figure 2.2. (a) **tb-RDA biplot of the fungal communities in relation to soil properties and site topography** using the Hellinger distance and displaying the Hellinger distance in terms of OTU composition using soil properties. (b) **Heatmap displaying interplot variability as the mean Hellinger distance within and between succession zones.** Different letters indicate significant differences between pairs of dune regions (for $P \leq 0.05$).

In comparison to bacteria, fungi, as exclusively heterotrophic organisms, are more dependent on the presence of organic matter, or on living organisms in the case of obligate parasites or symbionts. Vegetation could therefore play a key role in shaping soil fungal communities in this dune system. A more substantial shift in the composition of fungal communities than bacterial communities following plant host introduction, and based on plant identity, was already described in contaminated soils (Bell *et al.*, 2014). Because of the specificity of root exudates and litter quality, plant communities have been acknowledged as

important drivers of soil microbial community structure (Edwards *et al.*, 2006; Miniaci *et al.*, 2007). In fact, we found that plant species composition did explain a substantial proportion of the variation in fungal community data in this study that was left unexplained by soil parameters (unpublished data).

Visual examination of inter-plot variability of fungal communities within and between succession zones (Figure 2.2b) revealed that community structure was much more similar within the primary succession zones, and indicated increasing fungal community divergence at later succession stages. An ANOVA revealed highly significant differences in inter-plot distance ($P \leq 0.001$) across the different dune successional zones and regions. The post-hoc Tukey HSD test performed between pairs of regions confirmed that community structure is more variable in the latter succession stages. Inter-plot variability was lowest within the coastal region, and highest within the humid region and between the forested and humid regions. We also observed distinct fungal taxonomic signatures in each of the designated dune zones, with substantial variation in the relative abundance of most major fungal groups (Figure S2.2). For example, the relative abundance of *Glomeromycota* (in terms of number of reads) was higher in the coastal region, chytrids were found only in the coastal and humid regions, and the *Basidiomycota* (especially the *Agaricomycetes*) were more abundant in the heathland and dune forest.

2.4.4 Functional guilds and indicator taxa

Although we have learned a great deal from high-throughput taxonomic studies of microbial communities, we need to better understand how these shifts relate to ecosystem functioning. One approach is to correlate microbial taxa with measures of ecosystem productivity, while another is to project functions from previous data annotations. Metagenomic estimates of the functional diversity in bacterial communities rely heavily on such methods, as tools such as MG-RAST assign gene sequences to known functional categories (e.g. nitrogen fixation). While powerful and informative, metagenomics (which produces detailed functional and metabolic profiles by sequencing the vast majority of abundant genes in a community) requires deep sequencing and substantial analysis, and is still prohibitive due to its cost. The computational capacity required to assemble such datasets may limit its application to complex natural soil systems. By contrast, taxonomic profiles can

sometimes be used to reliably predict major shifts in soil microbial functional potential with minimal financial investment, allowing processing of many more samples, and the major functional abilities of bacterial communities can be predicted from 16S rRNA marker

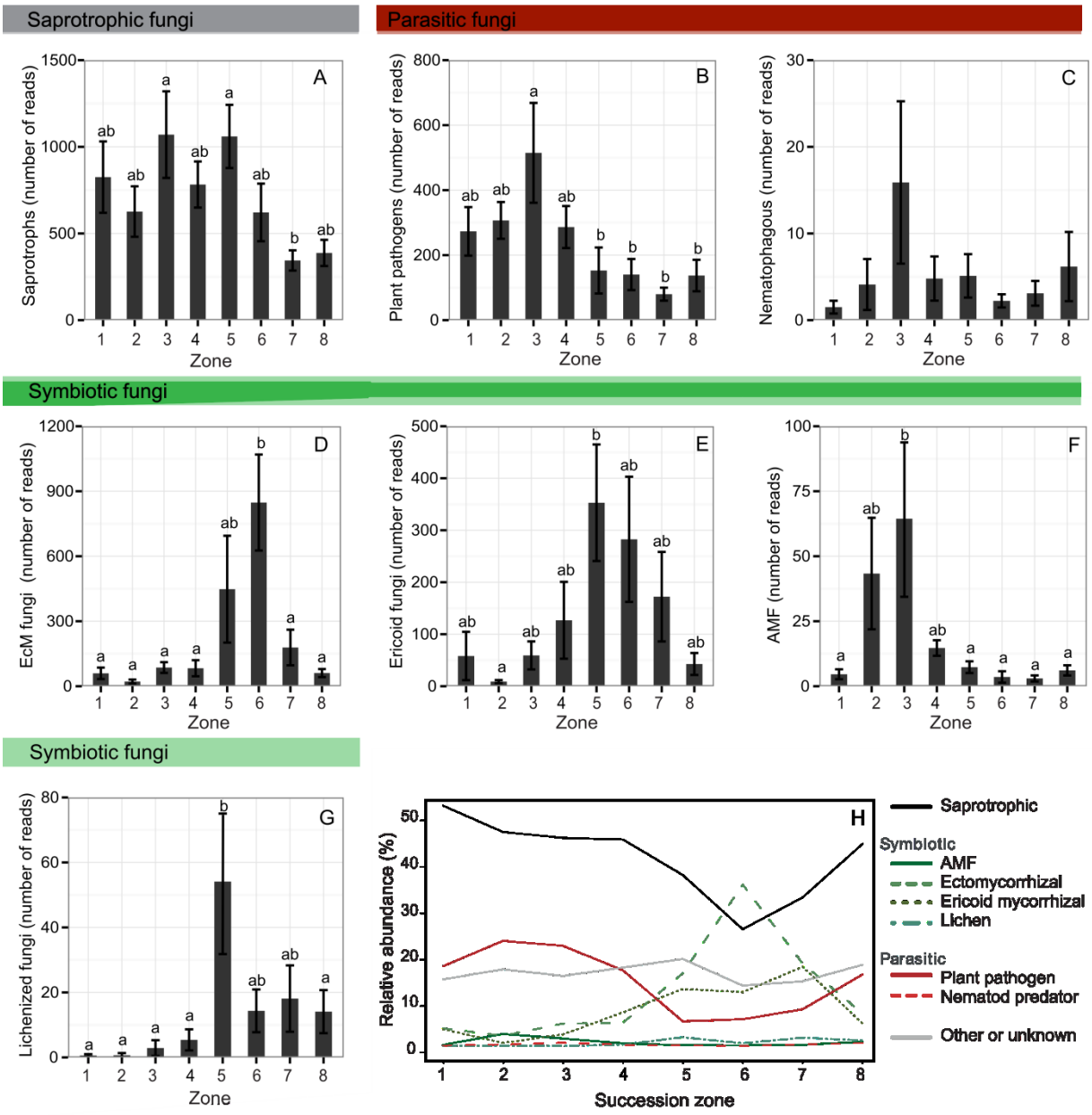

Figure 2.3. Changes in fungal life strategies across dune succession stages. Panels A-G show mean and standard error (n = 10) of the number of reads found in samples for each functional guild. Different lower case letters indicate indicate significant differences between

means as indicated by Tukey HSD post hoc tests ($P \leq 0.05$). H combines the relative abundance of all guilds.

sequences in various environments such as soil or the gut microbiome (Langille *et al.*, 2013; Barberán *et al.*, 2014). In addition, fungal reads are severely undersampled in metagenomes, due to the overwhelming dominance of prokaryotic genes. For example, gene annotation of soil metagenomes from the site of a former petrochemical plant showed that over 95% of annotated genes were prokaryotic, while less than 2% were fungal genes (Yves Terrat, unpublished data) and examination of public MG-RAST metagenomes from various soil environments reveals similar trends.

The ecological roles of many fungal genera have been described in the literature, and some ecological guilds are even catalogued in publically available databases (e.g. unite.ut.ee/EcM_lineages.php). Here we have projected fungal ecological roles from ITS-derived taxonomic information, as was done by Clemmensen *et al.* (2013; 2015) in boreal forests and Tedersoo *et al.* (2014) at a global scale. We aimed to determine how changes in fungal communities across a dune gradient are likely to correspond to changes in ecosystem function. By putatively assigning functions to each classified fungal genus based on the best-available literature, we found clear patterns in fungal lifestyles across dune zones (Figure 2.3). For example, the relative number of reads assigned to ectomycorrhizal (EM) and ericoid mycorrhizal fungi was higher in the forested region, while plant pathogens displayed the reverse pattern. Variations in the abundance of EM and ericoid fungi generally followed shifts in their preferred hosts. EM fungi are associated with a wide range of trees and shrubs, while ericoid mycorrhizal fungi form symbioses with the roots of plants from the *Ericaceae* family. All of these plants are found more frequently in the forested area of the dune, while the *Ericaceae* were also more abundant in the humid zone. By contrast, the arbuscular mycorrhizal fungi (AMF) reach their maximum relative abundance in the coastal region. The presence of AMF in the foredune of coastal dunes is well documented in the literature, and patterns similar to what we observed have been described in other studies (e.g. Koske & Halvorson, 1981; Gemma & Koske, 1997; Kowalchuk *et al.*, 2002). The decline in AMF abundance after the foredune, and the subsequent increase in EM and ericoid fungi correspond to the pattern described by Read (1989) and by Lambers *et al.* (2008) in his review of

variations in plant nutrient-acquisition strategies with soil age. Microbial patterns indeed reflect soil age, habitat maturation, and soil nutrient status. Very few nematode predator genera were present, and

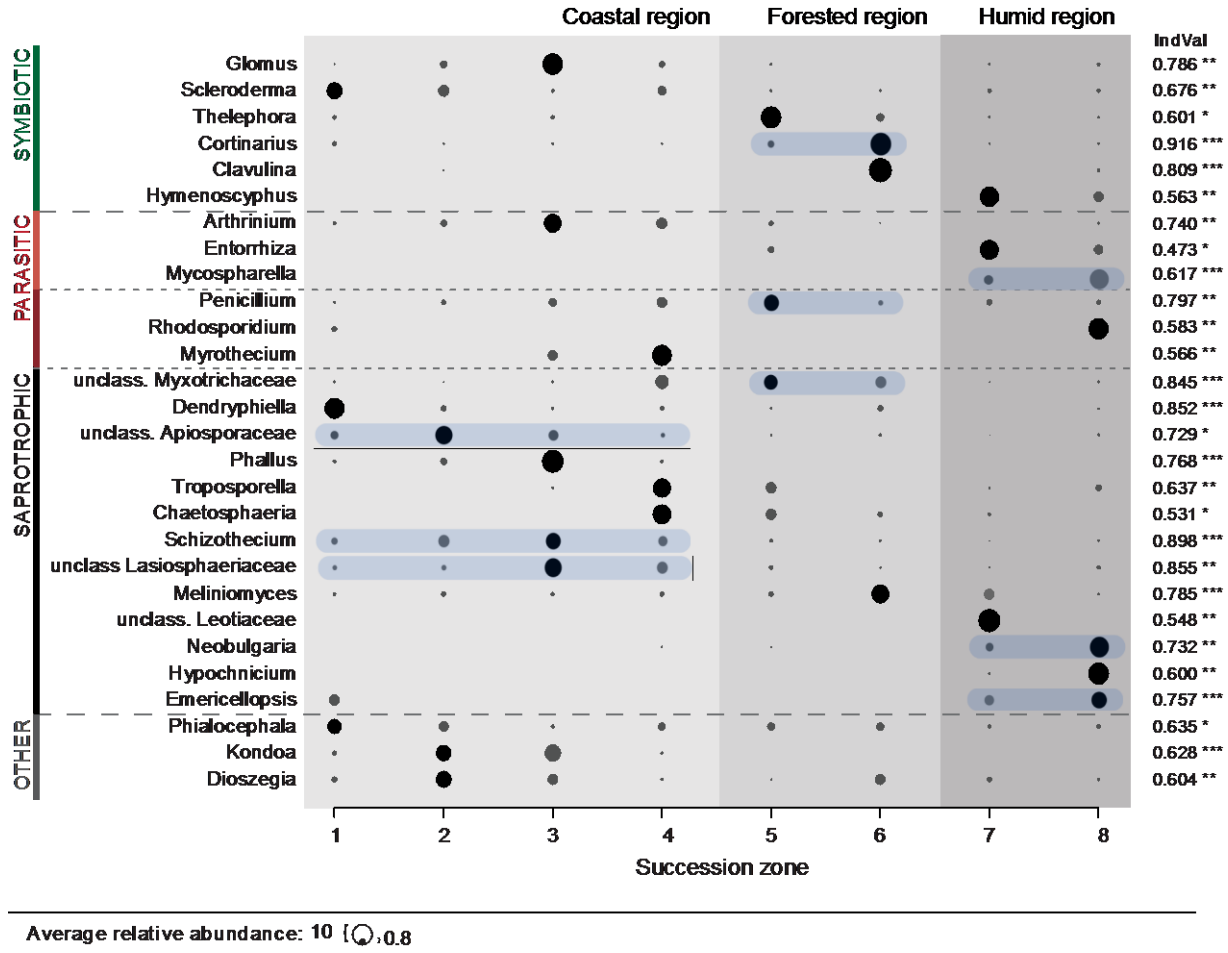

Figure 2.4. Bubble plot of the top indicator genera grouped by lifestyle for each dune succession zone and region. The size of the bubble reflects the average relative abundance (%) of each genus in a given succession stage in terms of number of reads. Black circles indicate the zone for which a given genus is an indicator, and the blue rectangles mark indicators of a dune region. Indicator values and significance level (*$P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$) are displayed on the right side of the plot.

there was no clear pattern in their relative abundance in terms of number of reads. Lichenized fungi were also uncommon, but peaked in the forested zone. Saprotrophy appears to be by far the dominant fungal lifestyle in almost all dune zones. Since we did not quantify fungal DNA with qPCR, it is not possible to draw conclusions about absolute fungal abundance, but our

approach does indicate which functional groups are likely to be most important at each successional stage. Analysis of the mean number of genera for each functional guild displayed very similar patterns (Supplemental Figure S2.3).

We also conducted an indicator species analysis and selected the best indicator genera (top three according to indicator value) for sites clustered into the eight designated zones and into the three regions. We calculated their relative abundance, and averaged these over each succession zone (Figure 2.4). All selected genera have high indicator values (ranging from 0.473 to 0.916), and these values were statistically significant, with a maximum *P*-value of 0.05. Within the selected genera, most ectomycorrhizal symbionts (*Thelephora*, *Cortinarius* and *Clavulina*) were indicators of sites in the forested region. *Glomus,* a common genus of AMF, had a high relative abundance in the coastal zone, particularly in the foredune area. *Hymenoscyphus*, an indicator of the inter-ridge swale, is a diverse genus within the *Ascomycota* that contains species that are known ectomycorrhizal and ericoid symbionts, and others that are known plant pathogens and saprobes. *Entorrhiza*, a genus of *Basiodiomycota*, and a known parasite of plants in the *Juncaceae* and *Cyperaceae* families (Cannon, 2007), was identified as an indicator taxa of the inter-ridge swale. Overall, most indicator genera were saprotrophs, but some, like the oleaginous red yeast *Rhodosporidium* (Li *et al.*, 2007)*,* can also act as parasites. *Kondoa* and *Dioszega* are two genera of yeast within the *Basidiomycota*, and were indicators of the pioneer zone. *Dioszega* has previously been isolated from soils, plant leaves, and roots, and may be interacting with AMF in soils (Takashima *et al.*, 2001; Renker *et al.*, 2004; Conell *et al.*, 2010).

The fact that many EM fungi are facultative saprotrophs could explain the presence of EM genera (e.g. *Scleroderma*) in the coastal region, as well as the concomitant decline in saprotrophic fungi (Figure 2.3 and Figure 2.4) in the forested region (Tedersoo *et al.*, 2003; Cullings & Courty, 2009). Through resource competition, EM fungi may replace certain decomposers in the forested region (Leake *et al.*, 2003). Interestingly, a co-dominance of saprotrophic and EM fungi, as we observed in the dune forest, was described previously in other forested ecosystems (Leake *et al.*, 2003). The decline in plant pathogens observed in the forested region can likely be explained by antagonistic interactions with EM fungi (Figure 2.3), as has been reported in other studies (e.g. Leake, *et al.* 2003; Xu *et al.*, 2012).

2.4.5 Conclusion

This study, as the first comprehensive ITS analysis of a dune ecosystem, revealed several interesting patterns in fungal community structure and the relative abundance of functional groups. We demonstrated the presence of a taxonomically and functionally diverse community across the dune sequence, including in the barren foredunes. The variations we observed in soil fungal community structure indicate the presence of fungal succession in both taxonomy and lifestyle in this sand dune ecosystem. As this system appears to be representative of other coastal sand dunes in terms of soil and environmental properties, it should provide reliable insights into the soil fungal dynamics in sand dune ecosystems. Direct testing of fungal metabolic potential and functional abilities, with measurements of enzymatic activities for example, will be required to explicitly demonstrate the functional importance of fungi.

2.5 Acknowledgments

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) to MH and by a doctoral grant from the Fonds québécois de la recherche sur la nature et les technologies (FQRNT) to ARB. Additional funding was provided by the Quebec Centre for Biodiversity Science and the Consortium en Foresterie Gaspésie-Les Îles. We would like to thank Sandrine Papageorges for her contribution to the field sampling, Drs Yves Terrat and Sébastien Halary for their assistance with data analysis, and Dr Pierre Legendre for his advice on statistical analyses.

2.6 Supplementary information

Appendix S2.1. Experimental procedures

Study site and field sampling

In order to describe the fungal communities associated with different sand dune successional stages, we performed an extensive field sampling of a coastal dune ecosystem within the Îles de la Madeleine archipelago, in Québec, Canada (47° 23′ 0″ N, 61° 52′ 0″ W). Using aerial photos and existing vegetation maps, the study site was divided into eight zones (see main text for detailed description). We used a stratified sampling design by randomly selecting ten sampling points from each zone for environmental data collection, vegetation surveys, and soil sampling within 1 x 1 m plots. Slope, soil texture, and organic layer thickness were determined at each sampling site (Table S2.2). In each plot, soil samples were composed of a mix of six soil cores from 0-25 cm depth that were collected randomly within the plot. The locations of the soil cores were determined by dividing the plot into a 10x10 grid and using a random number table to select the six cells to sample. The gravimetric water content was calculated as a ratio between the mass of water (weight of wet soil – weight of dry soil) and the dry weight of the sample. Conductivity, pH, soluble phosphorus (extracted by Mehlich-3), total phosphorus, carbon, and nitrogen were measured using an air-dried portion of the soil samples. Conductivity measures for older succession stages (5-8) were removed from the dataset since the presence of suspended particles in the soil solution caused excessively high reads, leading to an overestimation of salinity for all samples containing organic matter. Soil properties are summarized in Table S2.3. Approximately 15 mL of soil from each sample was conserved at -4°C until molecular analysis.

Molecular analysis

Total genomic DNA was extracted from 250-300 mg of soil material using the PowerSoil™ DNA Isolation Kit (MO-BIO Laboratories, Carlsbad, CA) following manufacturer's instructions with a few modifications: 3 x 20 sec cycles of homogenization with the FP120 FastPrep at speed 4 following the addition of the C1 solution, a 10 minute

incubation at 65°C before the first centrifugation, and incubation with the C2 solution at -20 °C instead of 4 °C.

We used the primers ITS1F and ITS4 (White *et al.*, 1990; Gardes and Bruns, 1993) to amplify the fungal ribosomal internal transcribed spacer (ITS) region, which includes two highly variable spacers (ITS1 and ITS2) and the intercalary 5.8S gene. The directional GS FLX Titanium adaptors A and B (including a four-base library key sequence) were attached at the 5' end of the primers. In addition, a unique 12-bp Multiplex Identifier (MID) was added between the library key and the template-specific sequence of the forward primer to allow sequences to be assigned to samples. PCR reactions were carried out in a volume of 20 µl in triplicate using 0.5 U of Qiagen Taq DNA Polymerase (Qiagen, Toronto, ON), 1X of the manufacturer's reaction buffer, 0.275 µM of each primer and dNTPs, a final concentration of 2.75 µM MgCl2, and 0.83 µL each of 1% Tween 20, DMSO and BSA, as well as 2 µL of diluted DNA (1:10). The cycling conditions were 94 °C for 5 min, followed by 32 cycles of 94 °C for 45 sec, 55 °C for 35 sec, and 72 °C for 1 min, and a final elongation of 72 °C for 7 min. The triplicates were pooled, purified with the NucleoMag 96 PCR clean-up kit (Macherey-Nagel, D-Mark Biosciences, Toronto, ON), and quantified using the Qubit® 2.0 Fluorometer (Life Technologies, Burlington, ON). An equal amount of amplified DNA from each sample was combined into a single pool, which was sent for pyrosequencing using Roche 454 GS-FLX+ chemistry at the Genome Quebec Innovation Center (McGill University, Montreal, QC).

Sequence processing

Sequences were pre-processed in Mothur v.1.29.2 (Schloss *et al.*, 2009) and then imported into Qiime (Caporaso *et al.*, 2010) for quality filtering. Low quality ends were trimmed using a moving window of 50 bp with a minimum quality score of 25. Reads shorter than 200 bp, longer than 1000 bp, with more than two ambiguities (Ns), with homopolymers longer than 8 bp, or with two or more mismatches in the primer or barcode sequences were eliminated. Reads were pruned to a fixed length (300 bp) with Mothur in order to avoid the partial coverage problem, which can result in erroneous or low-quality consensus sequences (Edgar, 2014). Usearch v7.0 (Edgar, 2010) was used for chimera checking and clustering sequences into Operational Taxonomic Units (OTUs) using 97% similarity. Singletons (i.e.

OTUs represented by only one read) were removed from analysis to avoid any artifacts that could be attributed to sequencing errors (Tedersoo *et al.*, 2010). Reads were mapped back into an OTU table, and all global singletons were eliminated. Taxonomy was assigned to each OTU consensus sequence using the UNITE database (Kõljalg *et al.*, 2013) in Mothur, which provides a Naïve-Bayes classifier, with a minimum bootstrap value of 60%. Raw sequence data were deposited in the NCBI Sequence Read Archive (Project number PRJNA269650).

Data analysis

All statistical analyses were conducted in R v.3.0.2 (R Foundation for Statistical Computing; available at http://www.R-project.org) and in Mothur. We first examined environmental variables and soil chemical properties by computing means and standard deviations, and after testing for normality (Shapiro-Wilkes, $P \leq 0.05$), we performed ANOVA and Tukey HSD post-hoc tests using the 'aov' and 'TukeyHSD' functions of the 'stats' package to identify significant differences between dune zones. To examine patterns in fungal diversity, we conducted OTU rarefaction analysis for individual samples. Chao total richness estimators (Chao, 1984), Shannon diversity index, and Good's coverage (Good, 1953) were all computed using the 'rarefaction.single' command in Mothur with 1000 iterations of re-sampling without replacement. A sampling effort curve showing the number of OTUs recorded in relation to the number of soil samples examined was computed in R with the 'iNEXT' package (Colwell *et al.*, 2012; Hsieh, 2013). Fungal diversity was compared across succession zones using One-Way ANOVA and Tukey HSD post-hoc tests. We also compared communities in terms of taxonomy by computing the relative abundance of major fungal classes.

We then assigned fungal genera to putative functional guilds based on best-available knowledge in the literature. Each classified genus was assigned to one of the following lifestyles: saprophytic, symbiotic (either arbuscular mycorrhizal, ectomycorrhizal, ericoid mycorrhiza, or lichenized fungi), or parasitic (plant pathogens or nematode predators). The complete classification as well as the literature consulted is provided in Annexe 1. We successfully classified 393 of the 409 fungal genera, which corresponded to 95.96% of reads.
After exploring general patterns in the diversity, lifestyle, and taxonomy of fungal communities, we used a canonical analysis to obtain an environmental interpretation of the

biological variation that we observed. A transformation-based redundancy analysis was chosen to assess the influence of soil and topographic variables on fungal community assemblage while preserving the Hellinger distance using the 'vegan' library (Oksanen *et al.*, 2013). The explanatory variables used and the transformations that we applied are described in Table S2.4.

The redundancy statistic (R^2) was computed and adjusted to the number of observations and the degrees of freedom. Its significance, as well as the significance of the relationship between the fungal community composition and the explanatory variables, was tested by permutation (999 permutations). The results of the RDA were represented in a biplot using scaling type 2 in order to preserve the distance between sites. A permutational analysis of variance (PERMANOVA) was conducted with 9999 permutations in order to test the significance of sites cluster according to their region and succession zone. Pairwise comparisons were computed and the *P*-values were adjusted for multiple comparison using the Holm correction with the 'p.adjust' function. The mean Hellinger distance (distance based on fungal community composition) between sites was computed for all pairs of succession zones, and was represented using a heatmap. ANOVA and Tukey HSD post-hoc tests were used to detect significant differences in interplot variability. Finally, in order to determine which classified fungal genera (using only OTUs which had been classified to at least the family level) best characterized the different dune zones and regions, we used indicator species analysis in the 'indicspecies' package in R (Cáceres and Legendre, 2009). Indicator species values provide a measure of how specialized or widespread a genus is within a given group of sites, independent of relative abundances. The indicator value calculation is based on specificity (the probability that the species is found only in a given group of sites) and fidelity (the probability that the species is found in all sites of a given group of sites) and yields a value between 0 and 1, with 1 describing a perfect indicator species. This analysis is particularly well suited to the study of species-habitat interactions (Dufrêne and Legendre, 1997; Cáceres and Legendre, 2009). The indicator values were group-equalized and their statistical significance was tested by a randomization procedure with 999 permutations.

Figure S2.1. Rarefaction curves of OTUs in each soil sample of the coastal (A), forested (B) and wetland (C) regions against the number of 454 reads excluding singletons. (D) Sampling effort as the number of OTUs against the total number of soil sample, the dashed line showing total richness extrapolation and the shaded areas representing the 95% confidence interval. Both analyses are based on 1000 iterations of re-sampling without replacement.

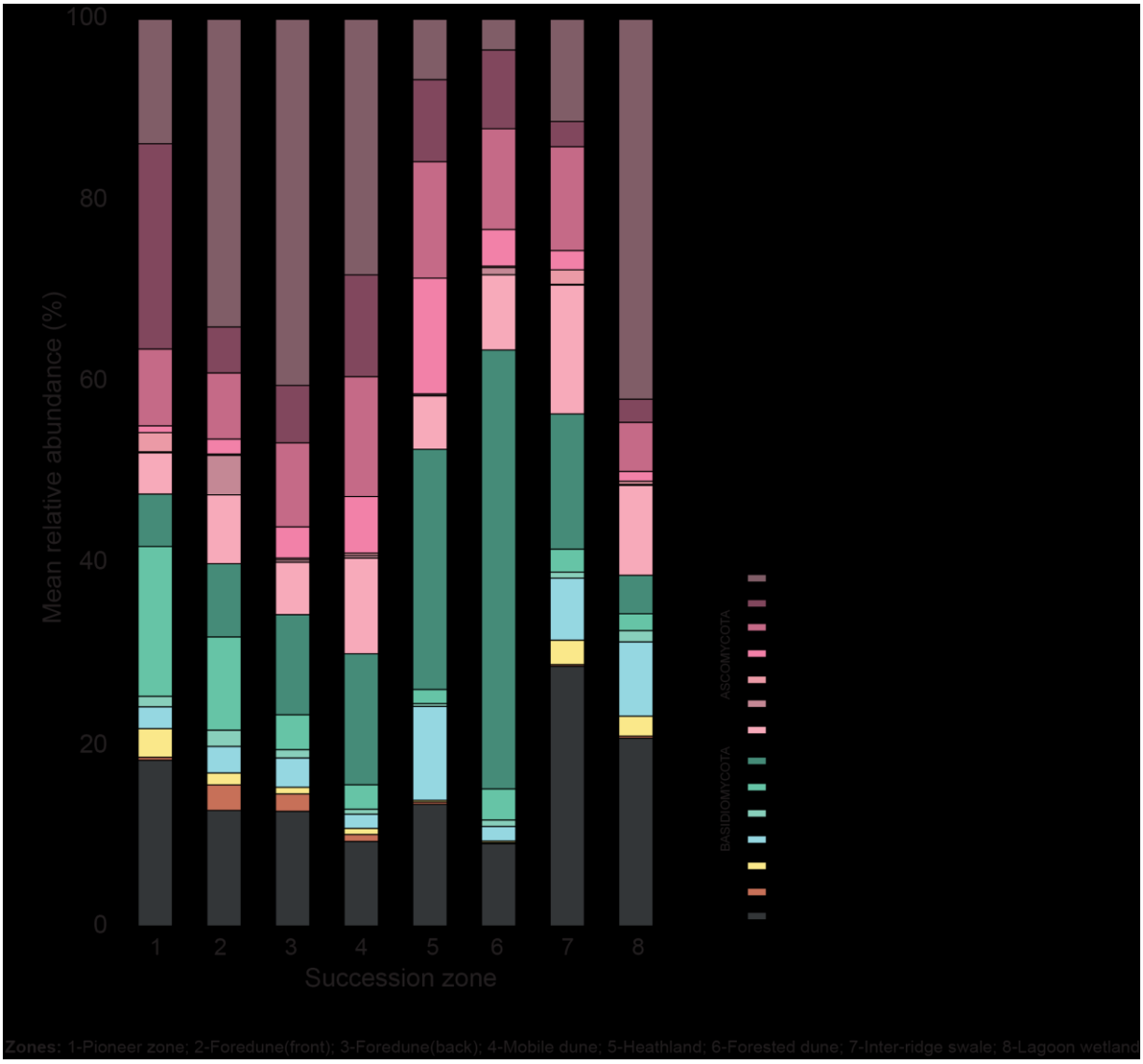

Figure S2.2. Mean relative abundance of the major classes of soil fungi in the different succession zones. The abundance is computed as the number of reads.

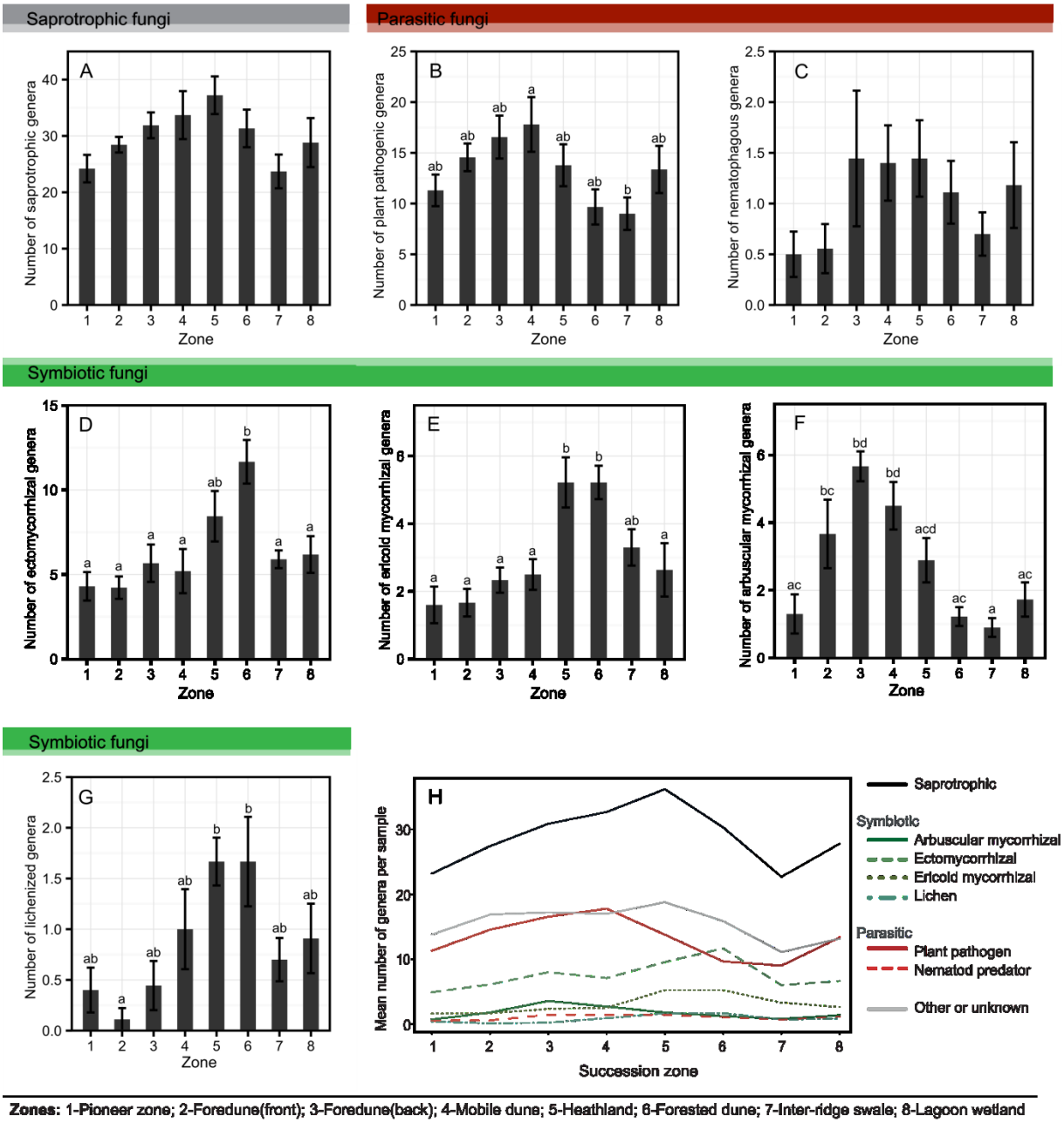

Figure S2.3. Changes in fungal life strategies across dune succession stages. Panels A-G show mean and standard error (n = 10) of the number of genera found in samples for each functional guild. Different lower case letters indicate significant differences between means as indicated by Tukey HSD post hoc tests ($P \leq 0.05$). Panel H combines the abundance of all functional guilds.

Table S2.1. Main plant species of each succession zone based on indicator species analysis.

		Representative plant species
1	**Pioneer zone**	*Ammophila brevigulata*
2	**Foredune(front)**	*Ammophila brevigulata* *Artemisia stelleriana*
3	**Foredune(back)**	*Ammophila brevigulata* *Festuca rubra* *Artemisia stelleriana*
4	**Mobile dune**	*Ammophila brevigulata* *Festuca rubra*
5	**Heathland**	*Deschampsia flexuosa* *Vaccinium vitis-idaea* *Juniperus communis* *Myrica pensylvanica* *Spiraea alba var. latifolia*
6	**Forested dune (ridges)**	*Myrica gale* *Chamaedaphne calyculata* *Picea mariana*
7	**Forested dune (hollows)**	*Vaccinium myrtilloides* *Abies balsamea* Bryophyte spp. *Spiraea alba var. latifolia* *Sphagnum* spp. *Carex* sp.
8	**Wetlands**	*Typha latifolia* *Lysimachia thyrsiflora* *Epilobium leptophyllum* *Menyanthes trifoliata* *Carex* sp. *Galium labradoricum* *Triglochin maritima* *Sphagnum* spp.

Table S2.2. Mean and standard error (n = 10) of topographical variables.

		Elevation (m)	Slope	Distance to the sea (m)	Soil type
1	Pioneer zone	3.70 ± 2.49	3.60 ± 3.88	20.50 ± 8.05	Sandy
2	Front foredune	8.00 ± 3.20	23.89 ± 19.26	34.00 ± 12.97	Sandy
3	Back foredune	9.22 ± 3.08	10.22 ± 12.75	49.88 ± 10.40	Sandy
4	Mobile dune	6.10 ± 3.18	6.50 ± 10.01	246.60 ± 136.22	Sandy
5	Heathland	3.33 ± 2.00	0.00 ± 0.00	723.89 ± 409.06	Sandy
6	Dune forest	3.89 ± 2.38	1.11 ± 3.14	750.56 ± 342.84	Sandy
7	Inter-ridge swale	1.90 ± 2.39	0.00 ± 0.00	821.60 ± 284.90	Sandy and organic
8	Lagoon wetland	0.91 ± 1.44	0.00 ± 0.00	1742.55 ± 270.66	Organic

Table S2.3. Variation in soil chemical properties across succession stages. Values are mean ± standard error (n = 10). Letters indicate significant differences between means assessed with Tukey HSD post hoc tests ($P \leq 0.05$). Soil samples were composed of a mix of six soil cores from 0-25 cm depth that were collected randomly within each 1 x 1 m plot. The gravimetric water content was calculated as a ratio between the mass of water and the dry weight of the sample. Conductivity, pH, soluble phosphorus, total phosphorus, carbon, and nitrogen were measured using an air-dried portion of the soil samples. All nutrient (C, N, and P) concentrations were measured on a dry-weight basis.

	Humus thickness (cm)		pH		Conductivity (mS/cm)		Water content (%w)		Organic carbon (%dw)		Total nitrogen (%dw)		Total phosphorus (ppm)		Soluble phosphorus (ppm)	
	Mean ± se	Tukey*	Mean ± se	Tukey	Mean ± se	Tukey	Mean ± se	Tukey	Mean ± se	Tukey	Mean ± se	Tukey	Mean ± se	Tukey	Mean ± se	Tukey
1	0.00 ± 0.00		7.25 ± 0.05	a	52.25 ± 7.24	a	2.07 ± 0.39	a	0.09 ± 0.04	a	0.08 ± 0.00	a	33.93 ± 1.69	a	6.43 ± 0.63	a
2	0.00 ± 0.00		7.13 ± 0.02	ab	41.34 ± 6.51	ab	2.18 ± 0.23	a	0.07 ± 0.02	a	0.07 ± 0.00	a	35.23 ± 1.44	a	7.28 ± 0.64	a
3	0.00 ± 0.00		7.05 ± 0.06	ab	28.30 ± 2.10	bc	1.65 ± 0.32	a	0.04 ± 0.01	a	0.07 ± 0.00	a	43.25 ± 4.99	a	6.97 ± 0.45	a
4	0.00 ± 0.00		6.76 ± 0.06	b	17.45 ± 1.23	c	2.59 ± 0.42	a	0.03 ± 0.00	a	0.08 ± 0.00	a	31.68 ± 1.69	a	6.54 ± 0.33	a
5	0.94 ± 0.39	a	5.64 ± 0.18	c	NA	NA	11.41 ± 4.45	ab	0.59 ± 0.22	a	0.10 ± 0.01	a	31.15 ± 2.51	a	6.35 ± 0.37	a
6	3.89 ± 0.68	ab	4.69 ± 0.10	d	NA	NA	15.12 ± 2.87	b	1.11 ± 0.18	a	0.11 ± 0.01	a	32.86 ± 2.51	a	6.72 ± 0.51	a
7	20.50 ± 9.18	b	5.43 ± 0.10	c	NA	NA	65.90 ± 4.95	c	12.79 ± 3.89	b	0.64 ± 0.20	b	216.82 ± 75.81	b	15.75 ± 3.83	a
8	20.00 ± 0.00	b	5.83 ± 0.12	c	NA	NA	85.36 ± 2.65	d	36.07 ± 3.75	c	1.51 ± 0.15	c	509.25 ± 52.06	c	56.05 ± 8.75	b

*Means of 0.00 ± 0.00 were excluded of the ANOVA and Tukey post hoc test.

Table S2.4. Data sets used as explanatory variables in the redundancy analysis.

Data set	Variable(s)	Transformation applied
Soil chemical properties	Humus thickness	Log
	Conductivity	-
	pH	-
	Gravimetric water content	Square root
	Carbon (organic matter) concentration	Log
	Nitrogen concentration	Log
	Total phosphorus concentration	Log
	Bio-available phosphorus concentration	Log
Topography	Altitude	-
	Slope	-

<u>Chapitre 3</u> | Liens entre les communautés végétales et fongiques dans un écosystème dunaire côtier

Nous avons observé, dans le chapitre précédent, un patron très clair au niveau de la structure des communautés fongiques du sol qui se distinguent selon le stade édaphique / successionnel des sites. Toutefois, ce patron n'était que modérément expliqué par les descripteurs environnementaux et les communautés fongiques ne semblaient pas fortement influencées par les propriétés physico-chimiques du sol. En tant qu'organismes hétérotrophes, les champignons sont dépendants de la présence de matière organique (décomposeurs) ou d'organismes vivants (symbiotes et parasites). La végétation pourrait donc jouer un rôle clé dans l'assemblage des communautés fongiques du sol. C'est ce que nous avons voulu investiguer dans le chapitre qui suit.

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Strong linkage between plant and soil fungal communities along a successional coastal dune system

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Author contributions: ARB MH and SB conceived and designed the study. MH and SB contributed reagents/materials/analysis tools. ARB collected and analysed the samples, performed bioinformatic processing and statistical analyses. EL provided advices on statistical analyses. ARB wrote the first draft of the paper, and all authors contributed to revisions and approved the final manuscript.

3.1 Abstract

The complex interactions existing between plants and soil microorganisms drive key ecosystem and community properties such as productivity and diversity. In nutrient-poor systems such as sand dunes, plant traits and fungal symbioses related to nutrient-acquisition can strongly influence vegetation dynamics. We investigated plant and fungal communities in a relic foredune plain located on an archipelago in Québec, Canada. We detected distinct communities across the edaphic and successional gradient. Our results showed a clear increase in plant species richness, as well as in the diversity of nutrient-acquisition strategies. We also found a very strong correlation between aboveground vegetation and soil fungal communities, and both responded to soil physicochemical properties. Soil pH importantly shaped plant and fungal communities, and could act as an important environmental filter along this relic foredune plain. On the other hand, the diversification of a belowground trait (nutrient-acquisition strategy) promoted functional diversity, which might favour resource partitioning and facilitation among co-occuring plant species. The coordinated changes in soil microbial and plant communities highlight the importance of aboveground-belowground linkages and positive biotic interactions during ecological succession in nutrient-poor environments.

3.2 Keywords

454-sequencing / coastal sand dunes / plant-soil feedback / soil fungal community / plant nutrient-acquisition / ecological succession

3.3 Introduction

There is growing recognition that interactions between plants and soil microorganisms drive important community and ecosystem properties such as diversity and productivity (Bever *et al.*, 2010; van der Putten *et al.*, 2013; Barberán *et al.*, 2015). In particular, soil fungi – as plant symbionts or as major regulators of decomposition, carbon sequestration and soil respiration – are key drivers of vegetation dynamics (Wardle *et al.*, 2004; Boddy *et al.*, 2007; van der Heijden *et al.*, 2008; Bever *et al.*, 2010). Along with other microorganisms, they

decompose dead organic material, ensure the recycling of essential nutrients in soils, and thus govern nutrient availability (Boddy *et al.*, 2007; van der Heijden *et al.*, 2008). Soil properties, particularly those related to nutrient availability, strongly influence plant growth and community composition (Lambers *et al.*, 2008; van der Heijden *et al.*, 2008).

Nutrient-acquisition by plants usually involves specialized root structures or symbiotic interactions with fungi (Lambers *et al.*, 2008; Zemunik *et al.*, 2015). These nutrient-acquisition strategies exist in the majority of plants species – over 90% of plant species have specialized features for nutrient uptake (Lambers *et al.*, 2008). Some plants developed specialized strategies such as parasitism or carnivory, or display a specialized root morphology such as the brush-like cluster roots, while others form symbiotic associations with nitrogen-fixing bacteria such as *Rhizobia* or *Frankia* or with mycorrhizal fungi. Arbuscular mycorrhizas (AM) are by far the most common strategy, occurring in more than 80% of vascular plant species (Brundrett, 2009). This symbiosis is the result of the colonization of the cortex of plant roots by Glomeromycota fungal hyphae where they form typical structures such as arbuscules, vesicules and, sometimes, intraradical spores. Fungal hyphae extend beyond the roots where they mobilize nutrients – in particular phosphorus (P) – and translocate it through their hyphae into arbuscules where P is exchanged for carbon (Smith & Read, 2008). There exist other types of mycorrhizal symbioses: ectomycorrhizas improve N uptake and involve a wide range of fungi in the Basidiomycota and Ascomycota in association with trees and shrubs, ericoid mycorrhizas are formed by specific soil ascomycetes partnered with Ericaceae, while orchid mycorrhizas are key to orchid seed germination (Smith & Read, 2008). Despite their ecological importance, soil fungal communities – and microbes in general – are often not considered in vegetation studies. However, a better understanding of plant-soil feedbacks and soil fungal community dynamics is imperative to improve our understanding of ecological processes such as succession (Wardle *et al.*, 2004; Kardol & Wardle, 2010). Aboveground and belowground components of ecosystems have traditionally been studied separately, but they must be studied together to understand ecosystem functioning (Wardle *et al.*, 2004).

In nutrient-poor systems such as sand dunes, plant traits and fungal symbionts related to nutrient-acquisition are expected to strongly influence vegetation dynamics and assembly (Lambers *et al.*, 2008). As such, early work indicated close association between the

development of vegetation and the abundance and identity of cultivable soil microbes (bacteria and fungi) along a sand dune successional gradient (Webley *et al.*, 1952). Yet, little is known about these belowground traits and the specific mechanisms linking above and below ground biota remain at least partially unresolved (Barberán *et al.*, 2015). Moreover, these feedbacks were only rarely considered in the context of ecological succession (Kardol *et al.*, 2013; Walker & Wardle, 2014). Coastal sand dunes are dynamic ecosystems presenting a sequence of vegetation spanning from low plant diversity in upper beach to fixed dunes with diverse vegetation. These habitats are typically characterized by different successional stages with distinct plant communities (Cowles, 1899; Olson, 1958; Lichter, 1998; Maun, 2009).

Here, we investigated the links between fungal and plant communities across a successional and edaphic gradient in a relic foredune plain (Giles & King, 2001). Building on our previous studies along this sequence where we investigated changes in soil microbial community composition (Roy-Bolduc *et al.*, 2015), we now explored linkages between soil microbial and plant communities across the dune system. Specifically, our study aimed to answer the following questions. Does the relative abundance of different plant nutrient-acquisition strategies vary along this edaphic and successional gradient? How does above-ground vegetation relate to belowground fungal communities in terms of diversity and community structure? Are changes in plant and/or fungal community structure related to variations in soil characteristics (e.g. pH, nutrients)? To address these questions, we performed a comprehensive sampling of soils and aboveground vegetation along a coastal dune system forming a successional and edaphic gradient (Giles & King, 2001; Roy-Bolduc *et al.*, 2015). Vegetation cover by species was recorded at each sampling site and belowground fungal communities were characterized using 454-sequencing. We studied patterns of plant and fungal diversity, and of plant nutrition-acquisition strategies. We also related plant and fungal community structure using co-inertia analysis and explored the effect of soil properties using multivariate analyses.

3.4 Materials and methods

3.4.1 Study site

The study was conducted along a relic foredune plain known as 'Les Sillons' (Giles & King, 2001). This sequence of dunes is located within the Îles de la Madeleine archipelago, in the southern Gulf of St. Lawrence in Québec, Canada (47° 23′ N, 61° 52′ W). The region is characterized by a maritime cold temperate climate with a mean annual temperature of 4.5 ^{o}C and annual average precipitation of 987 mm, of which approximately one third falls as snow (Houle, 2008). 'Les Sillons' is composed of a series of shore-parallel ridges forming a crescent-shaped area of 10.6 km^2. This dune system is a sandy depositional barrier that formed during the Holocene and now connects two bedrock islands (Giles & King, 2001). This relic foredune plain is notable because it harbours a diversity of vegetation type over a short distance (generally < 1 km), and includes a series of habitats ranging from the beach to forests (on dune crests) and wetlands (in dune swales) (Figure 1.2). The study site was divided into eight zones, moving away from the coastline: Z1) pioneer zone, Z2) front and Z3) back of the foredune, Z4) mobile dune, Z5) heathland, Z6) forest dune, Z7) inter-ridge swale, and Z8) lagoon wetland.

3.4.2 Field sampling

An extensive soil and vegetation field sampling was conducted in 'Les Sillons' in August 2011, in order to describe the plant and fungal communities associated with the different dune zones. We used a stratified sampling design by randomly selecting ten sampling points from each of the eight zones for environmental data collection, vegetation surveys, and soil sampling within 1 m x 1 m plots. Slope, soil texture, and organic layer thickness were determined at each sampling point. All vascular plants encountered in the plots were identified and their cover was estimated visually. In each plot, soil samples were composed of a mix of six soil cores of 0–25 cm depth that were collected randomly within the plot. The gravimetric water content was calculated as a ratio between the mass of water (weight of wet soil - weight of dry soil) and the dry weight of the sample. Conductivity, pH, extractable phosphorus (Mehlich-III), total phosphorus, carbon, and nitrogen were measured using an air-dried portion of the soil samples. Soil nutrients concentrations were converted from a dry weight basis to a

soil volume basis in order to make the values comparable across the different soil types (which included mineral sandy and organic soils). Soil properties are summarized in Table S3.1, and details about the conversion procedure can be found in Appendix S3.1 in supplementary information. Approximately 15 ml of previously homogenized soil samples were sub-sampled and frozen at -4^{o}C within 6 hours of sampling for subsequent molecular analysis. In the previous chapter, we completed our statistical analysis with soil properties expressed on a dry weight basis. This analysis might therefore lead to different results.

3.4.3 Soil fungal community description

We characterized soil fungal communities by 454-sequencing of the ITS region. The overall description of soil fungal diversity and community structure in this system was recently published (Roy-Bolduc et al., 2015), and the detailed molecular analysis and sequence processing methodology can be found in the supplementary materials of this publication. We used these fungal community data in some analysis presented here and will therefore quickly summarize the molecular and bioinformatics approach that was used. DNA was extracted from the soil samples, amplified using the ITS1F and ITS4 primers (White et al., 1990; Gardes and Bruns, 1993). The amplicons were multiplexed, purified and sequenced using Roche 454 GS-FLX+ chemistry at the Genome Quebec Innovation Center (McGill University, Montreal, Canada). We initially retrieved 435,124 reads and retained 182,242 after quality filtering in QIIME (Caporaso *et al*., 2010) and Mothur (Schloss *et al*., 2009). Sequences were clustered into Operational Taxonomic Units (OTUs) at a similarity level of 97% with Usearch v7.0 (Edgar, 2010). Taxonomy was assigned to each OTU consensus sequence using the UNITE database (Kõljalg *et al*., 2013). Raw sequence data were deposited in the NCBI Sequence Read Archive and are available under the project number PRJNA269650.

3.4.5 Statistical analyses

All statistical analyses were conducted in R v.3.0.2 (R Development Core Team, 2014). The relative cover (%) of all vascular plants was computed by normalizing their absolute cover against total cover in each plot. We computed plant species richness as the number of species recorded in every given plot, and plant diversity using the Shannon and

inverse Simpson diversity indices with the 'diversity' function in 'vegan' (Oksanen *et al.*, 2013). Our cover data and sampling design (i.e. no sub-plots) did not allow us to use individual or sample-based rarefaction to provide a better estimate of species richness (Gotelli & Colwell, 2001). We also assigned a nutritional strategy (facultative/obligate arbuscular mycorrhizal, ectomycorrhizal, ericoid mycorrhizal, arbutoid mycorrhizal, N-fixing, carnivorous, or unspecialized) to every plant species based on the literature (Table S3.3). We then computed the richness and diversity of the distinct plant nutritional strategies. Diversity was quantified using the Simpson index that take into account the abundance distribution of the different categories.

To examine patterns in fungal richness, we conducted OTU rarefaction analysis for individual samples. This analysis suggested that our sequencing depth was adequate since all curves reached or were close to reaching an asymptote and all samples had a Good's coverage value close to 1. The Chao total richness estimator (Chao, 1984) was computed using the 'rarefaction.single' command in Mothur with 1000 iterations of re-sampling without replacement. Fungal diversity was evaluated the same way as plant diversity, using the Simpson and Shannon diversity indices. We tested the Pearson correlation between fungal and plant richness or diversity. We used one-way ANOVA and Tukey HSD *post-hoc* tests to compare plant, nutritional strategies, and fungal richness and diversity among vegetation zones.

The relationship between plant and fungal community structure was tested using a co-inertia analysis. The analysis was performed on Hellinger transformed data using the 'coinertia' function in the 'ade4' library (Dray & Dufour, 2007) and significance of coefficients was tested with the 'randtest' function of the same package. The vector correlation coefficient (called RV coefficient) is a multivariate generalization of the Pearson correlation coefficient which gives a measure of the consistency of the two data sets and takes a value between 0 and 1 (1 being perfectly identical).

Canonical redundancy analysis (RDA) based on Hellinger-transformed data was used to assess the influence of soil and topographic variables on community assemblages, using the 'vegan' library (Oksanen *et al.*, 2013). The tests were performed on plant and fungal communities separately. Adjusted-R^2 values (Borcard *et al.*, 2011) were used to assess model fit. Statistical significance was assessed by permutation testing, using 999 permutations. The

results of the RDAs were represented in a biplot using scaling type 2 in order to preserve the distances between sites. We also modeled community variance for plant and fungal communities with permutational analysis of variance (PERMANOVA) using the 'adonis' function in 'vegan'. The significance of the variance explained by the different parameters was tested with 999 permutations.

3.5 Results

3.5.1 Plant and fungal community succession

Each of the eight zones sampled supported distinct vegetation (Figure 3.1; Supporting information, Table S3.2). The pioneer zone (Z1), foredunes (Z2-Z3) and mobile dunes (Z4) consisted of younger sites located close to the shore, which are characterized by dry, poorly-stabilised sandy soils and a plant community that was dominated by *Ammophila breviligulata* Fern., the American beachgrass. In areas that are not directly exposed to wind and salt spray, we also found a few herbaceous plants such as *Artemisia stellaria* L. and *Festuca rubra* L. Heathlands (Z5) are dry habitats and were dominated by shrubs such as *Myrica pensylvanica* Mirb., *Juniperus communis* L., and *Spiraea alba* Du Roi. This plant community transitioned progressively into black spruce, fir, and pine forests (Z6). The inter-ridge depressions and wetlands (Z7-Z8) harbour poorly-drained organic soils and support a diverse array of vegetation, including several Ericaceae, *Sphagnum*, and *Carex* species. We detected significant differences in plant richness and diversity across the eight zones (Richness: $F_{7,69}$ = 11.2, $P \leq 0.001$; Shannon: $F_{7,69} = 15.14$, $P \leq 0.001$; Simpson: $F_{7,69} = 7.82$, $P \leq 0.001$). Moving from the coastline to the wetland, we observed a generally increasing diversity with the heathland and lagoon wetland being the most diverse zones (Table 3.1). Observed plant richness by sampling plot ranged between 1 and 14. A total of 73 plant species were identified across all the plots sampled.

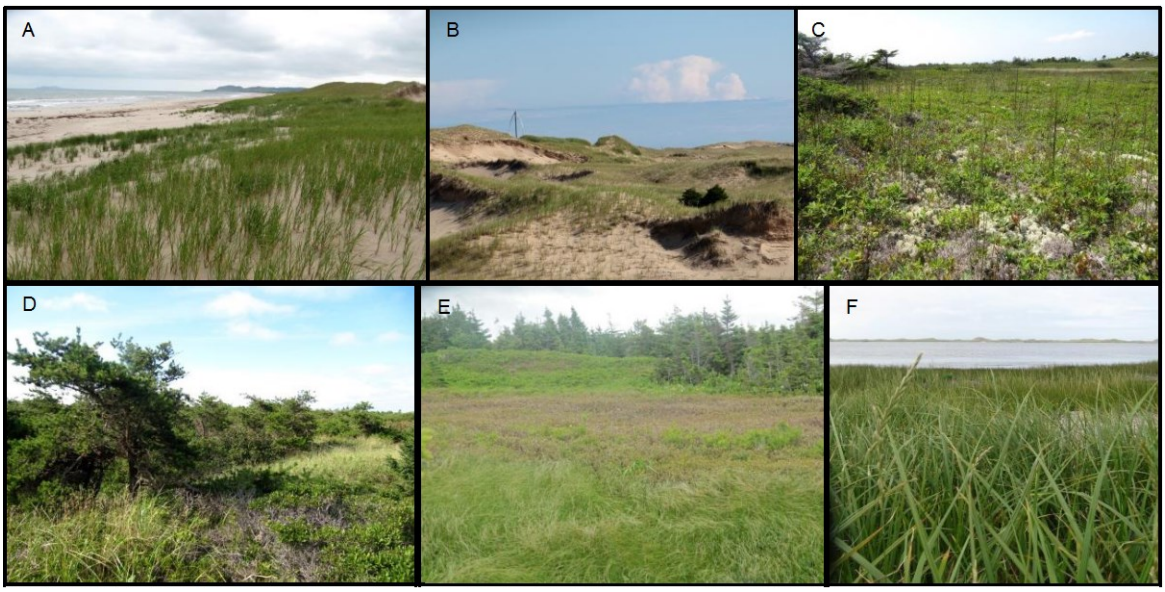

Figure 3.1. The different zones of our study site, from the pioneer zone and the foredune (a), to the mobile dune (b), the heathland (c) and the forested dune (d). At the end of the edaphic gradient, we can also observe wetlands in the inter-ridge swales (e) and the lagoon wetlands (f).

With regard to soil fungi, functionally and taxonomically distinct communities were found in each zone of the successional and edaphic gradient, as previously described (Roy-Bolduc *et al.*, 2015). Soil fungal richness was high across the succession gradient, with 3406 observed OTUs and a total richness of 4492 OTUs according the Chao estimator. Observed richness within individual sampling points ranged between 54 (in the pioneer zone) and 329 (in the lagoon wetland); however, there were no significant differences in either richness or diversity (Table 3.1). In addition, no significant correlations between fungal and plant richness nor alpha diversity were observed.

3.5.2 Plant nutrient-acquisition strategies

A clear increase in the number of strategies and their diversity was observed across the different zones (Figure 3.2). The arbuscular mycorrhizal (AM) strategy was the only plant nutrient-acquisition strategy present in all zones and the plants using this strategy were dominant in terms of relative cover in the first four successional stages, i.e. from the pioneer zone (Z1) to the mobile dune (Z4; Figure 3.3). Despite a high relative cover, AM plants

Table 3.1. Variation in plant and fungal alpha diversity across succession stages. Plant richness was measured as the number of observed species, while fungal richness was evaluated as the number of observed OTUs, total richness Chao estimator, and rarefied richness (subsampling of 200 reads per sample). Diversity was measured with the Shannon and the inverse Simpson indices. Values are mean ± standard error (n = 10), and represent average values in 1 x 1 meter plots by respective dune zone. The column on the right presents ANOVA P-values. Letters indicate significant differences between means according to Tukey HSD post hoc tests ($P \leq 0.05$). The last rows indicate the total richness, i.e. the total number of different plant species or fungal OTUs recorded in each zone.

ABOVE-GROUND VEGETATION

	Z1	**Z2**	**Z3**	**Z4**	**Z5**	**Z6**	**Z7**	**Z8**	
	Pioneer zone	Foredune front	Foredune back	Mobile dune	Heathland	Forest dune	Inter-ridge swale	Lagoon wetland	***P*-value**
Richness	1.00 ± 0.00 (a)	1.11 ± 0.11 (a)	2.44 ± 0.60 (ab)	2.6 ± 0.56 (ab)	6.44 ± 0.80 (cd)	4.56 ± 0.69 (abc)	5.20 ± 0.98 (bcd)	8.36 ± 1.30 (d)	≤ 0.001
Shannon	0.00 ± 0.00 (a)	0.07 ± 0.07 (a)	0.41 ± 0.18 (ab)	0.32 ± 0.12 (ab)	1.25 ± 0.11 (cd)	0.71 ± 0.16 (bc)	0.89 ± 0.18 (bcd)	1.39 ± 0.16 (d)	≤ 0.001
Simpson	1.00 ± 0.00 (a)	1.08 ± 0.08 (a)	1.66 ± 0.35 (ab)	1.29 ± 0.13 (a)	3.09 ± 0.43 (bc)	1.80 ± 0.29 (ab)	2.19 ± 0.32 (abc)	3.45 ± 0.56 (c)	≤ 0.001
Total richness	1	2	10	12	28	23	23	43	

SOIL FUNGI

	Z1	**Z2**	**Z3**	**Z4**	**Z5**	**Z6**	**Z7**	**Z8**	***P*-value**
Observed richness	107.30 ± 14.59	121.44 ± 9.74	151.00 ± 15.57	150.00 ± 22.80	181.67 ± 24.93	173.56 ± 22.69	152.80 ± 21.50	164.73 ± 23.07	0.174
Rarefied richness	99.28 ± 8.60	106.40 ± 6.78	112.40 ± 11.13	114.32 ± 13.17	124.93 ± 11.02	125.28 ± 13.62	118.32 ± 16.05	118.44 ± 12.88	0.895
Chao total richness	129.83 ± 18.24	148.06 ± 12.23	182.88 ± 18.65	187.38 ± 28.79	227.61 ± 30.65	223.29 ± 26.05	183.36 ± 26.18	198.94 ± 26.64	0.091
Simpson	12.78 ± 2.36	13.55 ± 2.58	13.12 ± 2.72	14.05 ± 2.92	15.55 ± 4.49	13.23 ± 4.44	13.18 ± 3.87	11.38 ± 3.50	0.997
Shannon	3.16 ± 0.20	3.31 ± 0.18	3.28 ± 0.25	3.33 ± 0.23	3.35 ± 0.25	3.15 ± 0.31	3.14 ± 0.29	2.92 ± 0.32	0.943
Total richness	607	519	685	892	1039	920	942	1157	

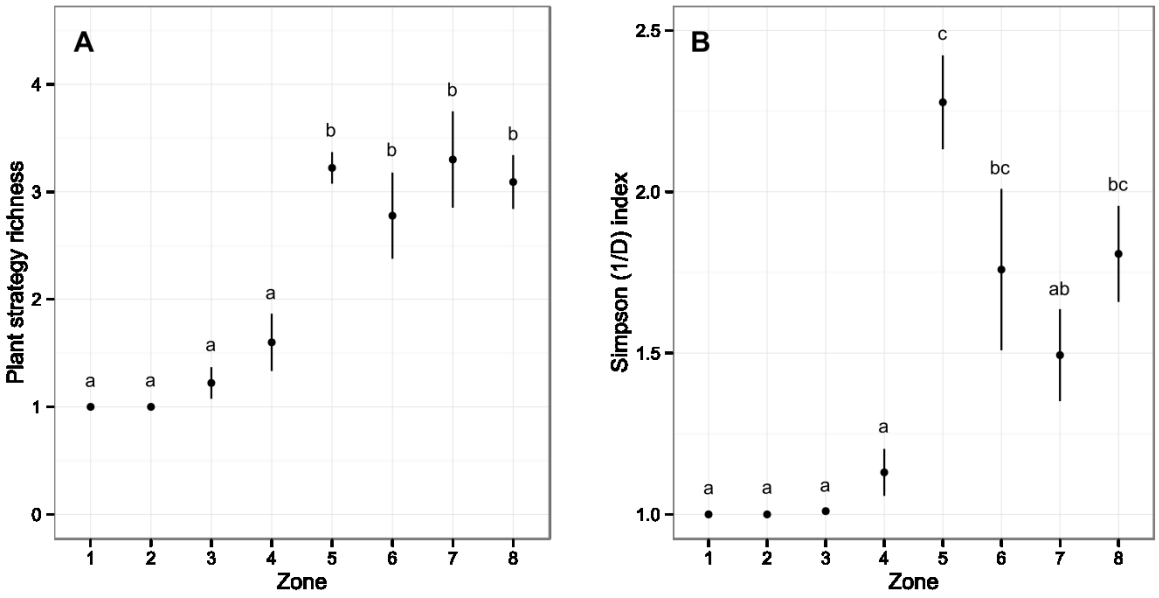

Figure 3.2. Richness (a) and diversity (b) of plants nutrient-acquisition strategy by sampling plots along the successional and edaphic gradient. Plants were classified according to their nutrient-acquisition strategy (arbuscular mycorrhizal, ectomycorrhizal, ericoid mycorrhizal, nitrogen-fixing or unspecialized). Obligate and facultative AM are considered as the same strategy, and unknown were removed from the dataset for this analysis. Points are means nutrient-acquisition strategies richness or diversity and bars are standard errors (n = 10). Different letters indicate significant differences ($P \leq 0.05$).

displayed low richness levels in the first zones because of the dominance of *Ammophila breviligulata* Fern. (Figure S3.1). The ectomycorrhizal strategy was first observed in the mobile dune zone (Z4) and the relative cover of EM plants peaked in the forested zone (Z6). Even though it was represented by a low richness (Figure S3.1), its relative cover was high especially in the forested zone. Ericoid mycorrhizal plants were only found in the last zones, i.e. from the heathland (Z5) to the lagoon wetlands (Z8). Plants forming symbioses with nitrogen-fixing bacteria were first observed in the mobile dune (zone 4) and their relative cover peaked in the inter-ridge swale (Z7). Plants without a specialized strategy were present mainly in the lagoon wetland, but also in zones 3, 5 and 7 but at very low densities (Figure 3.3). Some strategies were rare and are therefore not shown in Figure 3.3, such as carnivorous and arbutoid mycorrhizal (Supporting information, Figure S3.2). Two carnivorous plant species were identified in our sampling sites (*Drosera rotundifolia* L. and *Utricularia cornuta* Michx.) and were found mainly in the inter-ridge swale and in the lagoon wetland.

Arctostaphylos uva-ursi L., a species from the Ericaceae forming arbutoid mycorrhizal symbiosis, was found in the heathland (Z5) and in the forested zone (Z6). Finally, we did not find any parasitic plants nor plants forming orchid mycorrhizas.

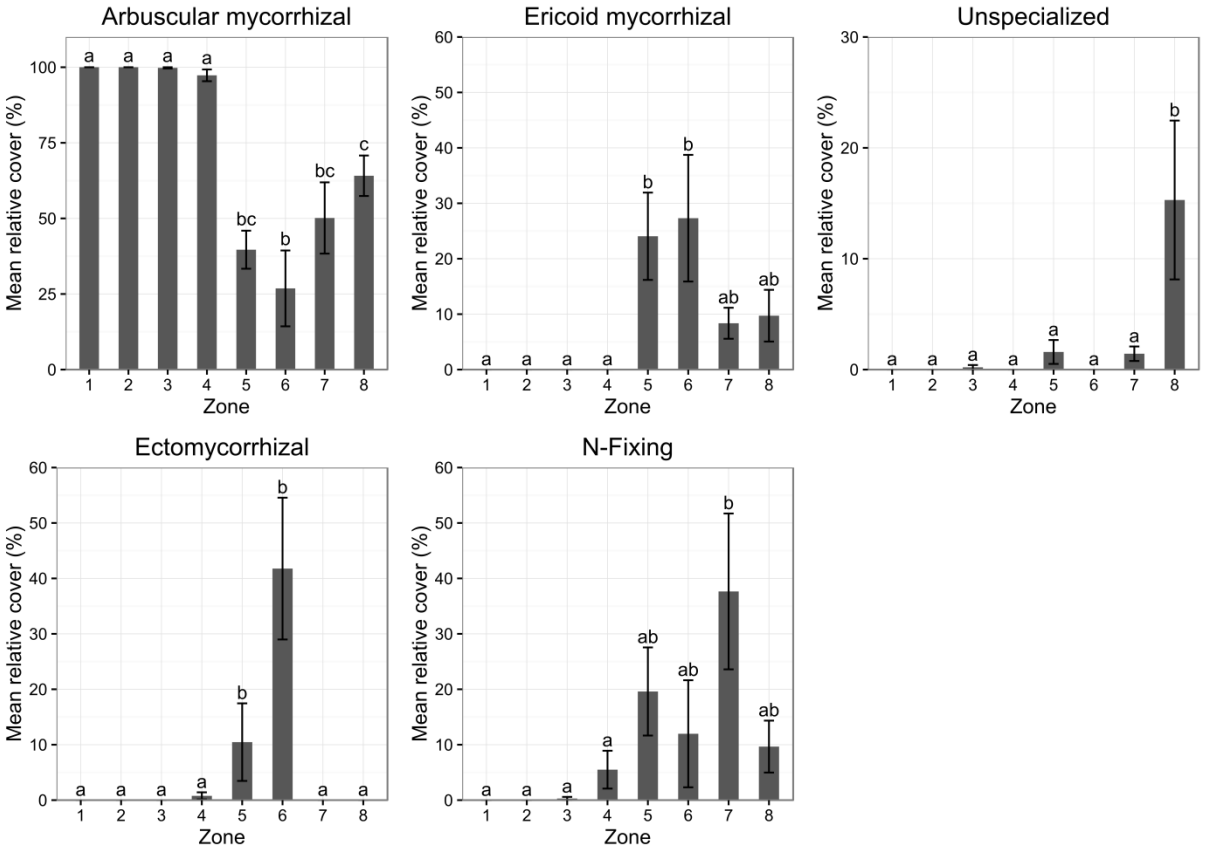

Figure 3.3. Mean relative cover of the main nutrient-acquisition strategies along the successional and edaphic gradient. Bars represent standard errors (n = 10) and different letters indicate significant differences ($P \leq 0.05$).

3.5.3 Linking plant and fungal communities

Despite the absence of a relationship between alpha diversity of plants and fungi, we observed coordinated changes in plant and soil fungal community structure. The RV coefficient measured in the co-inertia analysis was high (0.59) and highly significant (P = 0.0001 after 9999 permutations), indicating a very strong correlation between the two data sets (Figure 3.4). Panel A of Figure 3.4 shows the projection of the sampling sites in the co-inertia space with regards to both datasets. Sites from the first four successional stages are mainly

located on the right of co-inertia plot, while heathland (Z5) and forested sites (Z6) are grouped in the top left and the humid sites (Z7-Z8) at the bottom left. The first two axes represented respectively 73.85% and 7.12% of co-inertia. Arrows in panel B and C represent the importance and direction of the contribution of plant species and fungal genera to the distribution of sites in the co-inertia space. *Ammophila breviligulata* was the plant species with the most important contribution and was associated to the younger sites (Z1-Z4). *Juniperus communis*, *Myrica pensylvanica* and *Empetrum nigrum* were associated to Z5-Z6, and *Myrica gale*, *Carex* sp., *Typha latifolia*, *Sphagnum* sp. and *Menyanthes trifoliata* to Z7-Z8. Amongst fungi, Sordariomycetes spp. had the most important contribution to site distribution and was associated with humid sites (Z7-Z8).

3.5.4 Influence of soil physico-chemical properties on community structure

Humus thickness, gravimetric water content, and organic carbon significantly increased along the successional and edaphic gradient, while pH and soluble phosphorus generally decreased (Supporting information, Table S3.1). We observed a significant correlation between soil properties and plant community structure (Figure 3.5a). Our model explained 39.2% (adjusted R^2) of the variation in plant community ($F_{7,69}$ = 7.99; P = 0.001). Soil fungal community was also linked to soil properties but to a lesser extent, the model explaining 11.4% (adjusted R^2) of variation in soil fungal community composition ($F_{7,69}$ = 2.40; P = 0.001) (Figure 3.5b). The two first canonical axes explained 39.1% and 12.8% of the total model variance for plant and fungal communities respectively. Although soil physicochemical properties had a more important effect on vegetation (as determined by a higher adjusted R^2 value), their influence on plant and fungal communities were qualitatively similar (Table 3.2). Indeed, pH, humus thickness and water content were the variables explained most proportion of community variance; and soil pH had the highest R^2 value in both models. The proportion of variation explained was significant for humus thickness, pH, soil water content, total nitrogen, and total carbon for fungi. Neither total nor extractable phosphorus explained a significant proportion of the variation in plant and fungal community composition.

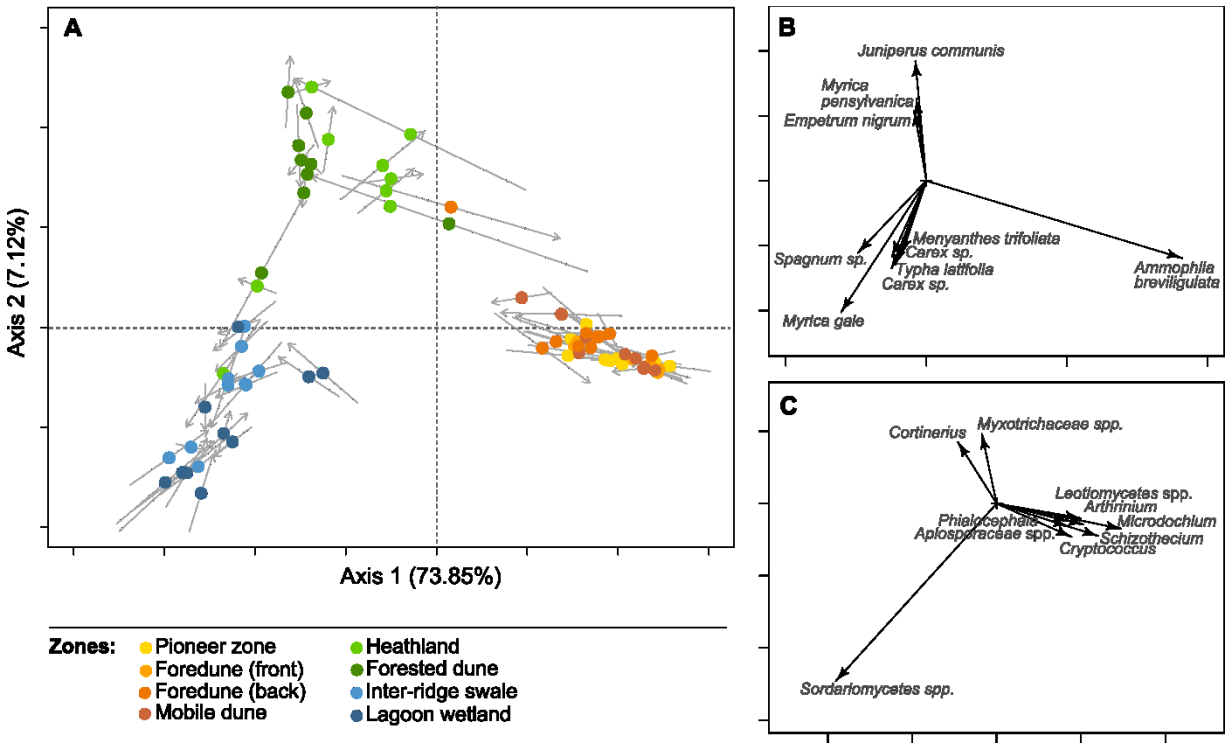

Figure 3.4. Joint site plot (a) depicting consensual ordination of sites in function of both plant and fungal community compositions preserving the Hellinger distances, and projection of the ten plant species (b) and fungal genera (c) with the highest scores. The two first canonical axes account for 80.97% of total co-inertia and the RV coefficient is 0.59 ($P = 0.001$). Arrow length in panel A is proportional to the difference between the ordinations of the two data sets: position of the arrow tails is determined by the ordination of plant community data, and the head by fungal community data.

Table 3.2. Community variance (portion of the model non-adjusted R^2) explained by soil physiochemical properties. Significant relationships (at $P \leq 0.05$) are in bold.

	PLANT COMMUNITY		**FUNGAL COMMUNITY**	
	R^2	*P*-value	R^2	*P*-value
Humus thickness	**0.11458**	0.001	**0.03766**	0.001
pH	**0.20532**	0.001	**0.05063**	0.001
Water content	**0.07731**	0.001	**0.04830**	0.001
Total carbon	0.01459	0.085	**0.01722**	0.022
Total nitrogen	**0.01698**	0.040	**0.01673**	0.018
Total phosphorus	0.01232	0.141	0.01289	0.235
Extractable phosphorus	0.00658	0.548	0.01238	0.291
Residuals	*0.55232*		*0.80418*	
Total	*1.00000*		*1.00000*	

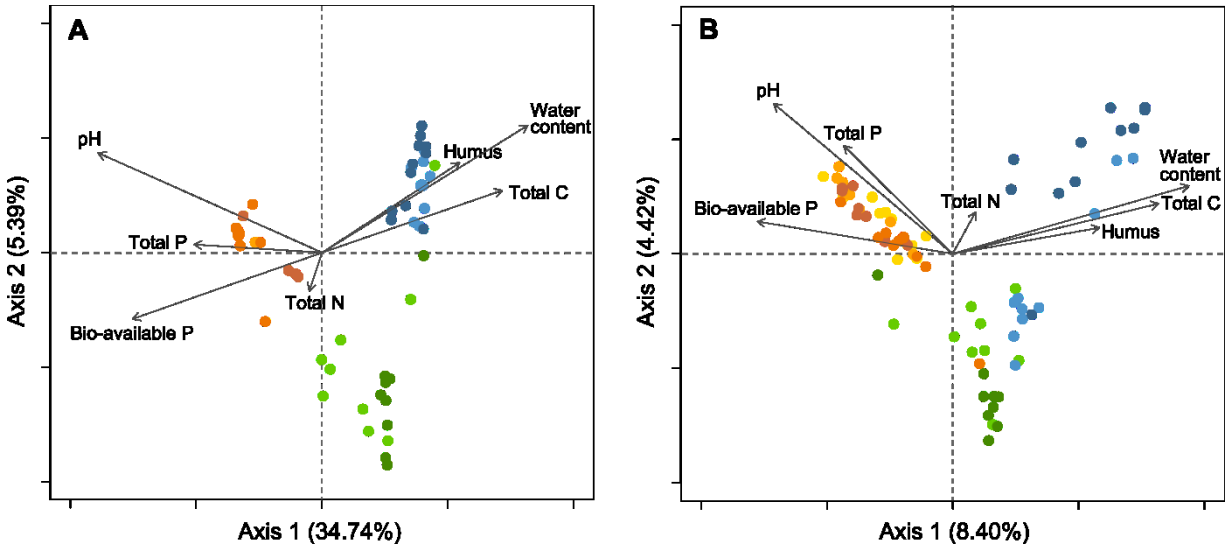

Figure 3.5. Transformation-based redundancy analysis biplots of plant (a) and fungal (b) communities in relation to soil properties using the Hellinger distance. Scaling type = 2. Adjusted R^2 is of 0.3916 ($P = 0.001$) for the analysis on vegetation and of 0.1142 ($P = 0.001$) for fungi.

3.6 Discussion

Our comprehensive sampling of the vegetation, combined with the characterisation of soil fungi using high-throughput sequencing, allowed us to explore changes in soil microbial and plant communities along a relic foredune plain forming a successional and edaphic gradient. Our results also showed a clear increase in plant richness and an increasing diversity of nutrient-acquisition strategies along the sequence. Finally, we found a significant correlation between plant and fungal communities, which were influenced by similar soil physicochemical properties.

First, we detected distinct plant and soil microbial communities across the successional and edaphic gradient. Given the contrasted abiotic conditions existing in the different dune zones (e.g. in terms of wind, salinity and soil water content), variations in plant communities were expected (Gurevitch *et al.*, 2002). We also expected that changes in soil fungal community structure would be strongly linked to vegetation dynamics (Roy-Bolduc *et al.*, 2015). As such, a co-inertia analysis produced a high and significant RV coefficient, demonstrating a strong correlation between plant and fungal community structure across the successional and edaphic gradient. These results are consistent with the current knowledge on the feedbacks existing between plants, microbes and soil (e.g. Barberán *et al.*, 2015; Wardle *et al.*, 2004). Vegetation, on one hand, is largely acknowledged as a key driver of soil microbial communities (e.g. Prescott & Grayston, 2013). Plants are hosts to many associations – both symbiotic and parasitic – with fungi (Broeckling *et al.*, 2008; Barberán *et al.*, 2015). Also, because saprophytic fungi are the main decomposers of plant dead material and rely directly on plant products such as leaf litter and root exudates, their community composition is often influenced by vegetation (Wardle *et al.*, 2004; Boddy *et al.*, 2007; Miniaci *et al.*, 2007). On the other hand, soil microbes have an impact on plant productivity, diversity and community composition through various processes implying direct (root-associated) or indirect (free-living) interactions that can be either positive or negative (van der Heijden, 2008). For example, soil pathogens such as *Fusarium* spp. can reduce plant productivity (a direct negative effect), while free-living soil saprotrophs are able to enhance soil nutrient availability by breaking down organic matter into inorganic forms available to plants (indirect positive effect) (Boddy *et al.*, 2007; van der Heijden, 2008).

Second, we observed a significant increase in plant richness and diversity along the gradient. This pattern of increasing plant diversity with soil age has been observed in several chronosequences under various climates (Laliberté *et al.*, 2013). Wardle *et al.* (2008) for example studied tree and plant diversity through succession and ecosystem retrogression (i.e. the decline phase of succession that is characterised by decreasing soil fertility and plant biomass) at six different locations. At all locations but one, they found a significant increase in vascular plant diversity through the successional gradient (including the retrogression stage) and observed an increasing number of plants with smaller statured life forms. Zemunik *et al.* (2015) also observed a clear increase in plant richness and diversity along a well-studied Australian dune chronosequence. Possible explanations for this pattern of increasing plant diversity with greater soil age are the decline of competition intensity among co-occurring plants during ecosystem retrogression, greater spatial heterogeneity of soil nutrients, and increased light availability because of fewer trees (Wardle *et al.*, 2008). However, most of these mechanisms remain incompletely understood and still need to be tested empirically (Laliberté *et al.*, 2013). Our data does not allow us to describe the mechanisms responsible for the pattern of plant diversity we observed. Still, the complementarity in belowground functional traits linked to resource acquisition along the successional gradient, which coincided with increasing plant richness and diversity, is consistent with results of a recent study (Zemunik *et al.*, 2015) and suggests a potential importance of belowground resource partitioning (Laliberté *et al.*, 2013). Indeed, access to distinct nutrient pools and diverse nutrient-uptake strategies can reduce interspecific competition for soil resources and thus promote local plant coexistence (Turner, 2008; Laliberté *et al.*, 2013). Complementarity of nutritional strategies could also perhaps facilitate the growth of neighbour plant species, which might promote plant species diversity. For example Teste *et al.* (2014) observed the best growth of *Melaleuca* seedlings in the presence of both ectomycorrhizal host plants and nutrient mining plants with cluster roots, suggesting a synergistic effect between the two strategies. The trends we observed in nutrient-acquisition strategies are consistent with the model proposed by Lambers *et al.* (2008), where AM plants are more abundant in young soils, followed by ectomycorrhizal, ericoid mycorrhizal and then non-mycorrhizal plants. The observation of AM as the dominant nutrient uptake strategy is consistent with the fact that the

majority of plant species associate with AM fungi (Smith & Read, 2008). Zemunik *et al.* (2015) also identified the AM strategy as the dominant one along a dune chronosequence.

We also found significant correlations of both plant and fungal community composition to soil physicochemical properties. From all soil properties investigated, pH was the one explaining the highest proportion of variance in plant and fungal community composition. Soil pH has previously been identified as one of the key drivers of plant community composition along an Australian dune chonosequence (Zemunik *et al.*, 2015; Laliberté *et al*., 2014). Even though fungi are thought to have a wide pH optima (Rousk *et al*., 2010), some fungal groups such as arbuscular mycorrhizal fungi are described as highly responsive to variations in soil pH (Dickie *et al*., 2013) which could have an indirect effect on the vegetation. Soil water content is another property that we found to be influential, as expected. Cornwell and Ackerly (2009) also found that gravimetric soil water content strongly affected plant functional trait distribution. For example, they predicted an increase in plant richness following increasing soil water, as we observed here. Also, fungi are known to be very sensitive to anoxic conditions (Bragazza *et al*., 2015; Lin *et al*., 2012). Water content was the second-most important environmental factor explaining variation in fungal community structure.

The various and complex factors shaping plant diversity along a gradient, such as the one we studied here, remain unresolved. One potential mechanism, called environmental filtering, explains local plant richness by the filtering of the species poorly adapted to local conditions from the regional species pool. Previous studies suggested that environmental filtering driven by soil acidification was responsible for plant assembly across a dune chronosequence (Laliberté *et al.*, 2014; Zemunik *et al.*, 2015). Similarly, our results also indicate that soil pH could act as an environmental filter along this relic foredune plain. Furthermore, we detected similar responses of plants and fungi to the same soil parameters. This could be the result of indirect interactions between above and belowground communities through modification of the soil environment. In other words, vegetation might be influencing (at least partially) soil microbiota through modification of soil properties. However, our data does not allow us to discriminate between this hypothesis or that of similar independent reactions to environmental variables.

These findings differ from the results obtained in chapter two, where we found that only water content and N concentration were the only soil properties having a significant influence on fungal community composition. This can be explained by the fact that we used dry-weight concentrations for the redundancy analysis conducted in the previous chapter and volume based equivalency in this one. Soil chemical properties are typically provided and used on a dry-weight basis, which is why we conducted our analysis as such at first. We realized later that it could represent a problem because we compared soils with very different structure and physical properties: sandy soils (Z1-Z6) to organic soils (Z7-Z8). Dried organic soils are extremely light, especially when compared to sand. We therefore converted nutrient concentrations to a volume basis. We think this ensures a more realistic representation of soil chemical properties, and of its relation to plant and soil fungal communities.

In conclusion, our study highlighted the strong linkage between plants and the soil biota in a nutrient-poor successional environment. The patterns we observed in nutrient-acquisition strategies indicate that symbiotic interactions with fungi have an influence on vegetation dynamics. As the positive effects of microbes are known to be most common in nutrient-poor habitats where they enhance the supply of growth limiting nutrients (van der Heijden *et al.*, 2008; Jonsson *et al.*, 2001; Teste *et al.*, 2014), plant-fungi symbiotic interactions could be important for plant establishment and survival in this relic foredune plain. The diversification of belowground traits promoted functional diversity, and might favour resource partitioning and facilitation among co-occuring plant species, which could be one of the mechanisms favoring local plant diversity (Zemunik *et al.*, 2015). Hence, local plant community assembly could be partly determined by mechanisms of resource competition and/or facilitation between co-occurring plant species. On the other hand, we also observed that soil properties, such as pH and water content, shapes community structure, which suggests that environmental filtering is also important. Our results suggest that both environmental filtering (exclusion of species outside their viable range) and limiting similarity (coexistence of functionally dissimilar species due to competition for limiting resources) can together drive plant community assembly. Our finding that changes in soil microbial and plant communities were strongly coordinated along this sequence highlights the importance of aboveground-belowground linkages during ecological succession (Walker & Wardle, 2014). Such aboveground-belowground linkages should be considered in conservation and ecological

restoration practices (Kardol & Wardle, 2010). This can be particularly important for ecosystems that are vulnerable to disturbance, such as coastal sand dunes.

3.7 Acknowledgments

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3.8 Supplementary information

Appendix S3.1.

Conversion of soil nutrients concentration from a dry-weight basis to a volume basis

Soil chemical properties are typically measured from air-dried portion of soil samples and nutrient concentrations are therefore provided on a dry-weight basis. This can represent a problem when comparing soils with very different structure and physical properties, as it is the case in this study. Indeed, we compared mineral sandy soils (Z1-Z6) to organic soils (Z7-Z8). Dried organic soils are extremely light, especially when compared to sand. To ensure a realistic representation of soil chemical properties and allow comparison across our study system, we converted nutrient concentrations to a volume basis. This conversion requires bulk density, i.e. the weight of soil in a given volume. Unfortunately, we did not measure it in the field. We were able to evaluate it a posteriori for the sandy soils (Z1-Z6) using 3-4 representative samples. The results are presented in the table below.

	Bulk density	**s.d.**	**n**
Zones 1-4	1.391	0.109	4
Zone 5	1.181	0.083	3
Zone 6	1.060	0.017	3

Because of the complex structure of organic soils, we could not follow the same procedure for zones 7 and 8. Therefore we used reference values from the literature. Based on field observation, we first classified our wetland sites in different types: bog (herbaceous or shrubby) or marsh. The table below shows the sites classification.

	Dominant plants	**Sites**
Herbaceous bog	*Sphagnum* spp., *Drosera rotundifolia*, *Sarracenia purpurea*	7_03, 7_09, 7_10, 8_03, 8_05, 8_09, 8_10, 8_11
Shrubby bog	*Myrica gale*, *Spiraea alba*, *Kalmia* spp., *Ledum groendlandicum*	7-01, 7_02, 7_04, 7_06, 7_07, 8_02
Marsh	*Typha* spp., *Carex* spp., *Juncus* spp., *Menyanthes trifoliata*	7_05, 7_08, 8_01, 8_06, 8_08

Then, we gathered data from the literature, targeting sites in eastern Canada (or north of US) that were ecologically similar to ours. We computed mean bulk density from different studies and used this value to calculate volume-based nutrient concentrations. The data available did not allow us to differentiate shrubby and herbaceous bogs so we attributed them

the same bulk density value. The data we used and the associated literature is presented in the table below. We used a bulk density value of 0.089 for all sites classified as shrubby or herbaceous bogs, and 0.051 for the marshes.

BOGS			
	mean	**s.d.**	**n**
Plaine plateau bog, Côte-Nord, QC, Canada (Loisel *et al.* 2013)	0.094	0.019	347
Lebel raised bog, Côte-Nord, QC, Canada (Loisel *et al.* 2013)	0.079	0.033	274
Petite bog, Nova-Scotia, Canada (Loisel *et al.* 2013)	0.066	0.019	415
Burnt village, Newfoundland, Canada (Loisel *et al.* 2013)	0.108	0.022	565
Mer Bleue peatland, 15 km East of Ottawa, ON, Canada (Scanlon and Moore 2000)	0.075	0.03	12
Average:	***0.089***	***0.022***	***1613***

MARSHES			
	mean	**s.d.**	**n**
Salt marshes in North-Western Atlantic (East coast of Canada and North of US) (Chmura *et al.* 2003)	0.036	0.011	57
Louisiana saline and freshwater coastal marsh (Dodla *et al.* 2008)	0.07	0.049	6
Brackish-water marsh on the Pamlico River estuary, near Aurora, North Carolina, US (Craft *et al.* 2002)	0.13	0.01	10
Average:	***0.051***	***0.013***	***73***

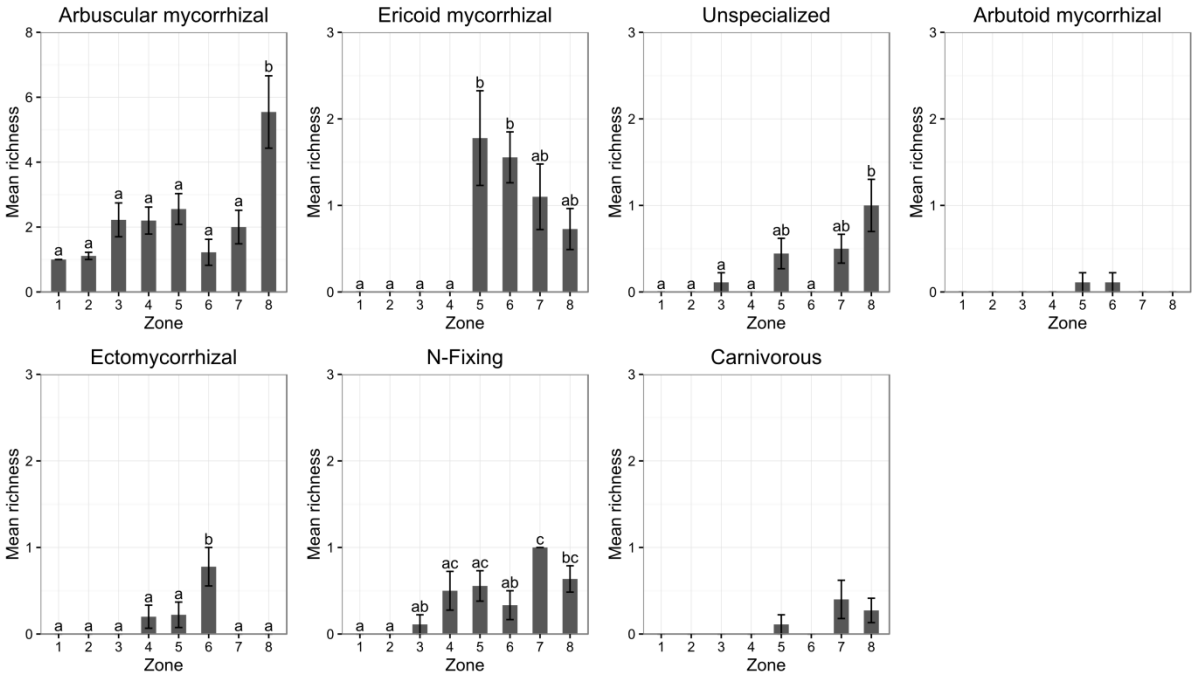

Figure S3.1. Mean richness of the main nutrient-acquisition strategies along the successional and edaphic gradient. Bars represent standard errors (n = 10) and different letters indicate significant differences according to ANOVA and Tukey HSD tests ($P < 0.05$).

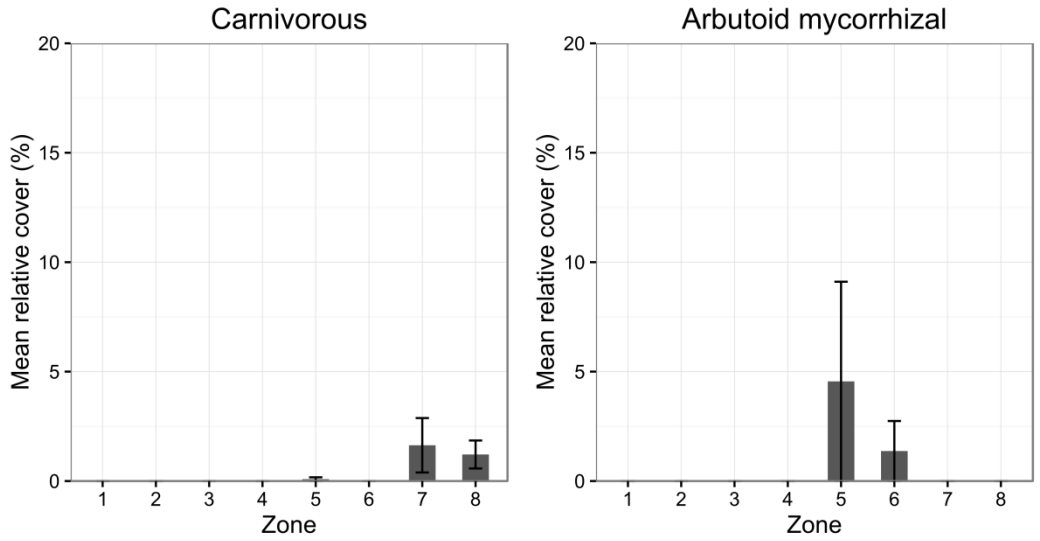

Figure S3.2. Mean relative cover of the carnivorous and arbutoid mycorrhizal nutrient-acquisition strategies along the successional and edaphic gradient. Bars represent standard errors (n = 10). No significant difference was observed according to ANOVA and Tukey HSD tests ($P < 0.05$).

Table S3.1. Variation in soil chemical properties across dune zones. Values are mean ± standard error (n = 10). Letters indicate significant differences between means assessed with Tukey HSD post hoc tests ($P \leq 0.05$). Soil samples were composed of a mix of six soil cores from 0-25 cm depth that were collected randomly within each 1 x 1 m plot. The gravimetric water content was calculated as a ratio between the mass of water and the dry weight of the sample. Conductivity, pH, soluble phosphorus, total phosphorus, carbon, and nitrogen were measured using an air-dried portion of the soil samples. All nutrient (C, N, and P) concentrations were converted to a volume basis.

	Humus thickness (cm)		pH		Conductivity (mS/cm)		Water content (%w)		Organic carbon (kg/m^3)		Total nitrogen (kg/m^3)		Total phosphorus (g/m^3)		Soluble phosphorus (g/m^3)	
	Mean ± se	Tukey*	Mean ± se	Tukey	Mean ± se	Tukey	Mean ± se	Tukey	Mean ± se	Tukey	Mean ± se	Tukey	Mean ± se	Tukey	Mean ± se	Tukey
1	0.00 ± 0.00		7.25 ± 0.05	a	52.25 ± 7.24	a	2.07 ± 0.39	a	1.28 ± 0.61	ab	1.05 ± 0.04	a	47.16 ± 2.35	ab	8.93 ± 0.87	ab
2	0.00 ± 0.00		7.13 ± 0.02	ab	41.34 ± 6.51	ab	2.18 ± 0.23	a	0.94 ± 0.23	ab	1.02 ± 0.04	a	48.97 ± 2.00	ab	10.11 ± 0.89	a
3	0.00 ± 0.00		7.05 ± 0.06	ab	28.30 ± 2.10	bc	1.65 ± 0.32	a	0.56 ± 0.10	a	0.94 ± 0.06	ab	60.11 ± 6.94	a	9.69 ± 0.62	ab
4	0.00 ± 0.00		6.76 ± 0.06	b	17.45 ± 1.23	c	2.59 ± 0.42	a	0.37 ± 0.06	a	1.08 ± 0.06	a	44.03 ± 2.35	ab	9.08 ± 0.46	ab
5	0.94 ± 0.39	a	5.64 ± 0.18	c	NA	NA	11.41 ± 4.45	ab	7.00 ± 2.60	abc	1.14 ± 0.06	a	36.76 ± 2.96	bc	7.50 ± 0.44	ab
6	3.89 ± 0.68	ab	4.69 ± 0.10	d	NA	NA	15.12 ± 2.87	b	11.76 ± 1.87	c	1.20 ± 0.05	a	34.83 ± 2.66	bc	7.12 ± 0.54	b
7	20.50 ± 9.18	b	5.43 ± 0.10	c	NA	NA	65.90 ± 4.95	c	10.78 ± 3.57	bc	0.55 ± 0.18	b	18.65 ± 6.88	c	1.30 ± 0.35	c
8	20.00 ± 0.00	b	5.83 ± 0.12	c	NA	NA	85.36 ± 2.65	d	28.76 ± 3.72	d	1.18 ± 0.13	a	40.05 ± 4.44	b	4.32 ± 0.66	d

se: standard error (n = 10); different letters indicate significant difference between succession zones ($P \leq 0.05$)
*Means of 0.00 ± 0.00 were excluded of the ANOVA and Tukey post hoc test.

Table S3.2. List of plant species found in sampling sites.

Coastal dune				**Forested dune**		**Wetlands**	
Pioneer zone	Foredune(front)	Foredune(back)	Mobile dune	Heathland	Forested dune	Inter-ridge swale	Lagoon wetland
Herbs and forbs : *Ammophila breviligulata*	**Herbs and forbs :** *Ammophila breviligulata* *Artemisia stelleriana*	**Herbs and forbs :** *Ammophila breviligulata* *Artemisia stelleriana* *Cakile edentula* *Carex* sp. *Convolvulus* sp. *Festuca rubra* *Hieracium pilosella* *Lathyrus japonicus* *Solidago bicolor* *Solidago sempervirens*	**Herbs and forbs :** *Ammophila breviligulata* *Carex silicea* *Carex* sp. *Chamerion angustifolium* *Festuca rubra* *Lathyrus japonicus* *Maianthemum stellatum* *Solidago bicolor* *Solidago canadensis* **Subshrubs and shrubs:** *Alnus incana* *Juniperus communis* *Myrica pensylvanica* **Trees :** *Picea mariana*	**Herbs and forbs :** *Ammophila breviligulata* *Atriplex* sp. *Carex* sp. *Deschampsia flexuosa* *Drosera rotundifolia* *Juncus balticus* *Rumex acetosella* *Smilacina trifolium* *Solidago bicolor* *Sphagnum* sp. *Stellaria alsine* Bryophytes Lichens **Subshrubs and shrubs:** *Andromeda polifolia* *Arctostaphylos uva-ursi* *Chamaedaphne calyculata* *Corema conradii* *Cornus canadensis* *Empetrum nigrum* *Hudsonia tomentosa* *Juniperus communis* *Kalmia angustifolia* *Ledum groendlandicum* *Myrica gale* *Myrica pensylvanica* *Spiraea alba* *Vaccinium angustifolium* *Vaccinium macrocarpon* *Vaccinium vitis-idaea* **Trees :** *Pinus mugo*	**Herbs and forbs :** Poaceae sp. *Sphagnum* sp. *Triadenum virginicum* *Trientalis borealis* Bryophytes Lichens **Subshrubs and shrubs:** *Arctostaphylos uva-ursi* *Chamaedaphne calyculata* *Corema conradii* *Cornus canadensis* *Empetrum nigrum* *Juniperus communis* *Kalmia angustifolia* *Ledum groendlandicum* *Myrica gale* *Myrica pensylvanica* *Photinia melanocarpa* *Spiraea alba* *Vaccinium angustifolium* *Vaccinium myrtilloides* *Vaccinium vitis-idaea* **Trees :** *Abies balsamea* *Picea mariana* *Pinus mugo*	**Herbs and forbs :** *Carex* spp. *Comarum palustre* *Drosera rotundifolia* *Dryopteris thelypteris* *Juncus balticus* *Lemna* sp. *Menyanthes trifoliata* Poaceae sp. *Sphagnum* sp. *Triadenum virginicum* *Typha latifolia* *Utricularia cornuta* *Xyris montana* **Subshrubs and shrubs:** *Andromeda glaucophylla* *Chamaedaphne calyculata* *Empetrum nigrum* *Kalmia angustifolia* *Myrica gale* *Photinia melanocarpa* *Rubus chamaemorus* *Spiraea alba* *Vaccinium macrocarpon*	**Herbs and forbs :** *Agropyron repens* *Ambrosia artemisifolia* *Asteraceae* sp. *Atriplex hastata* *Carex* spp. *Convolvulus* sp. *Deschampsia flexuosa* *Drosera rotundifolia* *Dryopteris thelypteris* *Epilobium leptophyllum* *Eriophorum vaginatum* *Fragaria virginiana* *Galium labradoricum* *Galium palustre* *Galium tinctorum* *Juncus balticus* *Lemna* sp. *Lysimachia thyrsiflora* *Lycopus americanus* *Maianthemum stellatum* *Matricaria maritima* *Menyanthes trifoliata* *Myriophyllum* sp. Poaceae sp. *Rumex acetosella* *Rumex* sp. *Saguisorba canadensis* *Salicorna europeae* *Scutelaria galericulata* *Sium suave* *Sphagnum* sp. *Triadenum virginicum* *Triglochin maritima* *Typha latifolia* *Viola* sp. **Subshrubs and shrubs:** *Andromeda polifolia* *Chamaedaphne calyculata* *Empetrum nigrum* *Lonicera canadensis* *Vaccinium macrocarpon*

Table S3.3. Assignement of nutritional acquisition strategies.

	Family	**Strategy**	**References**
Abies balsamea	Pinaceae	EM	Brundrett 2009; Peterson *et al.* 2004
Agropyron repens	Poaceae	AM	Peterson *et al.* 2004; Wang &Qiu 2006
Alnus incana	Betulaceae	EM / Nfix	Brundrett 2009; Peterson *et al.* 2004
Ambrosia artemisiifolia	Asteraceae	AM	Peterson *et al.* 2004; Wang & Qiu 2006
Ammophila breviligulata	Poaceae	AM	Koske 1981; Wang & Qiu 2006
Andromeda polifolia var. *glaucophylla*	Ericaceae	ErM	Wang & Qiu 2006
Arctostaphylos uva-ursi	Ericaceae	ArM	Peterson *et al.* 2004; Wang & Qiu 2006
Aronia melanocarpa	Rosaceae	Unknown	
Artemisia stelleriana	Asteraceae	AM	Peterson *et al.* 2004; Wang & Qiu 2006
Asteraceae sp.	Asteraceae	AM	Peterson *et al.* 2004; Wang & Qiu 2006
Atriplex prostata	Amaranthaceae	NM	Brundrett 2009; Wang & Qiu 2006
Atriplex sp.	Amaranthaceae	FacAM	Brundrett 2009; Wang & Qiu 2006
Cakile edentulata	Brassicaceae	NM	Brundrett 2009; Peterson *et al.* 2004
Carex silicea	Cyperaceae	FacAM	Brundrett 2009; Wang & Qiu 2006
Carex sp.	Cyperaceae	FacAM	Brundrett 2009; Miller *et al.* 1999; Wang & Qiu 2006
Carex sp. (2)	Cyperaceae	FacAM	Brundrett 2009; Miller *et al.* 1999; Wang & Qiu 2006
Chamaedaphne calyculata	Ericaceae	ErM	Malloch & Malloch 1982
Chamerion angustifolium	Onagraceae	FacAM	Wang & Qiu 2006
Comarum palustre	Rosaceae	Unknown	
Convolvulus sp.	Convolvulaceae	FacAM	Brundrett 2009; Veselkin *et al.* 2015
Corema conradii	Ericaceae	ErM	Read 1983
Cornus canadensis	Cornaceae	AM	Berliner & Torrey 1989; Peterson *et al.* 2004; Sylvia 1986
Deschampsia flexuosa	Poaceae	AM	Wang & Qiu 2006
Drosera rotundifolia	Droseraceae	Car	Brundrett 2009
Empetrum nigrum	Empetraceae	ErM	Moni *et al.* 2000; Wang & Qiu 2006
Epilobium leptophyllum	Onagraceae	AM	Wang & Qiu 2006
Eriophorum vaginatum	Cyperaceae	FacAM	Brundrett 2009; Wang & Qiu 2006
Festuca rubra	Poaceae	FacAM	Wang & Qiu 2006
Fragaria virginiana	Rosaceae	AM	Wang & Qiu 2006
Galium labradoricum	Rubiaceae	FacAM	
Galium palustre	Rubiaceae	FacAM	Wang & Qiu 2006
Galium tinctorium	Rubiaceae	FacAM	
Hieracium pilosella	Asteraceae	AM	Peterson *et al.* 2004; Wang & Qiu 2006
Hudsonia tomentosa	Cistaceae	EM	Brundrett 2009
Juncus artcticus	Juncaceeae	NM	Brundrett 2009; Maremmani *et al.* 2003

Juniperus communis	Cupressaceae	AM	Brundrett 2009, Peterson *et al.* 2004
Kalmia angustifolia	Ericaceae	ErM	Berliner & Torrey 1989; Wang & Qiu 2006
Lathyrus japonicus	Fabaceae	AM / Nfix	Koske 1981; Wang & Qiu 2006
Ledum groenlandicum	Ericaceae	ErM	Wang & Qiu 2006
Lemna sp.	Lemnaceae	NM	Brundrett 2009
Lonicera canadensis	Caprifoliaceae	AM	Maremmani *et al.* 2003; Wang & Qiu 2006
Lycopus americanus	Lamiaceae	AM	Wolfe *et al.* 2006
Lysimachia thyrsiflora	Primulaceae	AM	Maremmani *et al.* 2003
Maianthemum stellatum	Liliaceae	AM	Brundrett & Kendrick 1988
Maianthemum trifolium	Liliaceae	AM	Brundrett & Kendrick 1988
Menyanthes trifoliata	Menyanthaceae	NM	Wang & Qiu 2006
Myrica gale	Myricaceae	Nfix	Brundrett 2009
Myrica pensylvanica	Myricaceae	Nfix	Brundrett 2009
Myriophyllum sp.	Haloragaceae	NM	Beck-Nielsen & Vindbæk Madsen 2001; Brundrett 2009; Wang & Qiu 2006
Picea mariana	Pinaceae	EM	Brundrett 2009, Peterson *et al.* 2004
Pinus mugo	Pinaceae	EM	Brundrett 2009, Peterson *et al.* 2004
Poaceae sp.	Poaceae	AM	
Rubus chamaemorus	Rosaceae	NM	Wang & Qiu 2006
Rumex acetosella	Polygonaceae	NM	Brundrett 2009
Rumex sp.	Polygonaceae	NM	Brundrett 2009
Salicornia maritima	Chenopodiaceae	FacAM	Brundrett 2009, Wang & Qiu 2006
Sanguisorba canadensis	Rosaceae	AM	Wang & Qiu 2006
Scutellaria galericulata	Lamiaceae	FacAM	Wang & Qiu 2006
Sium suave	Apiaceae	FacAM	Šraj-Kržič *et al.* 2006; Wang & Qiu 2006
Solidago bicolor	Asteraceae	AM	Peterson *et al.* 2004; Wang & Qiu 2006
Solidago canadensis	Asteraceae	AM	Peterson *et al.* 2004; Wang & Qiu 2006
Solidago sempervirens	Asteraceae	AM	Peterson *et al.* 2004; Wang & Qiu 2006
Spiraea alba var. *latifolia*	Rosaceae	AM	Wang & Qiu 2006
Stellaria alsine	Caryophyllaceae	FacAM	Brundrett 2009; Wang & Qiu 2006
Thelypteris palustris	Thelypteridaceae	AM	Wang & Qiu 2006
Triadenum virginicum	Clusiaceae	AM	Rains *et al.* 2003; Wang & Qiu 2006
Trientalis borealis	Primulaceae	AM	Berliner & Torrey 1989; Malloch & Malloch 1982
Triglochin maritima	Juncaginaceae	FacAM	Brundrett 2009; Wang & Qiu 2006
Tripleurospermum maritimum	Asteraceae	AM	Peterson *et al.* 2004; Wang & Qiu 2006
Typha latifolia	Typhaceae	AM	Peterson *et al.* 2004
Utricularia cornuta	Lentibulariaceae	Car	Brundrett 2009
Vaccinium angustifolium	Ericaceae	ErM	Berliner & Torrey 1989; Malloch & Malloch 1982; Wang & Qiu 2006
Vaccinium macrocarpon	Ericaceae	ErM	Wang & Qiu 2006
Vaccinium myrtilloides	Ericaceae	ErM	Wang & Qiu 2006

Vaccinium vitis-idaea	Ericaceae	ErM	Wang & Qiu 2006
Viola sp.	Violaceae	AM	Peterson *et al.* 2004; Wang & Qiu 2006
Xyris montana	Xyridaceae	NM	Brundrett 2009

Abbreviations

AM : Obligate arbuscular mycorrhizal

ArM: Arbutoid mycorrhizal

Car: Carnivorous

EM : Ectomycorrhizal

ErM : Ericoid mycorrhizal

FacAM : Facultative arbuscular mycorrhizal

NM : Non mycorrhizal (unspecialized)

Nfix: Symbiotic with N-fixing bacteria

<u>Chapitre 4</u> | Associations ectomycorhiziennes dans les dunes fixées d'un écosystème dunaire côtier

Nous avons observé un fort lien entre les communautés végétales et fongiques. Les mécanismes responsables de cette corrélation demeurent toutefois incertains. Nous avons donc consacré le dernier chapitre de cette thèse à une étude plus détaillée des interactions entre les principales essences d'arbres des dunes (quatre pinacées) et les champignons ectomycorhiziens associés. Les symbioses mycorhiziennes à arbuscules ont été largement répertoriées en milieu dunaire, alors que très peu d'études se sont intéressées aux champignons ectomycorhiziens dans ce type de milieu. Pourtant, nous avons identifié dans les chapitres précédents la symbiose ectomycorhizienne comme principale stratégie d'acquisition de nutriments dans la zone fixée des dunes et il est reconnu que la nutrition des arbres repose principalement sur les champignons ectomycorhiziens dans les forêts tempérées et boréales.
Nous nous sommes intéressés au degré de spécificité des associations ectomycorhiziennes et avons utilisé l'approche des analyses de réseaux. Une grande diversité de champignons peut s'associer à une espèce d'arbre, ou même à un seul individu et il est possible de représenter ces interactions avec des réseaux bipartites. Dans ces réseaux, les nœuds appartiennent à deux classes (plante hôte ou champignon ectomycorhizien) qui peuvent être liés selon la force ou la fréquence d'interaction.

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High richness of ectomycorrhizal fungi and low host specificity in a coastal sand dune ecosystem revealed by network analysis

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4.1 Abstract

Ectomycorrhizal (EM) fungi are ubiquitous in temperate and boreal forests, comprising over 20,000 species forming root symbiotic associations with Pinaceae and woody angiosperms. As much as 100 different EM fungal species can coexist and interact with the same tree species, forming complex multispecies networks in soils. The degree of host specificity and structural properties of these interaction networks (e.g. nestedness and modularity) may influence plant and fungal community assembly and species coexistence, yet their structure has been little studied in northern coniferous forests, where trees depend on EM fungi for nutrient acquisition. We used high-throughput sequencing to characterise the composition and diversity of bulk soil and root-associated fungal communities in four co-occuring Pinaceae in a relic foredune plain located at Îles de la Madeleine, Québec, Canada. We found high EM fungal richness across the four hosts, with a total of 200 EM operational taxonomic units (OTUs), mainly belonging to the Agaricomycetes. Network analysis revealed an anti-nested pattern in both bulk soil and roots EM fungal communities. However, there was no detectable modularity (i.e. subgroups of interacting species) in the interaction networks, indicating a low level of specificity in these EM associations. In addition, there were no differences in EM fungal OTU richness or community structure among the four tree species. Limited shared resources and competitive exclusion typically restrict the number of taxa co-existing within the same niche. As such, our finding of high EM fungal richness and low host specificity highlights the need for further studies to determine the mechanisms enabling such a large number of EM fungal species to coexist locally on the same hosts.

4.2. Keywords

454-sequencing / coastal sand dunes / ectomycorrhizal fungal community / host preference / network analysis / plant-fungal interactions

4.3 Introduction

Ectomycorrhizal (EM) fungi are plant symbionts colonizing the roots of many tree and shrub species, for example conifers in the Pinaceae such as *Pinus*, *Picea*, and *Larix*. These fungal symbionts, ubiquitous in forested ecosystems of temperate and boreal biomes, are crucial for the growth and survival of their hosts as they enhance nutrient and water uptake and protect plant roots from infection by pathogens (Smith & Read, 2008). Ectomycorrhizal communities generally contain high numbers of fungal taxa associating with a low diversity of host plants (Tedersoo *et al.,* 2014; van der Heijden *et al.,* 2015). Northern forests, generally poor in tree species, can support several hundreds of EM fungal taxa (Trappe, 1977; Horton & Bruns, 2001). Furthermore, as much as 100 different EM fungal species can interact locally with one tree species in a single monospecific stand (Allen *et al.*, 1995), and over 15 EM fungi can be found in association with a single individual (Saari *et al.,* 2005). The properties of these complex species interaction networks can be characterized using bipartite network analysis, where the different species are represented as nodes belonging to two classes (EM fungi or host plant) that can be linked depending on the strength or frequency of the interspecies interactions (e.g. Bahram *et al.,* 2014). In such ecological networks, species may display various levels of specialization, which would result in different network structural properties.

The two most commonly characterized network structural properties are nestedness and modularity. Nestedness is a measure of the hierarchical organization of interactions (Bascompte *et al.,* 2003). In nested networks, specialized species are mainly associated with the generalist species of the other class but not with other specialists (Bascompte *et al.,* 2003). High nestedness is thought to enhance the diversity and resilience of ecological communities (Burgos *et al.,* 2007; Chagnon *et al.,* 2012), particularly by reducing interspecific competition and facilitating species coexistence (Bascompte *et al.,* 2003; Bastolla *et al.,* 2009). Modularity, on the other hand, is a measure of reciprocal specialization. It evaluates the presence of modules, i.e. subgroups of strongly interacting species. The modular organization of complex networks, resulting from functional complementarity and coevolutionnary dynamics, is believed to increase overall network stability particularly by containing the effects of perturbations within compartments and therefore buffering communities against secondary extinctions following disturbance (Guimera & Amaral, 2005; Stouffer & Bascompte, 2011).

The architecture of plant-fungal species interaction networks remains poorly studied, mainly because of the technical limitations to the accurate taxonomic description of microbial communities. The recent developments in next-generation sequencing techniques have greatly increased our ability to characterize soil microbial community composition and diversity (Nilsson *et al.,* 2011; Tedersoo *et al.,* 2014). Still, we know little about underground plant-fungal network architecture in most ecosystems. The structure of mutualistic networks (e.g. plant-pollinator networks) has been commonly described as highly nested (e.g. Bascompte *et al.,* 2003; Burgos *et al.,* 2007; Thebault & Fontaine, 2010). The general expectation for plant-fungal mutualistic associations would therefore be a nested network organisation. However, recent studies on the structure of belowground plant-fungal networks observed various results depending on environmental context and mycorrhizal type. For example, Jacquemyn *et al.* (2015) observed a high degree of specialization and modularity between orchids and their associated orchid mycorrhizal fungi. By contrast, network analysis of arbuscular mycorrhizal (AM) fungal communities showed significant nestedness (Chagnon *et al.,* 2012; Montesinos-Navarro *et al.,* 2012). With regard to EM fungal communities, Bahram *et al.* (2014) analyzed ten EM plant-fungal interaction networks and found significant negative relative nestedness (i.e. 'anti-nestedness') and, in some cases, significant levels of modularity.

Differences in the ecology of the distinct types of mycorrhizal symbioses may explain the differences observed in network properties. Arbuscular mycorrhizas are formed by the association of Glomeromycota fungi, a phylum currently comprising only 244 described species (Schüßler & Walker, 2010), with a high diversity of hosts – around 200,000 plant species (Brundrett, 2009). Ectomycorrhizal and orchid mycorrhizal symbioses are more balanced with regard to number of fungi and host plants, with respectively 20,000 and 25,000 fungal species associating with around 6,000 and 20,000-35,000 plant species (reviewed in van der Heijden *et al.,* 2015). Hence, we could expect a higher potential of preferred associations in EM fungi compared to AM fungi.

A few studies have characterised soil fungal communities in coastal sand dune ecosystems, but those have mainly focused on AM fungi (Koske & Halvorson, 1981; Corkidi & Rincón, 1997; Koske & Gemma, 1997; Kowalchuk *et al.,* 2002; Błaszkowski & Czerniawska, 2011; etc.). To our knowledge, EM communities and multispecies network have not previously been described in coastal dune ecosystems. In this study, we used high-

throughput sequencing to characterise soil and root-associated EM fungal communities in four co-occuring Pinaceae tree species in a relic foredune plain. We also used network analysis to further describe network structure and specificity of associations, and to compare network properties with that of previous studies in different systems.

Previous studies in Northern Hemisphere ecosystems observed overlapping communities, suggesting that most EM fungi have multi-host habits (Horton & Burns, 1998; Cullings *et al.* 2000; Horton & Burns, 2001; Kennedy *et al.*, 2003). However, recent work relying on high throughput sequencing were able to detect different degrees of association preferences (e.g. Ishida *et al.,* 2007; Morris *et al.*, 2008; Aponte *et al.,* 2010; Tedersoo *et al.,* 2010; Murata *et al.,* 2013), and tree species identity is increasingly recognized as a key factor shaping EM fungal communities (Smith *et al.*, 2009). As such, we expected to observe – in addition to some multi-host fungi – several specialists preferentially interacting with a single plant, resulting in a moderate to high effect of host identity on the structure of EM communities. We hypothesized that this host effect would translate into the absence of nested patterns and significant levels of modularity, as highlighted by Bahram *et al.* (2014) in other forested ecosystems dominated by EM trees.

It is important to note that the term 'network' is used throughout this chapter to designate virtual networks of ecological interactions, and not the physical mycelial network known as the "common mycelial network" (CMN). These so-called CMN phisically connect individuals (from the same or from distinct species) over tens of meters and facilitate seedlings establishment by the sharing of resources and the transfer of signalling compounds (Simard *et al.,* 2012). Ecological networks, on the other hand, are used to virtually represent interactions between species or individuals. They can be used to represent any ecological interactions, from predation and competition to symbiotic associations, and are very usefull to visualize and analyse complex systems.

4.4 Material and methods

4.4.1 Study area and site description

Our study system is a relic foredune plain known as 'Les Sillons' located within the Îles de la Madeleine, an archipelago situated in the southern Gulf of St. Lawrence in Québec,

Canada (47° 23′ N, 61° 52′ W). Îles de la Madeleine is characterized by a maritime cold temperate climate. Mean annual temperature on the islands is 4.5 ^{o}C and annual precipitation sums to 987 mm, of which approximately 30% falls as snow (Houle, 2008). 'Les Sillons' covers a crescent-shaped area of 10.6 km^2 and is composed of a series of shore-parallel ridges. Resulting from seaward growth, the system is a sandy depositional barrier that accumulated during the Holocene and now connects two bedrock islands (Giles & King, 2001). Soils are mainly sandy.

'Les Sillons' includes a succession of habitats from the coast, ranging from the beach to mobile dunes and heathlands, and then to forests (on dune crests) and wetlands (in dune swales) (see Figures 1.3 and 3.1). The mobile dunes are characterized a plant community that is largely dominated by *Ammophila breviligulata*, the American beachgrass, as well as a few herbaceous plant such as *Artemisia stellaria* and *Festuca rubra* in areas that are not directly exposed to wind and salt spray. Heathlands are dry habitats that are dominated by shrubs such as *Myrica pensylvanica*, *Juniperus communis,* and *Spiraea alba,* and these transition into black spruce, fir and pine forests in the older dunes. The inter ridge swales harbour a diverse array of vegetation, including several Ericaceae, *Sphagnum*, and *Carex* species. Details about the study system and its soil fungal communities are described in more detail in Roy-Bolduc *et al.* (2015).

Table 4.1. Mean environmental variables and soil physicochemical properties for the whole sampling area. Values are means ± standard deviation (n = 32).

Elevation (m)	3.9	±	0.7
pH	4.9	±	0.3
Water content (%w)	7.7	±	0.6
Organic horizons thickness (cm)	2.7	±	0.3
Total nitrogen (g kg-1)	1.16	±	0.04
Organic carbon (g kg-1)	7.1	±	1.5
Bioavalaible phosphorus (mg kg-1)	8.7	±	1.2
Total phosphorus (mg kg-1)	42.6	±	4.1

Our sampling was concentrated in a 3-4 km long portion of the forested dunes, which are roughly parallel to the coastline. The forest canopy is composed of mixed and distinct stands of Pinaeceae (e.g. *Abies balsamea, Picea mariana, Picea glauca, Pinus banksiana,* and

Pinus mugo), and the understory includes shrubs such as *Myrica gale* and *Chamaedaphne calyculata*. The forest floor is often covered with lichens (*Cladonia* spp.). Although the area was never intensively exploited for timber, the forest is mainly secondary because of small scale punctual logging, natural fires, and anthropogenic disturbances that occured during the construction of the highway and the development of the electricity network over the last century. We sampled fine roots (<2 mm diameter) and associated soil of four of the codominant tree species of this dune system, including two non-native species (*Pinus banksiana* and *Pinus mugo*) used in plantations for dune stabilisation in the 1940-1960 period (O'Carroll, 1998) and two native and naturally-occurring species (*Picea mariana* and *Abies balsamea*). *Pinus banksiana* is commonly found in north American boreal forests, but was not originally present on the Îles de la Madeleine. *Pinus mugo*, a small tree native from high elevation habitats in Europe, is well adapted to dry soils and low nutrient concentrations. Eight replicates of root and soil samples were sampled in August 2010 for each species in monospecific stands of at least 10 × 10 m, for a total of 32 soil samples and 32 root samples. These stands were randomly selected within the forested dune but were at least 100 m from each other to minimise spatial autocorrelation. Root identity was confirmed by tracing roots back to the main trunk. Fine roots from at least two main roots were collected and mixed together for each individual tree sampled. Each soil sample was composed of a mixture of six 0-25 cm deep soil cores collected randomly within a 1 m^2 plot located around the tree trunk. We measured gravimetric water content, conductivity, pH, extractable phosphorus (Mehlich-III), total phosphorus, organic carbon, and total nitrogen were measured on seven air-dried soil samples to characterize soil properties at the site (Table 4.1). Overall, soils were sandy and relatively low in available water and nutrients (Table 4.1). Roots were surface-cleaned with 70% ethanol, rinsed three times with deionized water and then cut in 1-5 mm long fragments. Samples of roots and approximately 15 ml of the soil samples were frozen at -4 ^{o}C within 6 hours of sampling for subsequent molecular analysis.

4.4.2 Description of ectomycorrhizal fungal communities using 454 pyrosequencing

Root fragments were disrupted using the TissueLyser (QIAGEN, Valencia, CA, USA) with four 30-second cycles. We extracted total genomic DNA from 100-200 mg of root

material using the NucleoMag 96 Plant DNA extraction kit (Macherey-Nagel, D-Mark Biosciences, Toronto, ON), and from 250-300 mg of soil material with the PowerSoil™ DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA) according to instructions by the manufacturer. The internal transcribed spacer (ITS) regions were then amplified using the ITS1F and ITS4 primers (White *et al.,* 1990; Gardes & Bruns, 1993). This region includes the two highly variable spacers ITS1 and ITS2, and the intercalary 5.8S gene. The directional GS FLX Titanium adaptors A and B (including a four-base library key sequence) were attached at the 5' end of the primers, and a unique 12-bp Multiplex Identifier (MID) was added between the library key and the template-specific sequence of the forward primer to allow sequences to be assigned to samples. We performed polymerase chain reaction (PCR) in triplicates using for each sample: 0.5 U of Qiagen Taq DNA Polymerase (Qiagen, Toronto, ON), 1X of the manufacturer's reaction buffer, 0.275 µM of each primer and dNTPs, a final concentration of 2.75 µM $MgCl_2$, and 0.83 µL each of 1% Tween 20, DMSO and BSA, as well as 2 µL of diluted DNA (1:10) in a total volume of 20 µl. The cycling conditions were 94 °C for 5 min, followed by 32 cycles of 94 °C for 45 sec, 55 °C for 35 sec, and 72 °C for 1 min, and a final elongation of 72 °C for 7 min. Triplicates were pooled, then purified with the NucleoMag 96 PCR clean-up kit (Macherey-Nagel, D-Mark Biosciences, Toronto, ON), and quantified with the Qubit® 2.0 Fluorometer (Life Technologies, Burlington, ON). An equal amount of amplified DNA from each sample was combined into a single pool, and sent for pyrosequencing using Roche 454 GS-FLX+ chemistry at the Genome Québec Innovation Center (McGill University, Montréal, QC).

We extracted and grouped *fasta* and *qual* files from the *sff* files provided by the sequencer using Mothur v.1.29.2 (Schloss *et al.,* 2009), and then imported sequences into Qiime (Caporaso *et al.,* 2010) for quality filtering and reassignment to samples. Low quality ends were trimmed using a minimum quality score of 25 within a moving window of 50 bp. We excluded sequences shorter than 200 bp, longer than 1000 bp, with more than two ambiguities (Ns), with homopolymers longer than 8 bp, or with two or more mismatches in the primer or barcode. Sequences were pruned to a fixed length (300 bp) with Mothur in order to avoid the partial coverage problem, which can result in erroneous or low-quality consensus sequences (Edgar, 2014). Chimera control and sequence clustering was done using Usearch v7.0 (Edgar, 2010). Operational Taxonomic Units (OTUs) were determined at a similarity

level of 97% and reads were then mapped back into an OTU table. All global singletons (i.e. OTUs represented by only one read in the whole dataset) were eliminated to avoid any artefacts that could be attributed to sequencing errors (Tedersoo *et al.,* 2010) and to improve the accuracy of diversity estimates (Ihrmark *et al.,* 2012). Taxonomy was assigned to each OTU consensus sequence using the UNITE database (Kõljalg *et al.,* 2013) in Mothur, which provides a Naïve-Bayes classifier with a minimum bootstrap value of 60%. Raw sequence data were deposited in the NCBI Sequence Read Archive and are available under the project number PRJNA286207. Identified OTUs were manually screened for potential EM interactions based on the more recent list of EM fungal taxa (Tedersoo & Smith, 2013) and retained for further analysis. Finally, using the UNITE database, we also attributed an exploration type (cord-forming or simple mycelia) as described by Agerer (2001) to each EM OTU.

4.4.3 Fungal diversity assessment

All statistical analyses were conducted in R v.3.0.2 (R Foundation for Statistical Computing; available at http://www.R-project.org), unless indicated otherwise. In order to evaluate the adequacy of sampling and sequencing depth, and to allow for comparison of richness among samples, a rarefaction analysis was performed using the 'iNEXT' package (Hsieh, 2013). Total OTU richness was evaluated with the Chao estimator (Chao, 1984) and sample coverage was computed as suggested by Chao and Jost (2012). We also used the approach of rarefying each sample to an equal number of sequences and computed coverage again. We rarefied at the level of 100 reads, and 5 samples (2 roots and 3 bulk soil) were discarded from the analysis because they were represented by less than 100 sequences. We conducted ANOVA and Tukey HSD post-hoc tests using the 'aov' and 'TukeyHSD' functions of the 'stats' package to identify significant differences among host tree species in terms of EM OTUs richness. We also assessed the taxonomical composition of roots and soil EM communities by computing relative read abundance and OTU richness of major fungal orders.

To explore the patterns of fungal OTU composition among samples, we used principal coordinate analysis (PCoA). The Hellinger distance was computed using the 'decostand' and 'vegdist' function in 'vegan'. This distance metric emphasises differences in relative rather than raw abundances (Anderson *et al.,* 2011; Ramette, 2007), which was deemed appropriate

in this case because the number of reads is not a direct measure of OTU abundance in the environment. For completeness, we also used the Sorensen distance, which is completely unweighted (i.e. relying on presence-absence data). Permutational multivariate analysis of variance was performed with the 'adonis' function in 'vegan' with 9999 permutations to evaluate the statistical significance of differences in OTU community structure among host species and sample type (i.e. roots vs soil). When a main term was significant, we performed post-hoc pairwise comparisons and corrected *P*-values for multiple comparisons with the Holm correction, using the 'p.adjust' function in the 'stats' package.

4.4.4 Quantification of network structure

A quantitative interaction matrix was constructed by computing the frequency (i.e. number of occurrences) of each EM OTU across the eight replicates for the four host species for both roots and bulk soil samples. To avoid describing patterns that are caused by very low frequency taxa, we only considered OTUs that were present in at least 10% of samples for the analysis (i.e. in at least four soil or root samples respectively). We computed two community-level properties that are widely applied in the analysis of bipartite ecological networks (Deng *et al.,* 2012; Fortuna *et al.,* 2010): nestedness and modularity. Nestedness gives a measure of the degree of hierarchy in the organization of the interactions. High nestedness occurs when the most specialized species of one class interact mainly with the generalist species of the other class (Bascompte *et al.,* 2003). Nestedness was measured as WNODF (Weighted Nestedness metric based on Overlap and Decreasing Fill), which is a quantitative adaptation of the NODF index (Almeida-Neto & Ulrich, 2011). This index, which ranges from 0 to 100, was computed using the 'FALCON' package (Beckett *et al.,* 2014).

Modularity is a network property evaluating the presence of modules, i.e. subgroups of closely connected species. A highly modular network would display a large number of modules and/or very well-defined and secluded modules. Weighted modularity was assessed using the QuanBiMo algorithm recently developed by Dormann and Strauss (2014). This algorithm uses a hierarchical random graph approach (Clauset *et al.,* 2008) which transposes the network into a dendrogram and randomly swaps branches to find the optimal division into modules to maximize Q. This approach is implemented in the 'bipartite' package and available through the 'computeModules' function. We used 10^7 steps (or swaps) after which the run was

terminated in the absence of further improvement. The degree *Q* of modularity, the number of detected modules and the affiliation of species to modules were recorded from the optimal run. Significance of both nestedness and modularity was tested by comparing observed values to those of 1000 permuted matrices generated from a conservative null model that preserves row and column sums (Beckett *et al.,* 2014).

Finally, we computed the paired difference index (PDI) with the 'getspe' function of the 'ESM' library in order to determine the degree of specificity of the different hosts and EM fungal OTUs. PDI is a robust specialization index that relies on continuous quantitative data, i.e. on the strength of links between species, to classify species as generalists or specialists (Poisot *et al.,* 2012). We also used the indicator species analysis to identify indicator OTUs of each host species, which is available in the 'indicspecies' package in R (Cáceres & Legendre, 2009). We conducted this analysis on presence-absence data. The indicator values were group-equalized and their statistical significance was tested by a randomization procedure with 999 permutations. The 'visweb' function of the 'bipartite' package was used to produce a visual representation of EM OTUs occurrence across replicates for each host species. We used Cytoscape (Shannon *et al.,* 2003) for visualizing our bulk soil and roots species interaction networks with the edge-weighted spring embedded layout and edge representation weighted by their betweenness.

4.5 Results

4.5.1 Patterns in EM fungal richness

454-sequencing of the ITS regions and quality filtering yielded a total of 190,600 sequences which clustered into 1613 fungal OTUs, excluding chimeras and singletons. We retained 200 of these OTUs identified as EM fungi for further analysis (Tedersoo & Smith, 2013); these were represented by 34,192 sequences, corresponding to approximately 18% of total reads (described in Annexe 2). One hundred and sixty-eight of these OTUs were associated with roots samples and 185 with bulk soil samples. Rarefaction analysis indicated that our sequencing depth was adequate: all curves reached or were close to reaching an asymptote (Figure S4.1, Supporting Information) and all samples had a Good's coverage value

close to 1 (Table S4.1, Supporting Information). Rarefaction analysis also showed that we accomplished sufficient sampling effort, i.e detection of over 50% of fungal taxa (Bahram *et al.* 2014). Based on the Chao estimator of total richness, we detected 65.28% of taxa in soil communities and 71.92% in root communities on average. We recorded 16 EM OTUs, on average, in each root sample and of 22 EM OTUs in each bulk soil sample.

There were no significant differences in rarefied OTU richness among hosts (Table S4.1, Supporting Information). However, there were some significant differences in Chao estimator values for total OTU richness (Figure 4.1). In particular, *Picea mariana* and *A. balsamea* supported a higher root-associated rarefied OTU richness than that found in *Pinus banksiana*. Also, as much as 50% lower average richness (both observed and total) was observed in soils associated with *Pinus mugo* compared to some other host species. Specifically, bulk soil EM fungal richness in *A. balsamea* and *Pinus banksiana* was significantly greater than that observed in *Pinus mugo* (Figure 4.1).

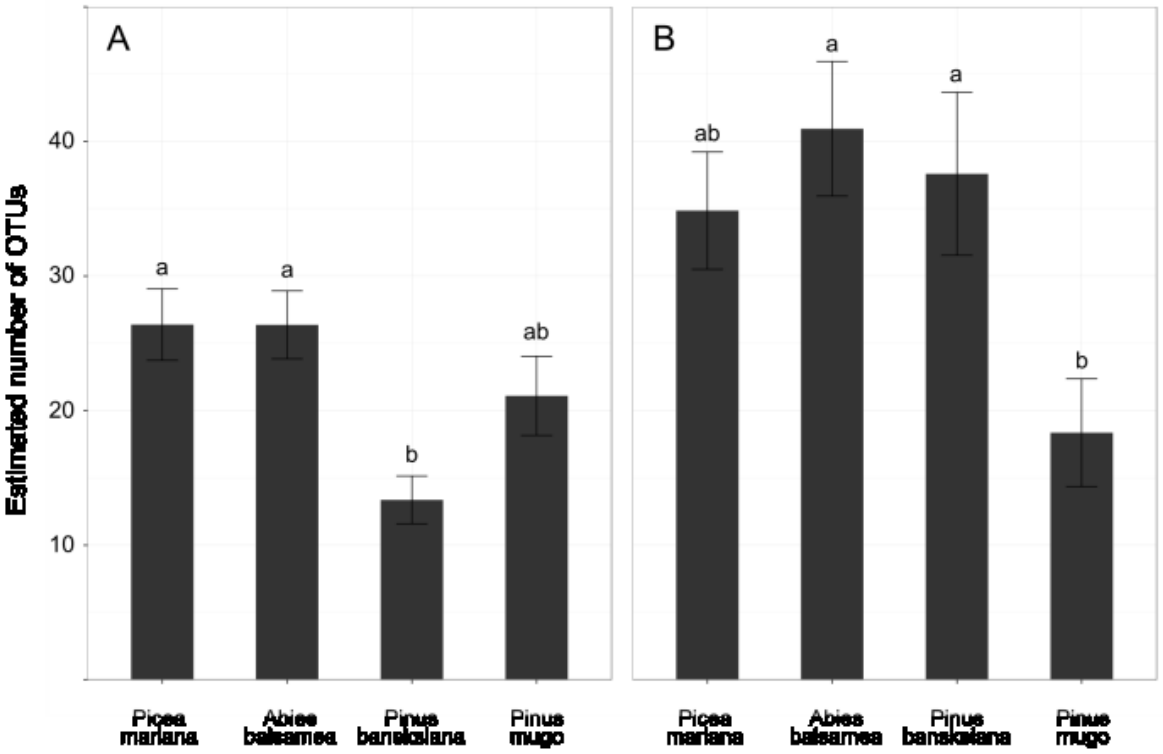

Figure 4.1. Estimated total EM OTU richness across the different host species for roots (a) and soil (b) samples. Total richness was evaluated with the Chao estimator. Letters indicate significant differences among hosts assessed with Tukey HSD post hoc tests ($P \leq 0.05$). Bars show mean values and error bars indicate standard error ($n = 8$).

4.5.2 Community structure

Ectomycorrhizal OTUs belonged to 51 different genera, 31 families, 14 orders and 5 classes (Agaricomycetes, Pezizomycetes, Leotiomycetes, Dothideomycetes, and Sordariomycetes; Figure 4.2). Agaricomycetes were the dominant class in terms of both relative sequence abundance and number of OTUs. Two families (Russulaceae and Atheliaceae) together accounted for over 50% of total sequences and contributed significantly to overall OTU richness, with 23 and 22 OTUs in each group, respectively (Table S4.2, Supporting Information). Russulaceae and Atheliaceae, together with Cortinariaceae and Sebacinaceae, represented half of the total EM richness observed (100 OTUs). There was considerable variation in the composition of root and bulk soil associated EM fungal communities within replicate individuals of the same tree species, and only a few significant differences among host species were detected, depending on the distance or dissimilarity metric used (Figure S4.2, Supporting Information). Analyses based on the Hellinger distance revealed that *Picea mariana* supported soil communities that were significantly different from those found in *Pinus banksiana* ($P = 0.009$) and *Pinus mugo* ($P = 0.0295$), while the analysis based on the Sorensen dissimilarity showed a significant difference between the root-associated communities of *Picea mariana* and those of *Pinus banksiana* ($P = 0.0390$).

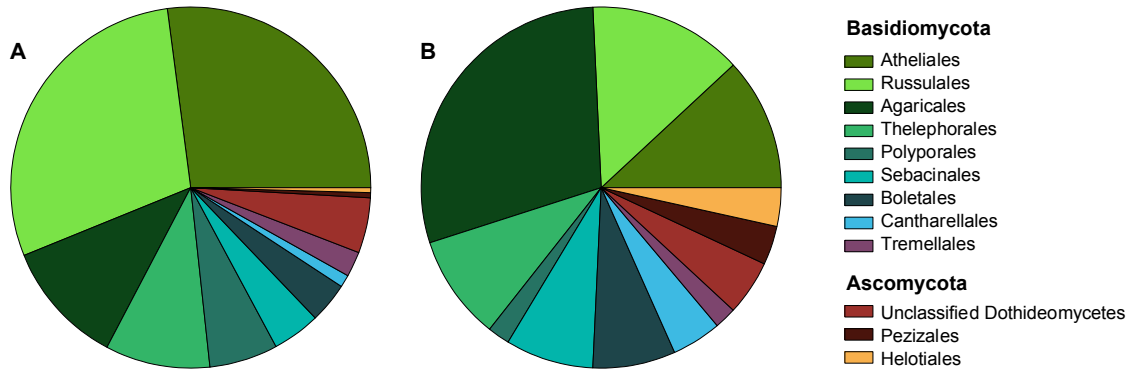

Figure 4.2. Frequency distribution of EM fungal taxa in terms of (a) number of reads and (b) number of OTUs. The charts display the combined data of all four hosts for both root and soil samples.

4.5.3 Architecture of belowground plant host-EM fungi interaction networks

Our analysis revealed significant anti-nestedness in the tree host-EM fungal interaction network. Indeed, we calculated a weighted NODF of 25.28 for roots and of 29.40 for bulk soil network, which corresponded to a significantly lower (corrected *P*-value $\leq$ 0.05) level of nestedness than that expected under a null model (roots, mean WNODF: 33.79, standard deviation: 2.18, *Z*-score: -3.90; soils, mean WNODF: 34.87, standard deviation: 1.91, *Z*-score: -2.86). On the other hand, modularity values for both roots and bulk soil were relatively low and did not differ significantly from the null expectation. We recorded modularity levels of 0.1914 and 0.1521 against expected means of 0.2005 and 0.1788 (standard deviations: 0.0169 and 0.0166; z-scores: -0.5422 and 1.6191 for roots and bulk soil network respectively (Figure S4.3, Supporting Information).

Despite the fact that ordination analysis highlighted only minor differences in EM community composition among hosts, a large proportion of OTUs was associated with only one host species (51.1% for roots and 39.5% for bulk soil) (Figure 4.3). There was also a large number of OTUs that were associated with three of the four host species (14.0% and 16.8%) and with all hosts (10.4% and 13.5%). We recorded slightly more OTUs associated with a simple mycelia exploration strategy (94 OTUs in the bulk soil samples and 86 in roots samples) than cord-forming fungi (73 in bulk soil and 65 in roots). Degree (i.e. the number of hosts to which EM OTUs are associated) distribution did not differ between the two exploration types (cord-forming and simple mycelia) as revealed by a chi-square test (χ^2= 7.2993, df = 6, *P*-value= 0.2941) (Figure S4.4, Supporting Information). This indicates that the two exploration types display similar patterns of specificity of association.

Our data revealed some phylogenetic patterns in host-EM interactions. In both roots and bulk soil samples, EM OTUs belonged predominantly to the Agaricomycetes. In addition, all observed Pezizomycetes were associated with *Pinus banksiana*, whereas some *Sebacina* were strictly associated with *Picea mariana* and *Abies balsamea.* Operational taxonomical units belonging to the Dothideomycetes always interacted with at least two different host species and often three or four, and thus could be considered as generalists. We recorded only one OTU from the Sordariomycetes.

4.5.4 Plant – EM fungal associations

Despite the low overall degree of host specialisation among EM fungal OTUs revealed by network analyses, we still observed a number of specialised EM fungal OTUs (Figure 4.4). From the 47 OTUs that occurred in at least 10% of samples (i.e. frequency > 0.1), 29 had a PDI above 0.5 and could therefore be considered as specialists. By contrast, only 13 OTUs had a PDI below 0.5 and could be defined as generalists. *Tylospora* sp., three *Cenococcum* OTUs, an unclassified *Amphinema*, and a *Lactarius deceptivus* were the generalist OTUs with the lowest PDI, as well as an unidentified Sebacinaceae. On the other hand, some OTUs were mostly restricted to particular hosts. For example, indicator species analysis designated *Piloderma* sp., *Tomentellopsis* sp., a *Cenococcum geophilum*, an unclassified *Amphinema* and an uncultured *Sistoderma* as privileged partners of *Picea mariana* (Figure 4.4). Operational taxonomy unit 124 (*Russula bicolor*) was identified as a specialist of *A. balsamea*, OTU 104 (uncultured *Sebacina*) of *Pinus banksiana*, and OTU 215 (*Suillus* sp.) of *Pinus mugo*. The PDI of all host trees was above 0.5, which classifies them as specialists and indicate that their roots support distinct EM fungal communities.

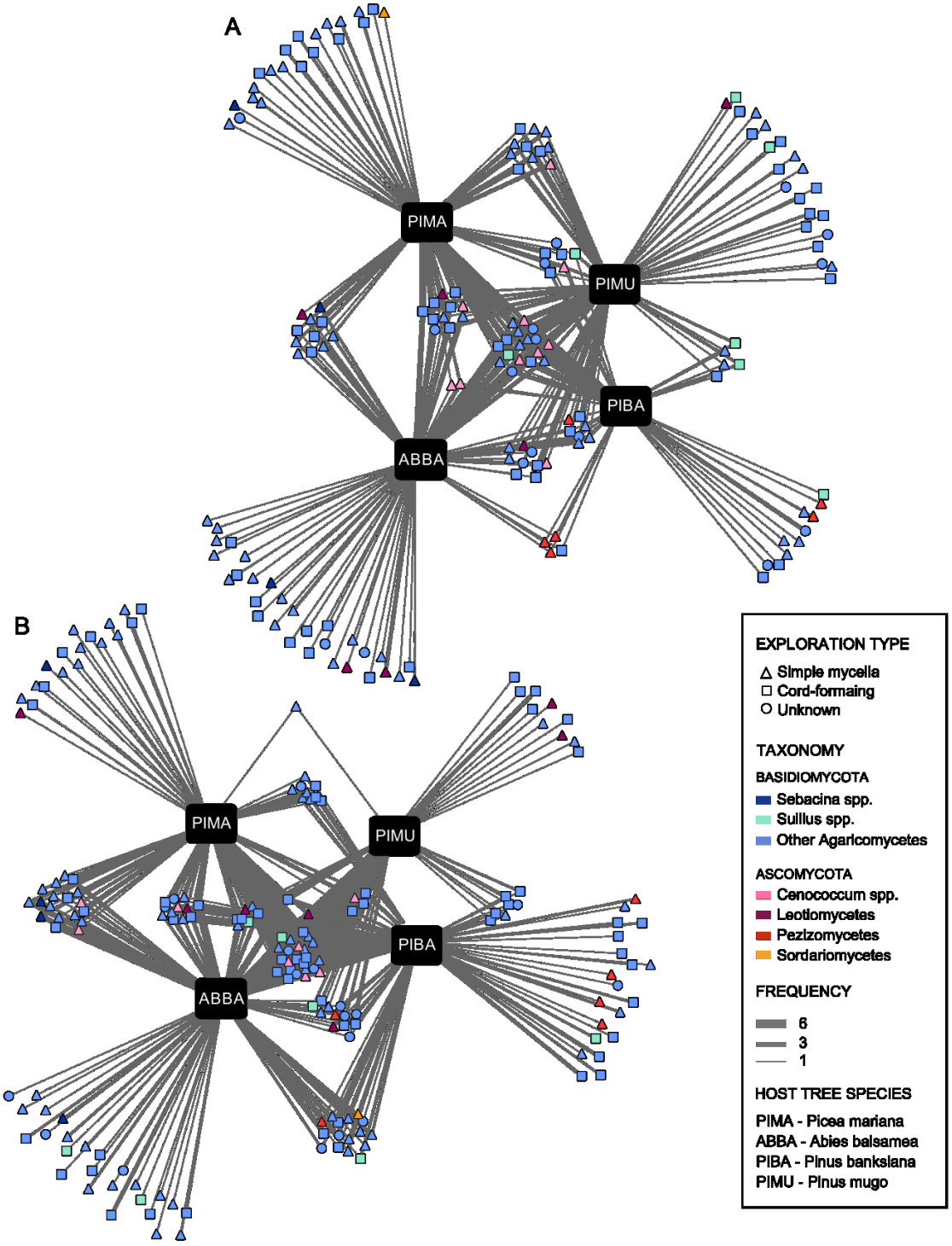

Figure 4.3. (a) Roots and (b) bulk soil EM interaction networks. The four hosts are represented by black boxes; EM fungal OTUs are coloured according to their taxonomy and their shape indicates exploration type. Edges (i.e. links between EM fungal OTUs and host trees) width is proportional to the frequency of observation.

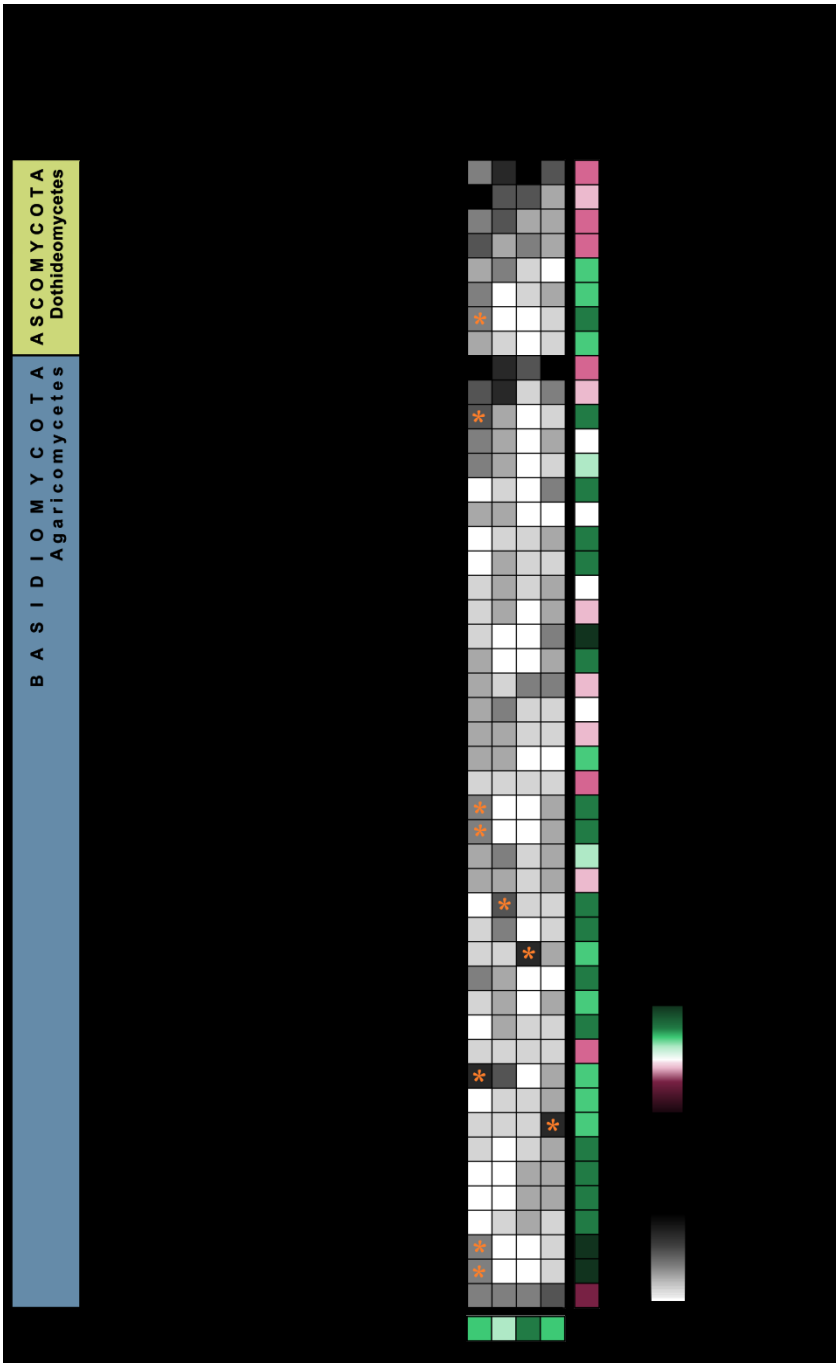

Figure 4.4. Association specificity of EM fungal OTUs with a frequency of at least 0.1 in tree roots showing frequency across replicates for each host species and PDI values. The total number of samples is 32 (four tree species with eight replicates). PDI is a specialization index ranging from 0 (generalist) to 1 (specialist). Orange stars indicate significant ($P \leq 0.05$) associations (i.e. OTUs having a significant indicator value of at least 0.5). Species attributed to each OTUs correspond to identity assigned using the UNITE database and number in parenthesis are the bootstrap values.

4.6 Discussion

Using an ITS-targeted pyrosequencing approach, we were able to describe EM fungal communities among four co-occurring tree hosts in a coastal dune boreal forest. Network analysis of plant-EM fungal interactions revealed an anti-nested pattern, but we could not detect significant modules of closely interacting species. Furthermore, we did not observe clear patterns of variations in OTU richness nor community composition among host species. Despite the relative low level of host preference observed, a number of less frequent OTUs nonetheless appeared to show some degree of specialization to a given host. The computation of PDI index as well as indicator species analysis allowed us to identify some specialist OTUs displaying specialized patterns of association. Generalists – i.e. OTUs associated with several or all hosts – still represented a large proportion of the EM fungal communities and might be important for community resilience as they improve connectivity in the network and enhance its stability. Overall, our findings point to relatively low levels of specialization of EM associations, contrary to our initial expectation of community distinctiveness and host preference.

Despite the generally low level of specialisation of EM association observed overall, OTUs interacting with only one host species represented an important proportion of recorded EM richness (51.1% of OTUs for roots and 39.5% for bulk soil). *Abies balsamea* supported the higher number of rare OTUs (OTUs encountered in only one root or soil sample) with 20 in soil samples and 28 in root samples. The number of rare OTUs per host species ranged between nine and 28. Even if it is difficult to discriminate OTUs that are preferentially associated with a given host from those infrequent species with undetected occurrences (Bahram *et al.* 2014), we are confident that most OTUs we identified as specialists are so, because rarefaction analysis indicated adequate sequencing depth. In addition, we met the 50% threshold of sampling effort, i.e. detection of at least 50% of taxon for the community, as recommended by Bahram *et al.* (2014). Operational taxonomic units of the *Sebacina* genus appeared to specialize on *Picea mariana* and *Abies balsamea.* Interestingly, *Picea*-*Sebacina* associations have been reported before (Warcup, 1988; Baier *et al.,* 2006). *Suillus* and other members of the sulloid group are known to display narrow association patterns, most species specializing on a single host genus, and are known as exclusive symbionts of Pinaceae

(Cairney & Chambers, 1999; Smith & Read, 2008). In this study, seven different OTUs were identified as members of the *Suillus* genus, which seemed to be found more frequently in association with the two *Pinus* – especially OTU 215, identified as a significant indicator of *Pinus mugo*. Association with specialists such as *Suillus* might be advantageous for the host tree if it reduces the risks of resource diversion to other competing tree species through the common mycelial network (Molina *et al.*, 1992; Smith & Read, 2008). Such functional outcomes of specialist plant-fungal interactions deserve to be further explored.

Despite the presence of these rare EM fungal OTUs, only a few minor significant differences in richness and community composition were detected. Overall, our results therefore indicate a relatively low level of host preference. We recorded a large proportion of fungal OTUs interacting with three or four different hosts. Even if no OTU was found in all roots or soil samples (the most widespread OTU, an *Amphinema* species, was present in around 70% of all samples), these taxa can interact with diverse host species and are potential generalists. In our network analysis, all OTUs from the *Cenococcum* genus were connected to several hosts and therefore could be seen as generalists. This genus is known to be a taxon with a very wide distribution and *C. geophilum* – one of the most widespread EM fungal species in the environment with an exceptionally wide habitat range – is considered as a "super-generalist" (Cairney & Chambers, 1999; Smith & Read, 2008). It is also known to act as a pioneer species and invade newly formed soils such as glacial moraines, volcanic ash and sand dunes during primary succession (Cairney & Chambers, 1999). In our study, four OTUs of the genus *Cenococcum* were classified as generalists (OTUs 146, 152, 130 and 41). The functions and benefits of cosmopolitan taxa such as *Cenococcum* are however still debated (Smith & Read, 2008). Generalist taxa might contribute to the resilience of ecological interaction networks by increasing connectivity within the network, which enhances its stability; whereas specialists enhance diversity (Bascompte & Jordano, 2007). Both categories contribute importantly to network structure.

Network analyses have gained recent interest to explore the structure of mutualistic interaction networks (Bascompte & Jordano, 2007; Bascompte *et al.,* 2003; Bastolla *et al.,* 2009), including mycorrhizal interactions (Bahram *et al.,* 2014; e.g. Chagnon *et al.,* 2012; Jacquemyn *et al.,* 2015). While mutualistic plant-pollinator networks generally display a nested structure, which minimizes interspecific competition and enhances diversity (Bastolla

et al., 2009), most studies on ectomycorrhizal networks have not detected such a nested pattern (Bahram *et al.,* 2014). As such, the anti-nested patterns we observed in both root-associated and bulk soil interaction networks are consistent with the findings of Bahram *et al.* (2014), where the authors found anti-nested networks in half of the data sets analysed. This pattern could have suggested the presence of subgroups of closely interacting species, i.e. modules. However, contrary to our hypothesis, we did not find significant modularity in EM interaction networks. The *Q* values of modularity that we recorded were much lower – with one exception – than the modularity levels recorded by Bahram *et al.* (2014) and were not significantly different from the null expectations. The absence of a positive nested pattern could be attributed to the fact that we sampled only four host species, which could be insufficient to statistically detect nestedness (Bascompte *et al.,* 2003). Larger networks were generally found to be more nested than smaller networks (Bascompte *et al.,* 2003), suggesting that our estimates of nestedness might have been conservative. Low and non-significant modularity, on the other hand, indicates the absence of specialized associations. Modularity measures are known to be sensitive to the total number of achieved links in a network, i.e. the existence of a specialized interactions could be obscured by the presence of a host with a high number of links. As such, Bahram *et al.* (2014) were able to detect higher and significant modularity by achieving equalized random sampling (i.e. randomly selecting the same number of EM OTUs for each host). Still, in the present study, the absence of modules is consistent with the observation of a large proportion of fungal OTUs associating with three or four different hosts. Moreover, OTUs interacting preferentially with one host species were infrequent – as discussed above, most of them were detected in a single sample – resulting in a non-modular network structure.

In recent studies, host identity has often been identified as the strongest predictor of EM fungal community composition and structure (e.g. Ishida *et al.,* 2007; Morris *et al.,* 2009 ; Tedersoo *et al.,* 2013), and has even been proposed as the main determinant of EM fungal community structure (Murata *et al.,* 2013). Still, the extent of this host effect remains incompletely understood, mainly because of the difficulty to unravel the complex interactions between host plants, microbial communities, and soil and environmental properties (Aponte *et al.,* 2010; Peay *et al.,* 2015).

A major challenge in the interpretation of field studies results is the confounding effect of environmental covariation affecting both tree hosts and EM fungi. In this study, we sampled a relatively small section of the dune system with relatively homogenous environmental, climatic and edaphic conditions, thus allowing us to examine EM fungal community structure and network architecture with minimal variations of the abiotic environment. Still, we did not observe clear effects of host identity on EM fungal richness or community composition. We recorded a total of 200 EM OTUs and the richness levels we observed in samples ranged from 4 to 46 and estimated total richness from 4 to 63, which is high but within the same range as other EM studies conducted in different ecosystems (e.g. Ishida *et al.,* 2007; Aponte *et al.,* 2010; Murata *et al.,* 2013; Tedersoo *et al.,* 2013; Peay *et al.,* 2015). Overall, our results indicate that while total EM fungal diversity was high, all hosts mostly showed similar levels of EM fungal diversity in their roots. Ordination analysis did not reveal clear segregation among host species in terms of EM fungal community structure either. The level of intra-host variability was high and we observed only few significant differences among EM communities associated with the different hosts.

One potential explanation of the low variation in community composition among host species would be that the four tree species all belong to the same family (Pinaceae), and as such plant-EM fungal interactions might be well conserved at the family level. Taxonomic relatedness was indeed found to be one of the main factor governing host-effect among Salicaceae trees (Bell *et al.,* 2014; Tedersoo *et al.,* 2013). Multi-host studies in temperate mixed forests also revealed positive correlations between host taxonomic distance and the distinctiveness of the EM communities they support (Ishida *et al.,* 2007; Morris *et al.,* 2009; Murata *et al.,* 2013). Peay *et al.* (2015) also studied closely-related hosts (13 genera of Dipterocarpaceae) and observed weak differentiation of EM communities. This study showed that edaphic specialisation (i.e. similar reaction of microbes and plants to edaphic conditions) accounted for the covariation observed amongst host taxonomy and EM community composition, not host specialization itself. Conversely, some plant families or genera such as *Alnus* are also known to display highly specific patterns of interactions, even at a regional scale (Roy *et al.,* 2013). Other studies also found that host phylogeny explained important proportions of variation in EM communities associated with members of the Salicaceae (Tedersoo *et al.,* 2013; Bell *et al.,* 2014). Moreover, the few significant differences that we

detected in both richness and community structure separated *Picea mariana* from the two *Pinus* species, reinforcing the idea that taxonomic relatedness is a key factor governing host effect and explaining variations in EM community composition. It is possible that genotypic variations of closely related host species trigger changes in EM fungal communities (Sthultz *et al.,* 2009) if associated phenotypes are ecologically divergent (Peay *et al.,* 2015), which might not be the case in our study. In the particular case of Pinaceae, there are several earlier reports of an important overlap in EM community composition among different tree species suggesting the predominance of multiple-host fungi (e.g. Horton & Burns, 1998; Kranabetter *et al.*, 1999; Cullings *et al.*, 2000). For example, Horton *et al.* (2005) found as much as 95% of EM species in common between western hemlock seedlings and co-occuring Douglas-fir. This could also explain the low and non-significant modularity we observed.

Two hosts (*Pinus mugo* and *Pinus banksiana*) were introduced in this dune system less than 75 years ago, when they were planted for dune stabilisation. Because they belong to the same genus, it is not possible to attribute any observed similarities to the fact that they are both non-native. Interestingly, we observed low level host specialization even between the native and introduced hosts, meaning that the soil and root fungal communities associated to the planted trees probably integrated rapidly native EM fungi and shifted to a local microbiota. This observation supports the idea that taxonomic relatedness governs host effect, as taxonomy seemed to have a more important effect than ecological history on EM fungal communities in these Pinaceae stands. Still, we identified OTUs that were specific to either *Pinus* species, of which some could be co-introduced EM fungi that persisted in plantations. Tedersoo *et al.* (2007) also observed overlapping EM communities associated to introduced eucalyptus and native dipterocarps in the forests of tropical African islands. It is also very likely that the introduction of new tree species (with their potting soil) modified the native soil fungal community, and some introduced EM may have become established in this system. The potential of EM fungi for host range expansion (Bahram *et al.*, 2013) would allow such adaptations and make possible the association of exotic EM fungi with native trees and vice versa, especially amongst closely related hosts.

In conclusion, our study revealed no strong effect of host identity on EM richness and community composition associated with four co-occuring Pinaceae. The examination of network structural properties suggests a relatively low level of host specialization in these EM

interactions. The lack of differences in EM fungal richness and community structure among hosts, as well as the absence of specialized subgroups of interactions (modules) could be attributed to the taxonomic relatedness and ecological similarity of our four host tree species, which all belong to the same family (Pinaceae). Therefore, important effects of host identity might operate at a higher taxonomic level. Low specificity might be advantageous for host trees by increasing their chance of finding suitable EM partners. This could be ecologically important, especially in a nutrient-poor environment such as sand dunes where trees rely strongly on EM fungi for nutrient uptake. We observed a high level of EM diversity (200 OTUs) despite the absence of fungal community differentiation amongst the four co-occuring hosts, raising the question of how such high fungal diversity is maintained if hosts have similar EM fungal community composition. Future studies should attempt to determine which mechanisms limit competitive exclusion among EM fungal species and allow the coexistence of a large number of EM fungal species in this habitat. Our study, as a first analysis of EM interactions in a coastal dune forest, provides further insights about the architecture of tree root-EM fungal species interaction networks, and also raises some questions about the mechanisms promoting EM fungal species coexistence.

4.7 Acknowledgments

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4.8 Supplementary information

Table S4.1. Number of reads, richness and coverage of EM fungal OTUs associated to the four different host trees. Values are mean ± standard error (n = 10) and different letters indicate significant differences ($P \leq 0.05$).

BULK SOIL

	Picea mariana	*Abies balsamea*	*Pinus banksiana*	*Pinus mugo*
Number of reads	507 ± 106	617± 124	362 ± 85	270 ± 68
Observed OTU richness	23.8 ± 1.4 (a)	27.8 ± 3.2 (a)	20.9 ± 2.7 (ab)	13.6 ± 2.2 (b)
Chao	34.9 ± 4.4 (ab)	40.9 ± 5.0 (a)	37.6 ± 6.1 (a)	18.4 ± 4.0 (b)
Good's coverage	0.98 ± 0.01	0.98 ± 0.00	0.96 ± 0.02	0.96 ± 0.03
Rarefied OTU richness *	15.0 ±1.1	16.4± 2.1	13.7 ± 1.5	11.3 ± 1.5
Rarefied coverage*	0.94 ± 0.01	0.94 ± 0.01	0.95 ± 0.01	0.97 ± 0.01

ROOT SAMPLE

	Picea mariana	*Abies balsamea*	*Pinus banksiana*	*Pinus mugo*
Number of reads	1102 ± 335	914 ± 330	266 ± 84	332 ± 85
Observed OTU richness	20.4 ± 2.1 (a)	18.0 ± 2.2 (a)	10.1 ± 1.4 (b)	14.4± 1.7 (ab)
Chao	26.4 ± 2.7 (a)	26.4 ± 2.5 (a)	13.4 ± 1.8 (b)	21.1 ± 2.9 (ab)
Good's coverage	0.99 ± 0.00	0.99 ± 0.00	0.96 ± 0.03	0.97 ± 0.01
Rarefied OTU richness *	11.9 ± 0.9	9.4 ± 0.7	8.7 ± 1.2	10.8 ± 1.3
Rarefied coverage*	0.97 ± 0,00	0.97 ± 0,00	0.97 ± 0,01	0.96 ± 0,00

All values: mean ± standard error.
Different letters indicate significant difference between host species in Tukey HSD test.
*Rarefied at 100 reads.

Table S4.2. Total relative abundance of the different EM fungal families encountered in roots or bulk soil samples in terms of number of OTUs and number of reads. Most families were observed in both roots and soil samples; stars (*) indentify the ones that were observed exclusively in soil samples.

	Number of OTUs	Number of reads	Genus included
Russulaceae*	23	9,644	*Lactarius, Russula*
Atheliaceae	22	9,257	*Amphinema, Piloderma, Tylospora*
Thelephoraceae	15	2,986	*Pseudotomentella, Tomentella, Tomentellopsis, Thelephora*
Sebacinaceae	20	2,245	*Sebacina*
Cortinariaceae	39	2,244	*Cortinarius, Inocybe*
Sistotremataceae	4	2,106	*Sistotrema*
Incertae sedis	10	1,695	*Cenococcum*
Suillaceae	7	988	*Suillus*
Bolbitiaceae	4	602	*Alnicola, Hebeloma*
Amanitaceae	3	370	*Amanita*
Hygrophoraceae	2	341	*Hygrophorus*
Clavulinaceae	3	319	*Clavulina*
Albatrellaceae	5	285	*Leucophleps*
Tricholomataceae	4	223	*Tricholoma*
Boletaceae	3	179	*Boletus*
Incertae sedis	7	153	*Cadophora*
Discinaceae	2	78	*Hydnotrya*
Hydnaceae	1	39	*Hydnum*
Sclerodermataceae	1	34	*Scleroderma*
Entolomataceae	4	32	*Entoloma*
Tuberaceae	2	32	*Tuber*
Helvellaceae	1	32	*Helvella*
Paxillaceae	1	18	*Alpova*
Cantharellaceae*	3	16	*unidentified*
Bankeraceae	4	13	*Hydnellum, Phellodon, Boletopsis, Sarcodon*
Pezizaceae	2	13	*Peziza*
Hysterangiaceae	2	11	*Hysterangium*
Gomphidiaceae	2	10	*Gomphidius, Chroogomphus*
Rhizopogonaceae*	1	5	*Rhizopogon*
Ceratobasidiaceae	2	4	*Ceratobasidium*
Chaetosphaeriaceae	1	4	*Chloridium*

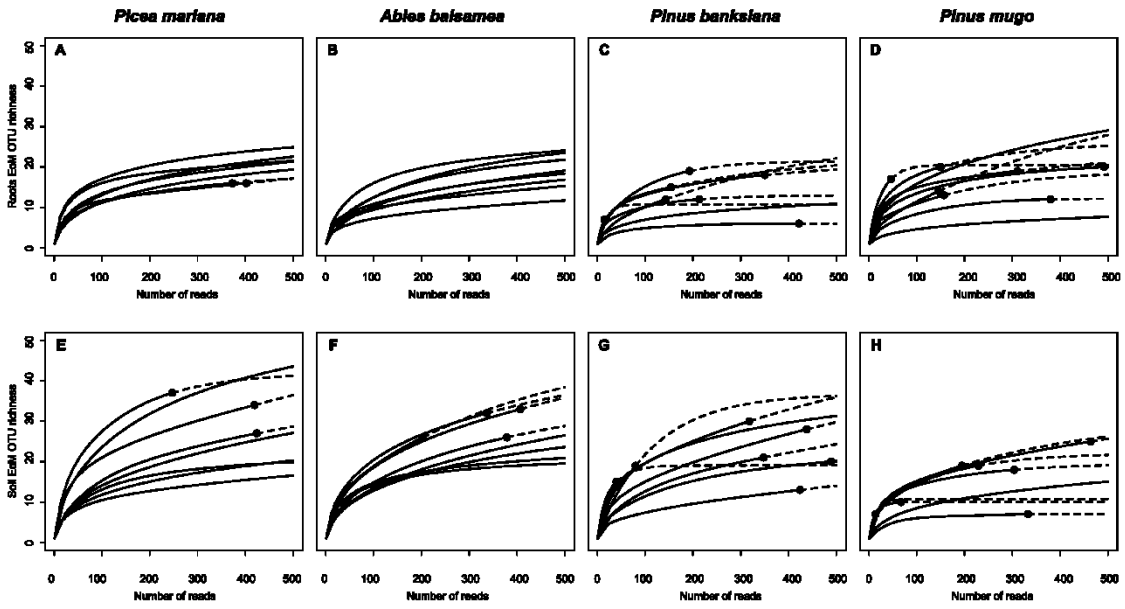

Figure S4.1. Rarefaction curves of EM fungal OTUs in roots (A-D) and soil (E-H) samples against the number of 454 reads excluding singletons for *Picea mariana* (A, E), *Abies balsamea* (B, F), *Pinus banksiana* (C, G), and *Pinus mugo* (D, H). Each curve represents the rarefaction for an individual sample. The dashed section of the curves represents richness extrapolations. The analyses are based on 1000 iterations of re-sampling without replacement.

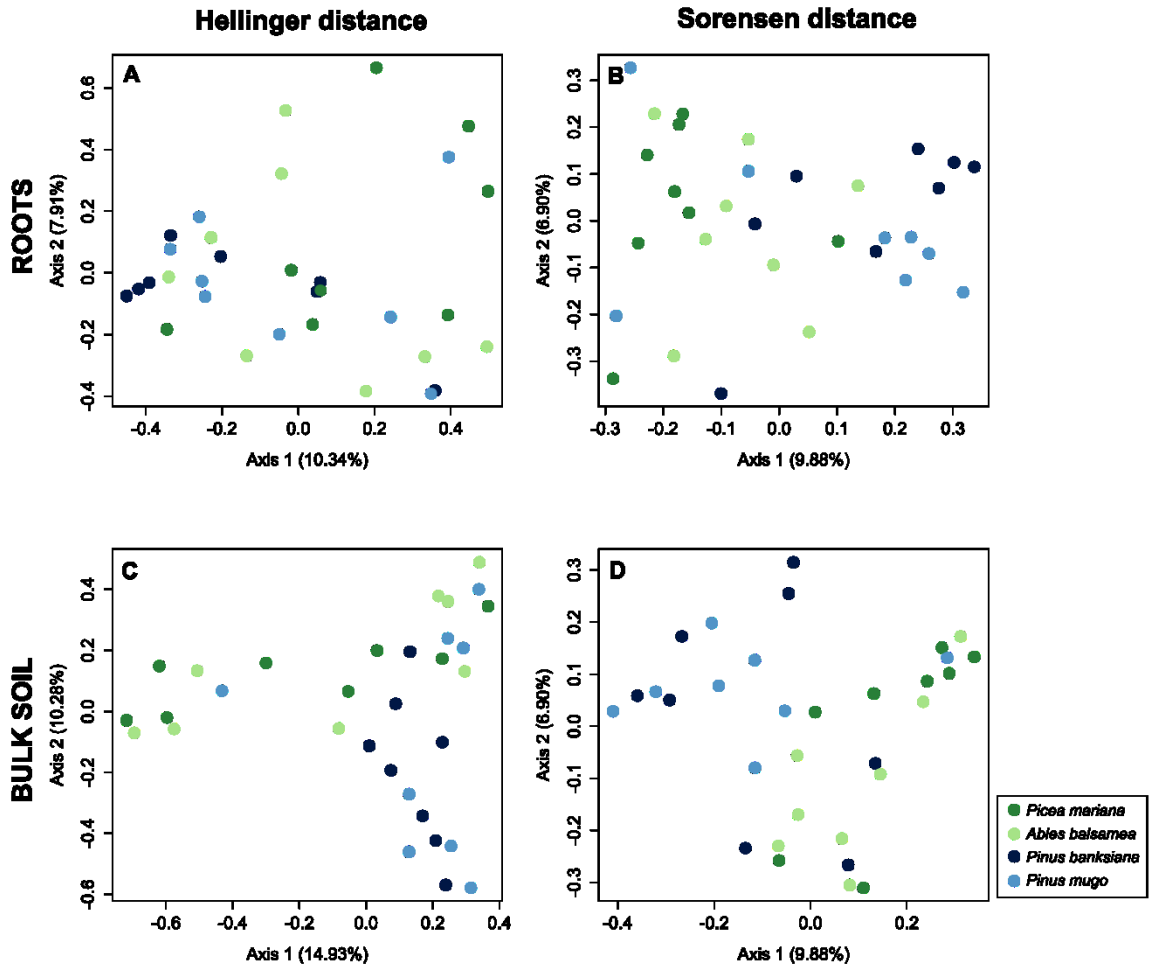

Figure S4.2. Principal coordinate analysis (PCoA) of roots (A-B) and bulk soil (C-D) associated EM fungal community based the Hellinger distance (A and C) and the Sorensen (B and D) dissimilarity.

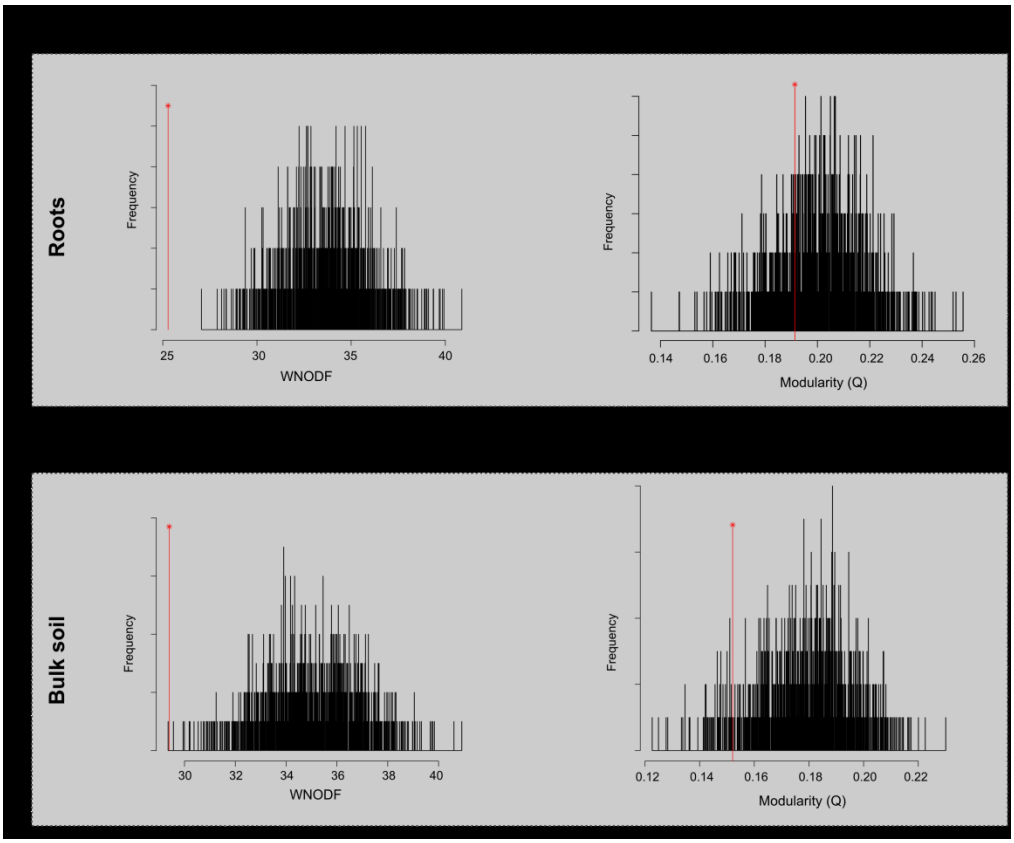

Figure S4.3. Modularity and nestedness of roots and bulk soil data in relation to the 1000 matrices generated with a null model preserving rows and columns sums. The red line indicates the value computed with our data.

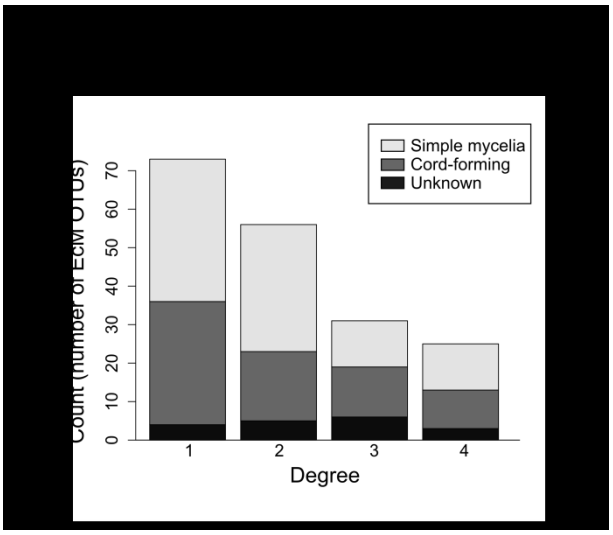

Figure S4.4. Degree distribution of EM fungal OTUs in function of exploration type. The degree is the number of interactions (i.e. the number of hosts to which each EM OTU is associated).

Chapitre 5 | Discussion générale

5.1 Résumé des principaux résultats et faits saillants

Cette thèse de doctorat représente une première description exhaustive des communautés fongiques du sol et de leurs interactions avec la végétation dans une plaine de dunes reliques. Le système des Sillons constitue un terrain d'étude de prédilection vu la succession d'habitat et le gradient de conditions édaphiques qu'il présente sur une superficie limitée, ce qui minimise les variations climatiques et de matériel parental. De plus, étant quasi isolé, l'influence des communautés des territoires adjacents est aussi minimisée.

Grâce au séquençage haut débit, j'ai caractérisé les champignons du sol à travers un gradient édaphique et successionnel (chapitre 2). J'ai pu observer des communautés taxonomiquement et fonctionnellement diversifiées dans toutes les zones de l'écosystème dunaire, incluant les jeunes dunes situées près du littoral. Bien qu'aucun patron clair n'ait été détecté au niveau de la richesse et de la diversité, mes résultats permettent de détecter des communautés distinctes en termes de taxonomie et de modes de vie. Une importante partie de la variance étant demeurée inexpliquée par les propriétés du sol, j'ai ensuite voulu lier ces observations aux dynamiques de la végétation (chapitre 3). Mes résultats ont démontré une très forte corrélation entre la structure des communautés végétales et fongiques. J'ai détecté des communautés végétales distinctes à travers les différentes zones des dunes, ainsi qu'une augmentation significative de la richesse et de la diversité. La diversification des stratégies d'acquisition de nutriments, qui représente une augmentation de la diversité fonctionnelle, pourrait favoriser le partitionnement des ressources et du fait même la coexistence des espèces et la diversité locale. Par contre, les plantes et les champignons du sol répondent de façons similaires aux variations des propriétés physico-chimiques du sol et il semble que des facteurs comme le pH du sol ou le contenu en eau pourraient agir comme un filtre environnemental régissant l'assemblage des communautés dans cet écosystème. Finalement, afin d'investiguer de façon plus approfondie les liens entre la végétation et les champignons du sol, une analyse centrée sur les dunes fixées par des forêts (chapitre 4) a été réalisée et a révélé une faible différentiation des communautés ectomycorhiziennes associées aux racines des principales

espèces arborescentes. J'ai tout de même observé un niveau de diversité élevé, avec 200 unités taxonomiques opérationnelles ectomycorhiziennes. L'identité de l'hôte ne semblait pas avoir une influence sur la structure et la diversité de la communauté microbienne qu'il supporte, ce qui pourrait être expliqué par le fait que les quatre arbres appartiennent à la famille des Pinaceae. Le faible niveau de spécificité des interactions ectomycorhiziennes pourrait augmenter les possibilités d'associations et avoir une importance écologique, particulièrement dans un système pauvre en nutriments comme les dunes de sable.

Globalement, j'ai observé, à travers la succession dunaire, des communautés diversifiées au niveau de la taxonomie et des guildes fonctionnelles, et des structures distinctes selon la zone de la dune, tant chez les champignons que chez les plantes. J'ai toutefois pu observer que la succession semble moins marquée au niveau des communautés fongiques, par rapport aux patrons observés chez les plantes (voir Figure 5.1). Tel que discuté dans le chapitre 2, les champignons pourraient être moins sensibles aux conditions environnementales et donc avoir une distribution plus large, malgré les contraintes abiotiques. Aussi, il est possible que les taxons fongiques détectés ne soient pas « actifs » dans les sols. En effet, l'approche moléculaire, plus précisément l'identification des champignons à partir d'ADN extrait d'échantillons environnementaux, détecterait des taxons présents dans un milieu sans que ceux-ci ne soient établis et métaboliquement actifs (Baldrian *et al*., 2012; Peay 2014). Par exemple, l'ADN d'un fragment de mycélium ou d'une spore tout juste déposée par le vent à la surface du sol serait séquencé et inclus dans notre description des communautés au même titre qu'un taxon bien établi et agissant comme décomposeur ou symbiote des plantes par exemple. Cela pourrait expliquer la présence de champignons ectomycoriziens ou éricoides dans des sites où je n'ai pas identifié d'hôte végétal associé. Quoi qu'il en soit, la présence de taxons fongiques, établis ou pas, représente un potentiel d'action et d'interactions. Ainsi, les sols d'une zone donnée abriteraient un plus large éventail de microorganismes et seraient donc aptes à accueillir les plantes une fois que celles-ci parviennent à passer le « filtre environnemental ». Cela pourrait aussi expliquer l'absence de différences significatives au niveau de la diversité fongique.

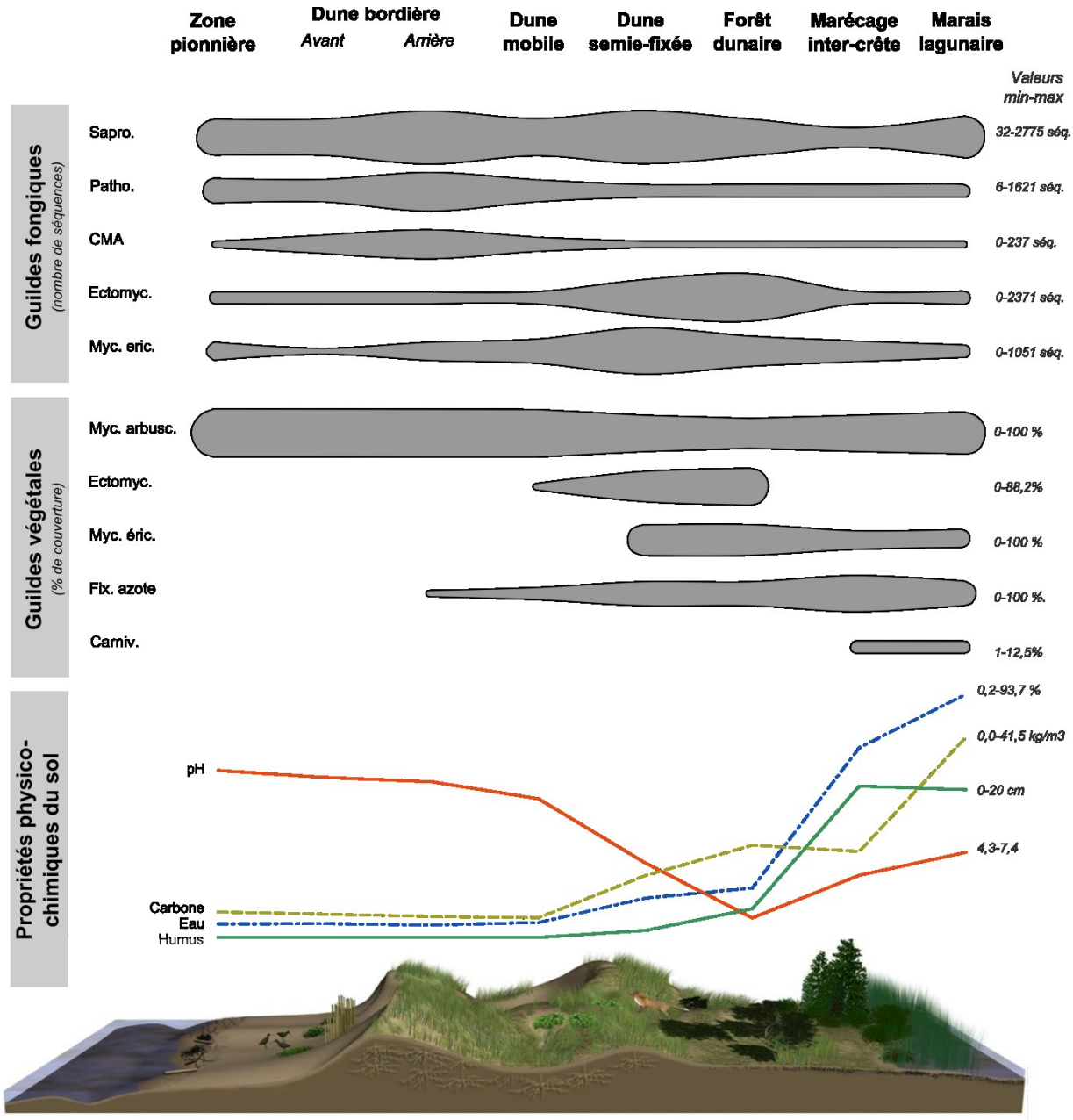

Figure 5.1. Résumé des principales observations au niveau des champignons du sol, de la végétation et des propriétés du sol. Cette figure se veut un aperçu qualitatif des patrons décrits précédemment, non pas une représentation quantitative rigoureuse. La largeur des barres donne un estimé de l'abondance des principales guildes fongiques (en fonction du nombre de séquences) et végétales (selon le pourcentage relatif de couverture au sol). Nous avons aussi représenté les propriétés du sol les plus significatives. Les minimum et maximum par site sont indiqués à droite afin de donner une idée de l'ordre de grandeur des valeurs des différentes variables présentées.

5.2 Applications et potentielles études complémentaires

Les communautés microbiennes du sol ont longtemps été négligées dans l'étude des écosystèmes. C'est toutefois une sphère de l'écologie qui est en pleine expansion grâce aux progrès récents dans le domaine des techniques de séquençage et des sujets comme les interactions plantes-sol obtiennent de plus en plus d'intérêt au sein de la communauté scientifique. L'importance des microorganismes du sol est maintenant reconnue, bien qu'encore intégrée de façon seulement marginale aux pratiques dans des domaines comme l'agriculture, la foresterie ou la restauration écologique. Or, une meilleure compréhension des interactions entre les plantes et les microorganismes pourrait certainement améliorer les pratiques dans ces domaines.

Les connaissances acquises dans le cadre de cette thèse pourraient d'ailleurs éventuellement être transférables à des projets d'aménagement du territoire, de conservation et de restauration écologique, ou au moins servir à guider des futures recherches pour générer les connaissances nécessaires. Les milieux dunaires sont effectivement des écosystèmes fragiles et particulièrement vulnérables face aux changements climatiques. Dans le cas des Îles de la Madeleine, un milieu insulaire donc particulièrement sensible, on commence déjà à observer les effets des changements climatiques : diminution (voire absence comme ce fut le cas en 2010 et 2011) de la période d'englacement, augmentation de la fréquence et de l'intensité des tempêtes et élévation du niveau de la mer (Bernatchez *et al.*, 2008). Ces changements ont comme conséquence d'augmenter les taux d'érosion, particulièrement dans les dunes côtières. Or, la plaine côtière revêt une importance particulière aux Îles de la Madeleine. Les dunes de sable couvrent environ 30% du territoire, représentent plus de 50% de la longueur de côte et remplissent plusieurs fonctions importantes (Bernatchez *et al.*, 2008). D'abord, ce sont les dunes qui relient les Îles entre elles et permettent la communication et le transport au sein de l'archipel. Elles forment aussi un écran protecteur réduisant l'érosion des côtes et prévenant l'ensablement et l'inondation des routes. De plus, les écosystèmes dunaires constituent un habitat essentiel à de nombreuses espèces végétales et animales, par exemple pour la nidification de plusieurs oiseaux migrateurs. Certaines dunes sont des ilots de diversité qui pourraient abriter des espèces endémiques. Des efforts devront continuer d'être déployés afin

d'assurer la conservation de ces milieux et, suite à des épisodes d'érosion ou de perturbations anthropiques, des interventions de restauration pourraient être nécessaires.

Cette étude a permis de constater l'importance des interactions plantes-champignons dans cette plaine de dunes reliques. Il serait intéressant d'étendre notre investigation à d'autres groupes de microorganismes, qui jouent aussi des rôles importants dans les sols. Par exemple, une investigation des populations de nématodes serait pertinente, étant donné qu'ils ont été proposés comme cause principale du déclin de l'ammophile à l'arrière des dunes bordières (Little & Maun, 1996). De plus, nous avons détecté quelques champignons nématophages, indiquant qu'une meilleure connaissance de ces interactions pourrait compléter le portrait que nous avons déjà dressé. Les bactéries fixatrices d'azote, stratégie très présente à partir de la dune semi-fixée, mériteraient aussi une attention particulière. C'est surtout la présence du myrique, un arbuste dominant dans les zones humides entre les crêtes dunaires (*Myrica gale*) et dans la dune semie-fixée et forestière (*M. pensylvanica*) qui explique ce patron. Ce sont des bactéries du genre *Frankia* qui s'associent aux myriques et entraînent une transformation morphologique des racines où se produit la fixation de l'azote. Les symbioses impliquant *Frankia* pourraient donc occuper une place importante dans les sols des plus vieilles dunes. Cette symbiose est d'ailleurs encore relativement méconnue; une meilleure compréhension de sa dynamique écologique serait donc bénéfique dans le cas particulier des Sillons, mais aussi de façon plus large.

5.3 Le séquençage haut débit pour la description des communautés microbiennes

5.3.1 Potentiel des approches « omiques »

Les communautés de microorganismes sont extrêmement complexes et diversifiées. La majorité des taxons microbiens ne pouvant être cultivés (Staley & Konopka, 1985; Whitman *et al.*, 1998; Quince *et al*., 2008), le séquençage d'ADN extrait à partir d'échantillons environnementaux présente un potentiel intéressant par rapport aux méthodes traditionnelles comme la collecte de spores, l'observation de sporocarpes ou l'isolation et la culture en

laboratoire. Les avancées technologiques récentes en biologie moléculaire, notamment le développement du séquençage haut débit, ont permis la caractérisation des communautés microbiennes comme jamais auparavant. L'approche massivement parallèle du séquençage haut débit permet la lecture rapide d'un grand nombre de séquences à faible coût, en comparaison au séquençage Sanger par exemple (Sogin *et al.*, 2006). En ciblant et amplifiant une région variable relativement courte qui peut être utilisée comme code barre pour le groupe d'organismes d'intérêt, il est possible d'obtenir une profondeur de lecture et une résolution très élevées, et de rapidement caractériser les communautés microbiennes. Un nombre croissant d'études utilisant cette approche, appelée « metabarcoding » ou « targeted metagenomics », pour décrire des communautés fongiques ont été publiées depuis 2009 (ex : Buée *et al*., 2009; Öpik *et al*., 2009; Tedersoo *et al.*, 2010). Il existe plusieurs plateformes de séquençage, mais c'est le 454 de Roche qui a été le plus fréquemment utilisé pour la caractérisation des communautés fongiques. Cette plateforme a longtemps été la seule capable de produire des séquences assez longues pour capter la région ITS (incluant ITS1 et ITS2) qui est utilisée comme code barre pour les champignons. Les plateformes alternatives sont toutefois constamment améliorées et certaines commencent à remplacer le 454 de Roche. Par exemple, la longueur des séquences produites par Illumina a considérablement augmenté dans les dernières années et des premières études utilisant le MiSeq d'Illumina pour du metabarcoding fongique ont été réalisées en 2013 (Schmidt *et al.*, 2013; McGuire *et al*., 2013). Les autres plateformes (Ion Torrent, PacBio) n'ont été utilisées jusqu'à maintenant que de façon marginale pour l'étude des communautés fongiques. Jusqu'à maintenant, le séquençage haut débit et les approches « omiques » ont permis d'apprécier l'ampleur, la complexité et l'importance fonctionnelle des communautés microbiennes dans une multitude de milieux comme les sols agricoles (ex : Fierer & Jackson, 2006), les sources hydrothermales (ex : Sogin *et al.*, 2006), les forêts (ex : Buée *et al.*, 2009), et le corps humain (ex : Weinstock, 2012), pour n'en nommer que quelques-uns. Ces avancées technologiques dans le domaine du séquençage, combinées à l'augmentation des capacités computationnelles, permettent aussi d'envisager des projets d'envergure comme le « Earth Microbiome Project » qui a comme objectif de catégoriser l'ensemble de la diversité taxonomique et fonctionnelle sur Terre (http://www.earthmicrobiome.org/).

5.3.2 Principales limitations et sources d'erreur

La caractérisation des communautés par « metabarcoding », peu importe la plateforme utilisée, introduit certains biais qu'il est important de reconnaître. Les effets de certaines de ces limitations peuvent toutefois être corrigés, ou du moins réduits, lors de la planification du travail de laboratoire et du traitement bio-informatique des séquences. J'ai tenté d'adopter les meilleures pratiques possibles afin de réduire les sources d'erreur et avons développé un pipeline d'analyse des séquences (contrôle de la qualité, groupement en OTUs, classification taxonomique) optimisé à l'aide d'une « mock community » générée par clonage-séquençage.

D'abord, la région d'ADN choisie et les marqueurs utilisés pour l'amplification peuvent avoir une grande influence sur la structure des communautés observées. Chez les champignons, les ITS ont été les plus largement utilisés puisqu'ils sont faciles à amplifier à cause de leur grand nombre de copies et parce qu'ils présentent un haut niveau de variabilité interspécifique. Par contre, le degré de variabilité intraspécifique fluctue considérablement d'une espèce à l'autre et n'est pas corrélé au lien taxonomique (Lindner *et al.*, 2013; Peay, 2014; Sota *et al*., 2014). Il est donc difficile de définir un seuil de similarité universel pour la différentiation des espèces. De plus, cette région ne permet pas de bien capter la diversité de certains groupes, comme les CMA. Il existe des paires de marqueurs ciblant les Gloméromycètes (ex : AML1 et AML2, NS31 et AM1) qui sont beaucoup mieux adaptés à l'étude des CMA (Lee *et al.*, 2008). C'est d'ailleurs probablement ce qui explique que je n'aie détecté que très peu de séquences de CMA, particulièrement en fin de succession, malgré l'importante présence des plantes mycorrhiziennes dans toutes les zones des dunes (voir Figure 5.1). À noter qu'il est fort improbable qu'une courte région d'ADN, quel qu'elle soit, ne présente un degré de variabilité suffisant pour séparer les espèces de tous les groupes du règne fongique (Lindner *et al.*, 2013). Il serait donc idéal, en l'absence de contraintes de temps et de ressources, d'utiliser une combinaison de plusieurs marqueurs. Ensuite, l'utilisation du metabarcoding tel qu'effectué dans le cadre de cette thèse ne permet malheureusement pas d'accorder une valeur quantitative aux différents taxons détectés. En effet, à cause de biais d'amplifications, le nombre de séquences attribuées à une unité taxonomique opérationnelle donnée n'est pas forcément représentatif de son importance dans l'échantillon de départ. Amend *et al.* (2010) a exploré le lien existant entre l'abondance biologique et l'abondance en

terme de nombre de séquences. Ses résultats indiquent que le nombre de séquences peut être informatif pour comparer l'abondance d'une même espèce à travers différents échantillons ou traitements, mais pas entre différentes espèces. Ce problème peut être partiellement résolu par l'utilisation de PCR quantitative (qPCR) lors de l'amplification (Amend *et al*., 2010), ce qui n'a pas été effectué dans le cadre de cette étude.

Bien qu'imparfaite, l'approche du metabarcoding demeure très intéressante et l'étude des communautés microbiennes a énormément progressé grâce au séquençage haut débit. Nous sommes maintenant capable d'inclure, dans la description des communautés, des taxons auparavant négligés puisque non-cultivables, et donc de capturer d'une façon de plus en plus complète la diversité microbienne.

5.3.3 Perspectives

Les analyses de communautés microbiennes ayant recours à la métagénomique génèrent typiquement plusieurs centaines à plusieurs milliers d'OTUs qui forment des communautés complexes. L'assignation taxonomique est déjà une pratique courante. Par contre, les différents modes de vie fongiques interagissent et répondent différemment à certaines conditions environnementales, ainsi cette approche peut passer à côté de tendances écologiques importantes. La classification des OTUs microbiens en guildes fonctionnelles, c'est-à-dire en groupes écologiquement logiques et cohérents définis selon les modes de vie et stratégies nutritionnelles, est toutefois peu répandue. Des avancées ont été faites dans ce domaine chez les procaryotes. Des outils comme PieCRUST (Langille *et al*., 2013) ont démontré que les informations taxonomiques tirées du séquençage de régions variables comme le 16S peuvent prédire les fonctions microbiennes de façon juste et efficace. L'assignation des OTUs fongiques à des guildes fonctionnelles demeure un défi de taille à cause du peu de génomes fongiques complètement séquencés, du grand nombre d'espèces fongiques encore non-décrites, et du manque d'information sur le mode de vie et les fonctions sur bon nombre des espèces connues (Peay, 2014). Un outil nommé FUNGuild qui a récemment été développé (Nguyen *et al*., 2016) pourra faciliter ce processus. Jusqu'à maintenant, dans les quelques études qui ont opté pour cette approche (ex : Clemmensen *et al*., 2013;, Taylor *et al.*, 2014, Tedersoo *et al*., 2014, Clemmensen *et al.*, 2015), la classification fonctionnelle semble avoir été réalisée manuellement. C'est aussi de cette façon que nous

avons procédé : en assignant manuellement un mode de vie à chacun des genres fongiques recensés selon les informations disponibles dans la littérature (voir chapitre 2). Cette classification, entre les champignons saprotrophes, pathogènes et symbiotiques, nous a permis de décrire la diversité fonctionnelle des communautés fongiques à travers les différentes zones des dunes. Bien que les contraintes méthodologiques discutées précédemment nous empêchent de quantifier l'importance des différents groupes fongiques à partir du nombre de séquences recensées pour chacun d'eux, cette approche nous a permis d'avoir un aperçu des variations des différents modes de vie à travers les dunes (voir Figure 5.1). Ainsi, les patrons détectés grâce aux analyses canoniques au niveau de la structure des communautés se sont confirmés au niveau fonctionnel. En effet, bien que toutes les zones des dunes supportent des communautés fongiques fonctionnellement différentes, certains groupes sont présents de façon plus importante dans une zone par rapport à une autre. Les champignons ectomycorhiziens, par exemple, étaient plus abondants dans la dune fixée. Une telle approche, c'est-à-dire l'attribution de guildes fonctionnelles aux OTUs identifiés par metabarcoding, est très intéressante puisqu'elle permet de donner un sens écologique à des données moléculaires et taxonomiques. Par contre, pour faire progresser le domaine de l'écologie microbienne et de la mycologie, il sera crucial d'augmenter les efforts et les ressources dédiés à caractériser l'histoire naturelle, l'écologie, les modes de vie et les fonctions des champignons. Bien que cela représente un défi de taille, ce travail est essentiel à une meilleure compréhension des dynamiques biotiques des sols. Nous avons d'ailleurs pu constater cette lacune en effectuant notre attribution fonctionnelle pour le premier chapitre de cette thèse. Il était parfois impossible de trouver dans la littérature une description fonctionnelle de certains genres et, dans plusieurs cas, le mode de vie d'une espèce ou d'un genre est déduit selon le contexte où il a été isolé sans être vérifié expérimentalement.

Il existe d'autres approches permettant d'acquérir des informations fonctionnelles. Dans le cas où on s'intéresserait à une ou plusieurs fonctions particulières, mesurer l'activité enzymatique serait une approche adéquate et très fiable. Autrement, l'analyse de métagénomes, par exemple, permet d'étudier l'ensemble des gènes se trouvant dans un échantillon sans se préoccuper de l'identité des organismes impliqués. Il en va de même pour l'étude des métatranscriptomes et des métaprotéomes, qui permet de profiler l'expression des gènes en ARN et protéines. Ces approches sont toutefois mal adaptées à l'étude des gènes

fongiques, puisque les métagénomes sont dominés par les gènes procaryotes. En effet, les métagénomes contiennent typiquement moins de 5% de gènes fongiques (base de données publiques de MG-RAST).

Un autre problème du metabarcoding est la difficulté d'attribuer avec certitude une identification taxonomique aux OTUs recensés. On attribue généralement à un OTU l'identité de la séquence la plus proche dans les bases de données publiques. Or, une importante proportion des séquences demeure souvent non-identifiée ou est identifiée comme « uncultured fungus ». En effet, la majeure partie de la diversité fongique reste à découvrir (probablement plus de 90%) et les bases de données sont incomplètes. De plus, avec le débit énorme de séquences déposées, il est difficile de contrôler la qualité des données. Le séquençage à haut débit présente un potentiel énorme pour l'amélioration des connaissances sur les communautés microbiennes; il est toutefois crucial que les efforts de description s'accompagnent d'une réflexion sur l'amélioration des outils d'analyses et la consolidation des bases de données.

5.4 Conclusions

Les récents développements technologiques des outils moléculaires pour l'identification des champignons, et particulièrement la métagénomique, ont définitivement révolutionné la façon de travailler dans le domaine de l'écologie fongique et microbienne. La quantité croissante de données d'observations produites, afin de pouvoir être interprétées et placées dans un contexte scientifique plus large, doit absolument s'accompagner d'un gain de connaissances fondamentales sur l'histoire naturelle, l'écologie et les fonctions des champignons. On oppose souvent les études d'observation aux études expérimentales. Or, les deux approches sont nécessaires et sont en fait non pas contradictoires, mais complémentaires (Jansson & Prosser, 2013). Cette thèse est définitivement de l'ordre de la description. J'ai exploré un système écologique diversifié, une plaine de dunes reliques, encore jamais décrit au niveau des champignons du sol. L'utilisation du séquençage haut débit m'a permis de décrire de façon exhaustive la diversité et la structure des communautés fongiques à travers un gradient de succession et de conditions édaphiques, et de lier ces patrons à la végétation et à des propriétés physico-chimiques du sol. Bien que j'ai réussi à décrire plusieurs patrons et

corrélations présentant un intérêt écologique – notamment le fort lien entre les communautés de champignons du sol et la végétation, les variations au niveau des guildes fonctionnelles fongiques et des stratégies d'acquisition de nutriments des plantes, ou le faible niveau de spécialisation des associations ectomycorhiziennes dans la dune fixée – plusieurs mécanismes spécifiques sont demeurés inexpliqués. Ces observations devraient servir à poser des hypothèses explicites sur les dynamiques et différents mécanismes opérants dans ce système, puis à développer des protocoles pour les tester. Le travail présenté ici soulève par exemple des questions sur la nature des mécanismes liant la végétation aux champignons du sol dans les dunes, notamment à savoir si les corrélations observées sont dues à un effet de l'hôte ou au phénomène de spécialisation édaphique, ou sur le rôle fonctionnel des taxons généralistes dans les réseaux d'interactions symbiotiques.

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Annexe 1- Putative functional assignment of fungal genera

Ascomycota

Genus	Family	Lifestyle	Ref
Sarea	Agyriaceae	Sym(li)	Cannon and Kirk 2007
Truncatella	Amphisphaeriaceae	Sap/Par(pl)	Cannon and Kirk 2007
Arthrinium	Apiosporaceae	Par(pl)	Crous and Groenewald 2013, Sharma *et al.* 2013
*Apiosporaceae*_spp.	Apiosporaceae	Sap	Cannon and Kirk 2007
Archaeorhizomyces	Archaeorhizomycetaceae	Sap	Rosling *et al.* 2011
*Arthopyreniaceae*_spp.	Arthopyreniaceae	Sym(li)/End	Cannon and Kirk 2007; Bacon and White 2000; Coppins 1988; Sipman and Aptroot 2005
Bionectria	Bionectriaceae	Par(pl)	Dodd *et al.* 2010
Capnodium	Capnodiaceae	Epi	Cannon and Kirk 2007; Reynolds 1999
Chaetomium	Chaetomiaceae	Sap	Cannon and Kirk 2007; Webster and Weber 2007
Humicola	Chaetomiaceae	Sap	Ko *et al.* 2011; Schulein 1997; Cannon and Kirk 2007
Thielavia	Chaetomiaceae	Sap	Cannon and Kirk 2007
Trichocladium	Chaetomiaceae	Sap	Cannon and Kirk 2007
*Chaetomiaceae*_spp.	Chaetomiaceae	Sap	Cannon and Kirk 2007
*Chaetosphaeriaceae*_spp.1	Chaetosphaeriaceae	Sap	Cannon and Kirk 2007
*Chaetosphaeriaceae*_spp.2	Chaetosphaeriaceae	Sap	Cannon and Kirk 2007
Cladonia	Cladoniaceae	Sym(li)	Esslinger 2012
Beauveria	Clavicipitaceae	Par(an)	Feng *et al.* 1994
Cordyceps	Clavicipitaceae	Par(my)/Par(an)	Webster and Weber 2007
Elaphocordyceps	Clavicipitaceae	Par(my)/Par(an)	Putri *et al.* 2010; Reynolds 2011
Harposporium	Clavicipitaceae	Par(ne)	Hodge *et al.* 1997
Pochonia	Clavicipitaceae	Par(ne)	Morton *et al.* 2003
*Clavicipitaceae*_spp.1	Clavicipitaceae	Par(pl)/Par(my)/Par(an)	Webster and Weber 2007; Cannon and Kirk 2007
*Clavicipitaceae*_spp.2	Clavicipitaceae	Par(pl)/Par(my)/Par(an)	Webster and Weber 2007; Cannon and Kirk 2007
Lecythophora	Coniochaetaceae	Par(pl)/Par(an)	Damm *et al.* 2010
Lecanicillium	Cordycipitaceae	Par(ne)/Par(an)	Yang *et al.* 2005; Cuthbertson and Walters 2005
*Davidiellaceae*_spp.	Davidiellaceae	Sap/Par(pl)/Par(an)	Schoch *et al.* 2006; Braun *et al.* 2003; Bensch *et al.* 2012
Cryptosporiopsis	Dermateaceae	Sym(er)/Sap/Par(pl)	Kowalski and Bartin 1995; Verkley *et al.* 2003; Sigler *et al.* 2005
Mollisia	Dermateaceae	Sap	MycoQuébec
*Dermateaceae*_spp.1	Dermateaceae	Sap/Par(pl)	Cannon and Kirk 2007
*Dermateaceae*_spp.2	Dermateaceae	Sap/Par(pl)	Cannon and Kirk 2007
Eutypa	Diatrypaceae	Par(pl)	Péros and Berger 1994
Eutypella	Diatrypaceae	Par(pl)	Jurc *et al.* 2006
Didymosphaeria	Didymosphaeriaceae	Sap	Kohlmeyer *et al.* 1990; Cannon and Kirk 2007
Dipodascus	Dipodascaceae	Sap	Webster and Weber 2007
Galactomyces	Dipodascaceae	Sap	Webster and Weber 2007
Geotrichum	Dipodascaceae	Sap	Webster and Weber 2007
*Dothideaceae*_spp.	Dothideaceae	Sap/Par(pl)	Cannon and Kirk 2007
Aureobasidium	Dothioraceae	Sap	Webster and Weber 2007
Hormonema	Dothioraceae	End	Bills *et al.* 2010; Pelaez 2000
Sydowia	Dothioraceae	Sap/Par(pl)	Cannon and Kirk 2007
Oidium	Erysiphaceae	Par(pl)	Jones *et al.* 2002
Geoglossum	Geoglossaceae	Sap	Reverchon *et al.* 2010
Thuemenidium	Geoglossaceae	Sym(er)/Sap	Cannon and Kirk 2007; Ohenoja *et al.* 2010
*Halosphaeriaceae*_spp.	Halosphaeriaceae	Sap	Cannon and Kirk 2007
Ascocoryne	Helotiaceae	Sap	Webster and Weber 2007
Crocicreas	Helotiaceae	Sap	Cannon and Kirk 2007
Cudoniella	Helotiaceae	Sap	Cannon and Kirk 2007
Dimorphospora	Helotiaceae	Sap	Cannon and Kirk 2007
Holwaya	Helotiaceae	Sap	Cannon and Kirk 2007
Hymenoscyphus	Helotiaceae	Sym(ec)/Sym(er)/Par(pl)	Queloz *et al.* 2011; Webster and Weber 2007; Tedersoo *et al.* 2010
Pezizella	Helotiaceae	Sym(er)	Bonfante-Fasolo 1984

Rhizoscyphus	Helotiaceae	Sym(er)	Webster and Weber 2007
*Helotiaceae*_spp.	Helotiaceae	Sym(er)/Sap	Cannon and Kirk 2007
Varicosporium	Helotiaceae	Sap	Park 1982
Capronia	Herpotrichiellaceae	Sym(li)	Esslinger 2012
Cladophialophora	Herpotrichiellaceae	Sap/Par(pl)/Par(an)	Badali et al 2008
Exophiala	Herpotrichiellaceae	Sap/Par(an)	Cooke 1977
Phaeomoniella	Herpotrichiellaceae	Sap/Par(pl)	Damm *et al.* 2010
Phialophora	Herpotrichiellaceae	Sap/Par(an)	Cannon and Kirk 2007
Rhinocladiella	Herpotrichiellaceae	Sap	Wagenaar et al 2000
*Herpotrichiellaceae*_spp.1	Herpotrichiellaceae	Sap/Par(an)	Cannon and Kirk 2007
*Herpotrichiellaceae*_spp.2	Herpotrichiellaceae	Sap/Par(an)	Cannon and Kirk 2007
Incrucipulum	Hyaloscyphaceae	Sap	Cannon and Kirk 2007
Lachnum	Hyaloscyphaceae	Sap	Cannon and Kirk 2007
*Hyaloscyphaceae*_spp.1	Hyaloscyphaceae	Sap	Cannon and Kirk 2007
*Hyaloscyphaceae*_spp.2	Hyaloscyphaceae	Sap	Cannon and Kirk 2007
Acrostalagmus	Hypocreaceae	Par(ne)/Par(an)	Drechsler 1942; Gray 1983
Hypocrea	Hypocreaceae	Par(my)	Webster and Weber 2007; Poldmaa 2000
Hypomyces	Hypocreaceae	Par(my)	Rogerson and Samuels 1994; Webster and Weber 2007
Trichoderma	Hypocreaceae	Sym(ec)/Sap/Par(my)	Harman *et al.* 2004; Webster and Weber 2007; Tedersoo *et al.* 2010
*Hypocreaceae*_spp.	Hypocreaceae	Sap/Par(my)	Cannon and Kirk 2007
Microdochium	Hyponectriaceae	Par(pl)	Loos *et al.* 2004; Murray 1981
*Hyponectriaceae*_spp.	Hyponectriaceae	Sap/Par(pl)	Cannon and Kirk 2007
Acremonium	Incertae_sedis	Sap/Par(pl)/Par(my)/End	Hoveland 1993; Breen 1994; Rivera-Varas *et al.* 2007
Cadophora	Incertae_sedis	Sym(ec)/Par(pl)	Dos Santos Utmazian *et al.* 2007; Almeida *et al.* 2010; Navarrette *et al.* 2011; Tedersoo *et al.* 2010
Calyptrozyma	Incertae_sedis	End	Bergero *et al.* 2003
Candida	Incertae_sedis	Sap/Par(an)/Epi	Webster and Weber 2007
Capnobotryella	Incertae_sedis	Sap	Sert *et al.* 2007; Sert *et al.* 2011
Cenococcum	Incertae_sedis	Sym(ec)	Pigott 2006
Cephalosporium	Incertae_sedis	Sap/Par(pl)/Par(an)	Quincke *et al.* 2012; Fincher *et al.* 1991
Chalara	Incertae_sedis	Par(pl)	Queloz *et al.* 2011
Dactylaria	Incertae_sedis	Par(ne)	Ahren *et al.* 1998
Dictyosporium	Incertae_sedis	Sap	Teik-Khiang *et al.* 1999
Emericellopsis	Incertae_sedis	Sap	Grum-Grzhimaylo *et al.* 2013
Geastrumia	Incertae_sedis	Par(pl)	Johnson and Sutton 1994
Geosmithia	Incertae_sedis	Sap	Kolarik *et al.* 2005
Gloeotinia	Incertae_sedis	Par(pl)	Alderman 1995
Gyoerffyella	Incertae_sedis	Sap	Marvanova 1975
Ilyonectria	Incertae_sedis	Par(pl)	Vitale *et al.* 2012; Cabral *et al.* 2012
Kendrickiella	Incertae_sedis	Sap	Kiyuna *et al.* 2012
Leptodontidium	Incertae_sedis	Par(pl)/End	Belding *et al.* 2000; Tsuneda *et al.* 1996; Fernando and Currah 1996
Meliniomyces	Incertae_sedis	Sap/Sym(ec)/Sym(er)/End	Hambleton and Sigler 2005; Ohtaka and Narisawa 2008; Tedersoo *et al.* 2010; Tedersoo *et al.* 2014
Microcyclospora	Incertae_sedis	Par(pl)Epi	Frank *et al.* 2010
Myrmecridium	Incertae_sedis	Sap/Par(pl)/Par(an)	Arzanlou *et al.* 2007
Myrothecium	Incertae_sedis	Sap/Par(pl)	Murakami *et al.* 2002; Updergraff 1971; Zhang *et al.* 2012
Neoaleurodiscus	Incertae_sedis	Sap	Wu *et al.* 2010
Phialea	Incertae_sedis	Par(pl)	Gray 1942; Calvert and Muskett 1945
Phoma	Incertae_sedis	Sap/Par(pl)	Webster and Weber 2007
Pilidium	Incertae_sedis	Par(pl)	Crous 1991; Rossman *et al.* 2004
Pyrenochaeta	Incertae_sedis	Sap/Par(pl)/Par(an)	Desnos-Ollivier *et al.* 2004; Golzar 2009; Dodd *et al.* 2010
Rachicladosporium	Incertae_sedis	Sap	Egidi *et al.* 2014
Savoryella	Incertae_sedis	Sap	Boonyuen *et al.* 2011, Ho *et al.* 1997
Schizoblastosporion	Incertae_sedis	Sap	Ramírez and González, 1984
Scolecobasidium	Incertae_sedis	Par(pl)/Par(an)/Epi/End	Mahmoud and Narisawa 2013; Ross and Yatsutake 1973; Barron and Bush 1962; Hao *et al.*

			2012
Setomelanomma	Incertae_sedis	Par(pl)	Rossman *et al.* 2002; Plewa *et al.* 2012
Spirosphaera	Incertae_sedis	Sap	Webster and Weber 2007; Voglmayr 2004
Stachybotrys	Incertae_sedis	Sap/Par(an)	Etzel *et al.* 1998; Haugland *et al.* 1999; Haugland *et al.* 2001
Stilbella	Incertae_sedis	Sap	Singh and Webster 1973; Lewis and Papvizas 1993; Seifert *et al.* 1995
Thermomyces	Incertae_sedis	Sap	Singh *et al.* 2006; Hedger and Hudson 1974
Troposporella	Incertae_sedis	Sap	Tsui and Berbee 2010; Voříšková and Baldrian 2012
Cercophora	Lasiosphaeriaceae	Sap	Whyte *et al.* 1997
Lasiosphaeris	Lasiosphaeriaceae	Sap	Miller and Huhndorf 2004
Podospora	Lasiosphaeriaceae	Sap	Cannon and Kirk 2007
Schizothecium	Lasiosphaeriaceae	Sap	Cannon and Kirk 2007
*Lasiosphaeriaceae*_spp.	Lasiosphaeriaceae	Sap	Cannon and Kirk 2007
Neobulgaria	Leotiaceae	Sap	Webster and Weber 2007; Cannon and Kirk 2007
Pezoloma	Leotiaceae	Sap	Descals *et al.* 1998
*Leotiaceae*_spp.	Leotiaceae	Sap	Cannon and Kirk 2007
Leptosphaeria	Leptosphaeriaceae	Par(pl)	Fit *et al.* 2006
*Leptosphaeriaceae*_spp.1	Leptosphaeriaceae	Sap/Par(pl)	Cannon and Kirk 2007
*Leptosphaeriaceae*_spp.2	Leptosphaeriaceae	Sap/Par(pl)	Cannon and Kirk 2007
Lipomyces	Lipomycetaceae	Sap	Zhao *et al.* 2008; Angerbauer *et al.* 2008
Lophiostoma	Lophiostomataceae	Sap	Hirayama and Tanaka 2011
Clavispora	Metschnikowiaceae	Sap	Cannon and Kirk 2007; Rodrigues de Miranda 1979; Lachance 1990
Metschnikowia	Metschnikowiaceae	Par(an)	Cannon and Kirk 2007; Nguyen and Panon 1998; Türkel and Ener 2009
Cephalotrichum	Microascaceae	Sap	Cannon and Kirk 2007
Kernia	Microascaceae	Sap/Par(pl)	Thirumalachar 1946; Malloch and Cain 1971
Wardomyces	Microascaceae	Sap	Cannon and Kirk 2007; Hennebert 1962
Paraconiothyrium	Montagnulaceae	Par(pl)	Damm *et al.* 2008
Paraphaeosphaeria	Montagnulaceae	Par(pl)	Damm *et al.* 2008; Câmara *et al.* 2001
Cercospora	Mycosphaerellaceae	Par(pl)	Zhimo *et al.* 2013
Cladosporium	Mycosphaerellaceae	Sap/Par(pl)	De Vries 1952; Bensch *et al.* 2012
Mycosphaerella	Mycosphaerellaceae	Par(pl)	Crous *et al.* 2006; Crous *et al.* 2007
Ramularia	Mycosphaerellaceae	Par(pl)	Crous *et al.* 2006; Crous *et al.* 2007; Hildebrand 1935
Septoria	Mycosphaerellaceae	Par(pl)	Eyal 1987
*Mycosphaerellaceae*_spp.	Mycosphaerellaceae	Sap/Par(pl)	Cannon and Kirk 2007
*Myriangiaceae*_spp.	Myriangiaceae	Par(pl)	Cannon and Kirk 2007
*Mytilinidiaceae*_spp.1	Mytilinidiaceae	Sap	Cannon and Kirk 2007
Myxotrichum	Myxotrichaceae	Sap	Orr *et al.* 1963
Oidiodendron	Myxotrichaceae	Sym(er)/Sap	Webster and Weber 2007
*Myxotrichaceae*_spp.2	Myxotrichaceae	Sym(er)/Sap	Cannon and Kirk 2007
*Myxotrichaceae*_spp.*3*	Myxotrichaceae	Sym(er)/Sap	Cannon and Kirk 2007
Cylindrocarpon	Nectriaceae	Sap/Par(pl)	Webster and Weber 2007; Dodd *et al.* 2010
Fusarium	Nectriaceae	Sap/Par(pl)	Webster and Weber 2007
Gibberella	Nectriaceae	Par(pl)	O'Donnell *et al.* 1998
Neonectria	Nectriaceae	Sap/Par(pl)	Halleen *et al.* 2006; Langrell 2001; Dodd *et al.* 2010
*Nectriaceae*_spp.1	Nectriaceae	Par(pl)	Cannon and Kirk 2007
*Nectriaceae*_spp.2	Nectriaceae	Par(pl)	Cannon and Kirk 2007
Volutella	Nectriaceae	Par(pl)	Stevens and Hall 1907; Ofong 1974
Aphanoascus	Onygenaceae	Sap/Par(an)	Cannon and Kirk 2007
Castanedomyces	Onygenaceae	Sap	Cano *et al.* 2002
Chrysosporium	Onygenaceae	Sap/Par(an)	Allender *et al.* 2011; Chabasse 1989
Chaunopycnis	Ophiocordycipitaceae	Unknown	Bills *et al.* 2002; Moller *et al.* 1995
Hirsutella	Ophiocordycipitaceae	Par(ne)	Jaffee and Zehr 1985
Ophiocordyceps	Ophiocordycipitaceae	Par(an)	Yang and Yao 2011; Pontoppidan *et al.* 2009
Tolypocladium	Ophiocordycipitaceae	Sap/Par(an)	Strasser *et al.* 2000; Bandani *et al.* 2000
*Ophiocordycipitaceae*_spp.	Ophiocordycipitaceae	Unknown	
Arthrobotrys	Orbiliaceae	Sap/Par(ne)	Ahren *et al.* 1998; Tunlid *et al.* 1991
Dactylella	Orbiliaceae	Par(ne)	Cooke and Dickinson 1965; Ahren *et al.* 1998

Monacrosporium	Orbiliaceae	Par(ne)	Liu and Zhang 1994; Cooke and Dickinson 1965; Ahren *et al.* 1998
*Orbiliaceae*_spp.	Orbiliaceae	Sap/Par(ne)	Cannon and Kirk 2007
Myelochroa	Parmeliaceae	Sym(li)	Esslinger 2012
Parmelia	Parmeliaceae	Sym(li)	Cannon and Kirk 2007
Peziza	Pezizaceae	Sap	Reverchon *et al.* 2010
Phaeosphaeria	Phaeosphaeriaceae	Par(pl)	Halama and Lacoste 1995; Cannon and Kirk 2007
*Phaeosphaeriaceae*_spp.	Phaeosphaeriaceae	Sap/Par(pl)	Cannon and Kirk 2007
Pichia	Pichiaceae	Sap/Par(an)	Dodd *et al.* 2010; Chakrabarti *et al.* 2001; Bergman *et al.* 1998
*Pichiaceae*_spp.	Pichiaceae	Unknown	
Plectosphaerella	Plectosphaerellaceae	Par(pl)	Dodd *et al.* 2010
Verticillium	Plectosphaerellaceae	Sap/Par(pl)/Par(my)/Par(an)	Nazar *et al.* 1991; Barbara and Clewes 2003; Nilanonta *et al.* 2003; Verhaar *et al.* 1996
Alternaria	Pleosporaceae	Sap/Par(pl)/Par(an)	Webster and Weber 2007
Dendryphiella	Pleosporaceae	Sap	Wainwright and Sherbrock-Cox 1981; Edwards *et al.* 1998
Epicoccum	Pleosporaceae	Sap/Par(pl)	Dodd *et al.* 2010; Samson *et al.* 2004; Weber 2006; Domsch *et al.* 1993; (Institut national de santé publique du Québec)
Leptosphaerulina	Pleosporaceae	Sap/Par(pl)	Abler 2003; Thal 1986
Lewia	Pleosporaceae	Par(pl)	Kwasna and Kosiak 2003; Vieira and Barreto 2005
Pithomyces	Pleosporaceae	Sap/Par(an)	Brook 1963; di Menna and Bailey 1973
Scutellinia	Pyronemataceae	Sap	Denison 1959; Yao and Spooner 1996
*Pyronemataceae*_spp.	Pyronemataceae	Sap	Perry *et al.* 2007
Ramalina	Ramalinaceae	Sym(li)	Cannon and Kirk 2007
Lophodermium	Rhytismataceae	Sap/Par(pl)	Cannon and Kirk 2007
Torulaspora	Saccharomycetaceae	Unknown	Cannon and Kirk 2007; Watanabe *et al.* 1994
Williopsis	Saccharomycetaceae	Unknown	Cannon and Kirk 2007; James *et al.* 1998
*Sclerotiniaceae*_spp.1	Sclerotiniaceae	Par(pl)	Cannon and Kirk 2007
*Sclerotiniaceae*_spp.2	Sclerotiniaceae	Par(pl)	Cannon and Kirk 2007
Gelasinospora	Sordariaceae	Sap	Cannon and Kirk 2007
Preussia	Sporormiaceae	Sap	Cannon and Kirk 2007; Dodd *et al.* 2010
Lalaria	Taphrinaceae	Par(pl)	Cannon and Kirk 2007
Devriesia	Teratosphaeriaceae	Sap/Par(pl)/Par(an)	Seifert *et al.* 2004; Martel *et al.* 2008; Li *et al.* 2012
Teratosphaeria	Teratosphaeriaceae	Par(pl)	Crous *et al.* 2009
*Teratosphaeriaceae*_spp.	Teratosphaeriaceae	Par(pl)	Pérez *et al.* 2013
Thelebolus	Thelebolaceae	Sap	Cannon and Kirk 2007
*Thelebolaceae*_spp.	Thelebolaceae	Sap	Cannon and Kirk 2007
Phaeoacremonium	Togniniaceae	Par(pl)/Par(an)	Cannon and Kirk 2007
Aspergillus	Trichocomaceae	Sap/Par(pl)	Thom *et al.* 1926; Thom *et al.* 1945; de Vries and Visser 2001; Houbraken and Samson 2011
Eupenicillium	Trichocomaceae	Sap	Hodges and Perry 1973; Cannon and Kirk 2007; Houbraken and Samson 2011
Eurotium	Trichocomaceae	Sap	Butinar *et al.* 2005; Cannon and Kirk 2007; Houbraken and Samson 2011
Paecilomyces	Trichocomaceae	Par(ne)	Wraight *et al.* 1998; Houbraken and Samson 2011
Penicillium	Trichocomaceae	Sap/Par(an)	Houbraken and Samson 2011
Sagenomella	Trichocomaceae	Par(an)	Houbraken and Samson 2011; Gené *et al.* 2003
Thysanophora	Trichocomaceae	Sap	Houbraken and Samson 2011; Cannon and Kirk 2007; Van Maanen and Gourbière 1997
*Trichocomaceae*_spp.	Trichocomaceae	Sap	Cannon and Kirk 2007
Fusicladium	Venturiaceae	Par(pl)	Schubert *et al.* 2013
*Venturiaceae*_spp.1	Venturiaceae	Sap/Par(pl)	Cannon and Kirk 2007; Crous *et al.* 2007; Barr 1968
*Venturiaceae*_spp.2	Venturiaceae	Sap/Par(pl)	Cannon and Kirk 2007; Crous *et al.* 2007; Barr 1968
*Verrucariaceae*_spp.	Verrucariaceae	Sym(li)	Cannon and Kirk 2007; Gueidan *et al.* 2007
Acephala	Vibrisseaceae	Sap/End	Cannon and Kirk 2007; Grünig *et al.* 2008; Grünig and Sieber 2005
Phialocephala	Vibrisseaceae	End	Cannon and Kirk 2007; Jumpponen *et al.* 1998; Jacobs *et al.* 2003

Annulohypoxylon	Xylariaceae	Sap/End	Cheng *et al.* 2011; Raei 2012
Anthostomella	Xylariaceae	Sap/Par(pl)	Francis *et al.* 1975; Kohlmeyer and Volkmann-Kohlmeyer 2002; Lee and Crous 2002
*Xylariaceae*_spp.	Xylariaceae	Sap/Par(pl)/End	Cannon and Kirk 2007; Rogers 2000
Chaetosphaeria		Sap	Fernandez *et al.* 2006
Chloridium		Sap/End	Kharwat *et al.* 2009; Wang and Wilcox 1985; Omvik 1970

Basidiomycota

Genus	Family	Lifestyle	Ref
*Agaricaceae*_spp.	Agaricaceae	Sap	Moncalvo *et al.* 2002; Webster and Weber 2007
Bensingtonia	Agaricostilbaceae	Unknown	Qi-Ming *et al.* 2006
Amanita	Amanitaceae	Sym(ec)	Godbout et Fortin 1985; Tedersoo *et al.* 2010
Amphinema	Atheliaceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Athelia	Atheliaceae	Par(pl)	Cannon and Kirk 2007; Baminger *et al.* 2001; Gilbert *et al.* 1988; Harlton *et al.* 1995
Piloderma	Atheliaceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Tylospora	Atheliaceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
*Atheliaceae*_spp.1	Atheliaceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
*Atheliaceae*_spp.2	Atheliaceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Boletopsis	Bankeraceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Phellodon	Bankeraceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Sarcodon	Bankeraceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Agrocybe	Bolbitiaceae	Sap	Cannon and Kirk 2007
Alnicola	Bolbitiaceae	Sym(ec)	Moreau *et al.* 2006; Tedersoo *et al.* 2010
Hebeloma	Bolbitiaceae	Sym(ec)	Godbout et Fortin 1985; Tedersoo *et al.* 2010
Panaeolus	Bolbitiaceae	Sap	Reverchon *et al.* 2010
Boletus	Boletaceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Chalciporus	Boletaceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Chamonixia	Boletaceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Leccinum	Boletaceae	Sym(ec)	Godbout et Fortin 1985; Cannon and Kirk 2007; Tedersoo *et al.* 2010
Botryobasidium	Botryobasidiaceae	Sap	Cannon and Kirk 2007
Craterellus	Cantharellaceae	Sym(ec)	Webster and Weber 2007; Cannon and Kirk 2007; Tedersoo *et al.* 2010
*Cantharellaceae*_spp.	Cantharellaceae	Sym(ec)	Webster and Weber 2007; Cannon and Kirk 2007; Tedersoo *et al.* 2010
Ceratobasidium	Ceratobasidiaceae	Sym(or)/Sap/Par(pl)/Par(my)	Mosquera-Espinosa *et al.* 2013; Medina *et al.* 2012
*Ceratobasidiaceae*_spp.	Ceratobasidiaceae	Sap/Par(pl)	Cannon and Kirk 2007
*Chionosphaeraceae*_spp.	Chionosphaeraceae	Sap/Par(my)	Cannon and Kirk 2007
Clavaria	Clavariaceae	Sym(er)/Sap	Moncalvo *et al.* 2002; Webster and Weber 2007
*Clavariaceae*_spp.1	Clavariaceae	Sym(li)/Sap	Cannon and Kirk 2007
*Clavariaceae*_spp.2	Clavariaceae	Sym(li)/Sap	Cannon and Kirk 2007
Clavulina	Clavulinaceae	Sym(ec)	Uehling *et al.* 2011; Tedersoo *et al.* 2010
Coniophora	Coniophoraceae	Sap/Par(pl)	Cannon and Kirk 2007
Jaapia	Coniophoraceae	Sap/Par(pl)	Cannon and Kirk 2007
Leucogyrophana	Coniophoraceae	Sap	Stone *et al.* 1989, Jarosh and Besl 2001
Serpula	Coniophoraceae	Sap/Par(pl)	Cannon and Kirk 2007
Coprinus	Coprinaceae	Sap	Mohamed and Dix 1988; Reverchon *et al.* 2010
*Corticiaceae*_spp.1	Corticiaceae	Sym(li)/Sap/Par(pl)	Cannon and Kirk 2007
*Corticiaceae*_spp.2	Corticiaceae	Sym(li)/Sap/Par(pl)	Cannon and Kirk 2007
Cortinarius	Cortinariaceae	Sym(ec)	Godbout et Fortin 1985; Tedersoo *et al.* 2010
Galerina	Cortinariaceae	Sap	Gulden *et al.* 2005; Reverchon *et al.* 2010
Gymnopilus	Cortinariaceae	Sap	Reverchon *et al.* 2010; Guzman-Davalos 2003
Inocybe	Cortinariaceae	Sym(ec)	Matheny 2005; Tedersoo *et al.* 2010
*Cortinariaceae*_spp.1	Cortinariaceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
*Cortinariaceae*_spp.2	Cortinariaceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Fellomyces	Cuniculitremaceae	Sap/End	Prinllinger *et al.* 1997; Hamamoto 1998
*Cuniculitremaceae*_spp.	Cuniculitremaceae	Par(my)	Cannon and Kirk 2007
Occultifur	Cystobasidiaceae	Sap	Sampaio et al 1999
Cystofilobasidium	Cystofilobasidiaceae	Sap	Cannon and Kirk 2007
Mrakia	Cystofilobasidiaceae	Sap	Messner *et al.* 1994

Echinodontium	Echinodontiaceae	Sap/Par(pl)	Etheridge and Craig 1976; Yu *et al.* 2009
Clitopilopsis	Entolomataceae	Sym(ec)/Sap	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Clitopilus	Entolomataceae	Sap	Redhead and Baroni 1986; Cannon and Kirk 2007
Entoloma	Entolomataceae	Sym(ec)/Sap/Par(my)	Noordeloos and Hausknecht 2007; Czederpiltz *et al.* 2001; Agerer and Waller 1993; Tedersoo *et al.* 2010
Nolanea	Entolomataceae	Sym(ec)/Sap	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Rhodocybe	Entolomataceae	Sym(ec)/Sap	Cannon and Kirk 2007; Tedersoo *et al.* 2010
*Entolomataceae*_spp.	Entolomataceae	Sym(ec)/Sap	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Entorrhiza	Entorrhizaceae	Par(pl)	Cannon and Kirk 2007
Exidia	Exidiaceae	Sap	Cannon and Kirk 2007
Sebacina	Exidiaceae	Sym(ec)/Sym(er)/Sym(or)	McKendrick *et al.* 2002; Selosse *et al.* 2007; Ghimire *et al.* 2009; Tedersoo *et al.* 2010
*Exidiaceae*_spp.1	Exidiaceae	Sap/Par(pl)	Cannon and Kirk 2007
*Exidiaceae*_spp.2	Exidiaceae	Sap/Par(pl)	Cannon and Kirk 2007
Exobasidium	Exobasidiaceae	Par(pl)	Cannon and Kirk 2007
*Exobasidiaceae*_spp.	Exobasidiaceae	Par(pl)	Cannon and Kirk 2007
Cryptococcus	Filobasidiaceae	Sap	Cannon and Kirk 2007; Dodd *et al.* 2010
Filobasidium	Filobasidiaceae	Sap	Moore and Rij 1972; Cannon and Kirk 2007
*Filobasidiaceae*_spp.	Filobasidiaceae	Sap	Cannon and Kirk 2007
Postia	Fomitopsidaceae	Par(pl)	Martinez *et al.* 2009
Ganoderma	Ganodermataceae	Sap/Par(pl)	Moncalvo *et al.* 1995
*Ganodermataceae*_spp.	Ganodermataceae	Sap/Par(pl)	Cannon and Kirk 2007
Ramariopsis	Gomphaceae	Sap	Cannon and Kirk 2007
Ceriporiopsis	Hapalopilaceae	Sap	Rüttimann *et al.* 1992; Cannon and Kirk 2007
Mucronella	Hericiaceae	Sap	Cannon and Kirk 2007
Laccaria	Hydnangiaceae	Sym(ec)	Godbout et Fortin 1985; Tedersoo *et al.* 2010
Camarophyllopsis	Hygrophoraceae	Sym(ec)/Sap	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Cuphophyllus	Hygrophoraceae	Sym(ec)/Sap	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Hygrocybe	Hygrophoraceae	Sap	Reverchon *et al.* 2010; Cannon and Kirk 2007
Hygrophorus	Hygrophoraceae	Sym(ec)	Webster and Weber 2007; Tedersoo *et al.* 2010
*Hygrophoraceae*_spp.1	Hygrophoraceae	Sym(ec)/Sap	Cannon and Kirk 2007; Tedersoo *et al.* 2010
*Hygrophoraceae*_spp.2	Hygrophoraceae	Sym(ec)/Sap	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Hymenochaete	Hymenochaetaceae	Sap	Burt 1918
Aegerita	Hyphodermataceae	Sap/Par(ne)	Webster and Weber 2007; Cannon and Kirk 2007
Hyphoderma	Hyphodermataceae	Sap	Larsson 2007; Sheng-Hua 1997; Nilsson *et al.* 2003
Hypochnicium	Hyphodermataceae	Sap	Reverchon *et al.* 2010
Hysterangium	Hysterangiaceae	Sym(ec)	Malajczuk 1987; Tedersoo *et al.* 2010
Erythrobasidium	Incertae_sedis	Unknown	Suh *et al.* 1993
Holtermanniella	Incertae_sedis	Unknown	Wuczkowski *et al.* 2011
Malassezia	Incertae_sedis	Par(an)	Webster and Weber 2007
Naohidea	Incertae_sedis	Par(my)	Swann and Taylor 1995; Platek 2002
Rhodosporidium	Incertae_sedis	Sap/Par(my)	Yonghong *et al.* 2007; Sampaio *et al.* 2001; Caoa and Utkhedea 2005; Sampaio *et al.* 2003
Rhodotorula	Incertae_sedis	Sap/Par(an)/Epi	Webster and Weber 2007; Diekema 2005; Sampaio *et al.* 2003
Sakaguchia	Incertae_sedis	Unknown	Mycobank; Yamada *et al.* 1994
Sporobolomyces	Incertae_sedis	Sap/Par(an)/Epi	Webster and Weber 2007
Kondoa	Kondoaceae	Unknown	Fonseca *et al.* 2000
Leucosporidiella	Leucosporidiaceae	Unknown	Sampaio 2003
Lycoperdon	Lycoperdaceae	Sap	Reverchon *et al.* 2010; Moncalvo *et al.* 2002
Calocybe	Lyophyllaceae	Sap	Brunner and Miller 1988
*Lyophyllaceae*_spp.	Lyophyllaceae	Unknown	
Armillaria	Marasmiaceae	Sap/Par(pl)	Anderson and Stasovski 1992; Baumgartner and David M. Rizzo 2001
Clitocybula	Marasmiaceae	Sap	Antonin *et al.* 2011
Gymnopus	Marasmiaceae	Sap/Par(pl)	Reverchon *et al.* 2010; Moncalvo *et al.* 2002
Lachnella	Marasmiaceae	Sap	Seaver 1951
Marasmius	Marasmiaceae	Sap	Reverchon *et al.* 2010; Moncalvo *et al.* 2002
Phlebia	Meruliaceae	Sap	Cannon and Kirk 2007; Cooke 1956
Mycena	Mycenaceae	Sap	Smith 1947; Reverchon *et al.* 2010; Moncalvo *et al.* 2002

Roridomyces	Mycenaceae	Sap	Cannon and Kirk 2007
Nidularia	Nidulariaceae	Sap	Mycoquébec
Alpova	Paxillaceae	Sym(ec)	Massicotte *et al.* 1986; Nouhra *et al.* 2005; Tedersoo *et al.* 2010
Paxillus	Paxillaceae	Sym(ec)	Godbout et Fortin 1985; Tedersoo *et al.* 2010
Entomocorticium	Peniophoraceae	Par(pl)	Scott *et al.* 2008; Whitney *et al.* 1987
Mutinus	Phallaceae	Sap	Godbout et Fortin 1985
Phallus	Phallaceae	Sap	Cannon and Kirk 2007
Nematoctonus	Pleurotaceae	Par(ne)	Barron and Dierkes 1977; Cannon and Kirk 2007
Pluteus	Pluteaceae	Sap	Reverchon *et al.* 2010
*Podoscyphaceae*_spp.	Podoscyphaceae	Sap	Cannon and Kirk 2007
Oligoporus	Polyporaceae	Sap	Cannon and Kirk 2007; Schmidt and Moreth 2005
Perenniporia	Polyporaceae	Sap	Cannon and Kirk 2007; Decock and Ryvarden 2003
Polyporus	Polyporaceae	Sap/Par(pl)	Cannon and Kirk 2007; Eriksson and Goodell 1974; Bohaychuk and Whitney 1973
Trametes	Polyporaceae	Sap/Par(pl)	Cannon and Kirk 2007; Mehrotra et al 1996; Osono *et al.* 2003
Trichaptum	Polyporaceae	Sap/Par(pl)	Dai *et al.* 2009; Kauserud and Schumacher 2003; Kobro 2001
Tyromyces	Polyporaceae	Sap	Cannon and Kirk 2007; Brotzman and Gilbertson 1967; Yao *et al.* 1999
Coprinopsis	Psathyrellaceae	Sap	Reverchon *et al.* 2010
Psathyrella	Psathyrellaceae	Sap	Reverchon *et al.* 2010
Rhizopogon	Rhizopogonaceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Lactarius	Russulaceae	Sym(ec)	Godbout et Fortin 1985; Tedersoo *et al.* 2010
Russula	Russulaceae	Sym(ec)	Avis *et al.* 2003; Tedersoo *et al.* 2010
*Russulaceae*_spp.	Russulaceae	Sym(ec)/Sap	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Rectipilus	Schizophyllaceae	Sap	Cannon and Kirk 2007
Schizophyllum	Schizophyllaceae	Sap	Cannon and Kirk 2007
Hyphodontia	Schizoporaceae	Sap	Cannon and Kirk 2007
Scleroderma	Sclerodermataceae	Sym(ec)	Godbout et Fortin 1985; Tedersoo *et al.* 2010
*Sebacinaceae*_spp.	Sebacinaceae	Sym(ec)/Sym(er)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Sistotrema	Sistotremataceae	Sym(ec)	Nilsson *et al.* 2006; Tedersoo *et al.* 2010
Trechispora	Sistotremataceae	Sap	Cannon and Kirk 2007
Sporidiobolus	Sporidiobolaceae	Sap	Cannon and Kirk 2007
Antrodiella	Steccherinaceae	Sap	Cannon and Kirk 2007
Junghuhnia	Steccherinaceae	Sap	Hood and Dick 1988, Cannon and Kirk 2007
Stereum	Stereaceae	Sap	Cannon and Kirk 2007
Hypholoma	Strophariaceae	Sap	Valášková *et al.* 2009; Pearce *et al.* 1995
Kuehneromyces	Strophariaceae	Sap	Cannon and Kirk 2007
Pholiota	Strophariaceae	Sap/Par(pl)	Reverchon *et al.* 2010; Cannon and Kirk 2007
Psilocybe	Strophariaceae	Sap	Cannon and Kirk 2007
*Strophariaceae*_spp.	Strophariaceae	Sap/Par(pl)	Cannon and Kirk 2007
Suillus	Suillaceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
Syzygospora	Syzygosporaceae	Par(my)	Ginns 1986
Amaurodon	Thelephoraceae	Sap	ČÍŽEK *et al.* 2007
Pseudotomentella	Thelephoraceae	Sym(ec)	Tedersoo *et al.* 2010
Thelephora	Thelephoraceae	Sym(ec)/Sap	Agerer and Weiss 1989; Selvam *et al.* 2003; Tedersoo *et al.* 2010
Tomentella	Thelephoraceae	Sym(ec)	Lilleskov and Bruns 2005; Barrett *et al.* 2010; Kõljalg *et al.* 2002; Tedersoo *et al.* 2010
Tomentellopsis	Thelephoraceae	Sym(ec)	Kõljalg *et al.* 2002; Tedersoo *et al.* 2010
*Thelephoraceae*_spp.	Thelephoraceae	Unknown	Cannon and Kirk 2007
Bullera	Tremellaceae	Unknown	Nakase 2002
Dioszegia	Tremellaceae	Unknown	Renker *et al.* 2006; Takashima *et al.* 2001; Connell *et al.* 2010
Filobasidiella	Tremellaceae	Sap/Par(my)	Rodriguez-Carres *et al.* 2010
Tremella	Tremellaceae	Par(my)	Bezerra and Kimbrough 1978; Roberts 1995
Arrhenia	Tricholomataceae	Sap	Barrasa and Rico 2003; Barrasa ans Rico 2010
Clitocybe	Tricholomataceae	Sap	Reverchon *et al.* 2010
Melanoleuca	Tricholomataceae	Sap	Mohamed and Dix 1988
Ripartites	Tricholomataceae	Unknown	

Tricholoma	Tricholomataceae	Sym(ec)	Godbout et Fortin 1985; Cannon and Kirk 2007; Tedersoo *et al.* 2010
*Tricholomataceae*_spp.	Tricholomataceae	Unknown	Cannon and Kirk 2007
Asterotremella	Trichosporonaceae	Sap/Par(my)	Prillinger *et al.* 2007; Liu *et al.* 2010
Cryptotrichosporon	Trichosporonaceae	Unknown	Okoli *et al.* 2007
Trichosporon	Trichosporonaceae	Sap/Par(an)	Middelhoven *et al.* 2001; Walsh *et al.* 1990; Wolf *et al.* 2001
Tubulicrinis	Tubulicrinaceae	Sap	Cannon and Kirk 2007
Urocystis	Urocystidaceae	Par(pl)	Cannon and Kirk 2007; Wennström and Ericson 1990; Borgen and Kristensen 2001; Nus and Hodges 1990
Pseudozyma	Ustilaginaceae	Sap/Epi	Cannon and Kirk 2007; Buxdorf *et al.* 2013
*Ustilaginaceae*_spp.	Ustilaginaceae	Sap/Par(pl)/Epi	Cannon and Kirk 2007
Ustilago	Ustilaginaceae	Par(pl)	Cannon and Kirk 2007; Kamper *et al.* 2006; Kolk 1930

Chytridiomycota

Genus	Family	Lifestyle	Ref
Hyaloraphidium	Incertae_sedis	Unknown	Forget *et al.* 2001
Lobulomyces	Lobulomycetaceae	Sap	Simmons *et al.* 2009; Kagami *et al.* 2011; Willoughby and Townley 1961
Monoblepharis	Monoblepharidaceae	Sap	Unestam 1965; Cannon and Kirk 2007
*Monoblepharidaceae*_spp.	Monoblepharidaceae	Sap	Cannon and Kirk 2007
Olpidium	Olpidium	Par(pl)	Barr and Hartmann 1977
Rhizophlyctis	Rhizophlyctidaceae	Sym(ec)	Cannon and Kirk 2007; Tedersoo *et al.* 2010
*Spizellomycetaceae*_spp.	Spizellomycetaceae	Sap/Par(pl)	Cannon and Kirk 2007

Glomeromycota

Genus	Family	Lifestyle	Ref
Acaulospora	Acaulosporaceae	Sym(am)	Webster and Weber 2007
*Archaeosporaceae*_spp.1	Archaeosporaceae	Sym(am)	Cannon and Kirk 2007
*Archaeosporaceae*_spp.2	Archaeosporaceae	Sym(am)	Cannon and Kirk 2007
*Diversisporaceae*_spp.	Diversisporaceae	Sym(am)	Palenzuela *et al.* 2010
Entrophospora	Entrophosporaceae	Sym(am)	Li *et al.* 2013; Palenzuela *et al.* 2010
Scutellospora	Gigasporaceae	Sym(am)	Cannon and Kirk 2007; Jansa *et al.* 2002
*Gigasporaceae*_spp.	Gigasporaceae	Sym(am)	Cannon and Kirk 2007; Jansa *et al.* 2002
Glomus	Glomeraceae	Sym(am)	Cannon and Kirk 2007; Jansa *et al.* 2002
Rhizophagus (syn. *Rhizoglomus*)	Glomeraceae	Sym(am)	Cannon and Kirk 2007
*Glomeraceae*_spp.1	Glomeraceae	Sym(am)	Cannon and Kirk 2007; Jansa *et al.* 2002
*Glomeraceae*_spp.2	Glomeraceae	Sym(am)	Cannon and Kirk 2007; Jansa *et al.* 2002
*Diversisporales*_spp.1	unclassified	Sym(am)	Webster and Weber 2007
*Glomeromycetes*_spp.	unclassified	Sym(am)	Webster and Weber 2007
*Glomeromycota*_spp.1	unclassified	Sym(am)	Webster and Weber 2007
*Archaeosporales*_spp.	unidentified	Sym(am)	Webster and Weber 2007
*Diversisporales*_spp.2	unidentified	Sym(am)	Webster and Weber 2007
*Glomeromycota*_spp.2	unidentified	Sym(am)	Webster and Weber 2007

Zygomycota

Genus	Family	Lifestyle	Ref
Basidiobolus	Basidiobolaceae	Sap	Cannon and Kirk 2007
Mortierella	Mortierellaceae	Sap	Cannon and Kirk 2007
*Mortierellaceae*_spp.	Mortierellaceae	Sap	Cannon and Kirk 2007
Umbelopsis	Umbelopsidaceae	Unknown	Cannon and Kirk 2007; Meyer and Gams 2003; Amos and Barnett 1966

Lifestyle abreviations

Sap: saprotrophic fungi
Par: parasitic fungi
    an: animal parasite; my: mycoparasite; ne: nematophagous; pl: phytoparasite
Sym: symbiotic fungi
    am: arbuscular mycorrhizal fungi; ec: ectomycorrhizal fungi; er: ericoid mycorhizal fungi; li: lichenized fungi; or: orchidoid mycorrhizal fungi
End: endophytic fungi
Epi: epiphytic fungi

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Annexe 2 - List of EM fungal OTUs detected in roots or bulk soil of the four host species.

	Family	Genus	Total number of reads	Total number of occurrence	Occurrence in roots samples				Occurrence in bulk soil samples			
					PIMA	ABBA	PIBA	PIMU	PIMA	ABBA	PIBA	PIMU
Basidiomycota- Agaricomycetes												
OTU_230	Albatrellaceae	*Leucophleps*	163	6	0	1	0	0	0	3	2	0
OTU_3152	Albatrellaceae	*Leucophleps*	75	4	0	1	0	0	0	2	1	0
OTU_913	Albatrellaceae	*Leucophleps*	35	3	0	1	0	0	0	1	1	0
OTU_1948	Albatrellaceae	*Leucophleps*	4	2	0	0	0	0	0	1	1	0
OTU_3061	Albatrellaceae	*Leucophleps*	8	2	0	0	0	0	0	1	1	0
OTU_335	Amanitaceae	*Amanita*	70	6	2	1	0	0	2	1	0	0
OTU_204	Amanitaceae	*Amanita*	158	4	0	0	1	1	1	0	0	1
OTU_237	Amanitaceae	*Amanita*	142	4	0	1	0	0	1	1	1	0
OTU_10	Atheliaceae	*Amphinema*	2623	46	6	5	4	6	7	8	5	5
OTU_1915	Atheliaceae	*Amphinema*	782	31	4	5	1	3	6	5	4	3
OTU_62	Atheliaceae	*Amphinema*	1218	21	3	2	0	2	5	6	3	0
OTU_78	Atheliaceae	*Amphinema*	558	16	0	1	1	2	1	4	4	3
OTU_103	Atheliaceae	*Amphinema*	333	14	0	1	0	3	2	1	3	4
OTU_2706	Atheliaceae	*Amphinema*	58	12	3	2	0	1	1	1	2	2
OTU_2366	Atheliaceae	*Amphinema*	11	9	2	2	0	0	1	0	1	3
OTU_1874	Atheliaceae	*Amphinema*	15	8	4	2	0	1	0	0	1	0
OTU_483	Atheliaceae	*Amphinema*	50	6	0	0	1	0	1	2	2	0
OTU_94	Atheliaceae	*Amphinema*	231	6	0	1	1	0	1	2	1	0
OTU_1357	Atheliaceae	*Amphinema*	27	4	0	0	0	0	1	2	1	0
OTU_2051	Atheliaceae	*Amphinema*	29	3	0	0	0	0	0	3	0	0
OTU_2200	Atheliaceae	*Amphinema*	18	2	0	0	0	1	0	0	0	1
OTU_68	Atheliaceae	*Piloderma*	416	10	3	0	0	2	4	0	1	0
OTU_2609	Atheliaceae	*Piloderma*	84	9	3	0	0	2	3	0	1	0
OTU_123	Atheliaceae	*Piloderma*	380	4	1	1	0	0	1	1	0	0

OTU_117	Atheliaceae	*Piloderma*	30	3	0	0	0	0	2	1	0	0
OTU_179	Atheliaceae	*Piloderma*	1	1	0	0	0	0	0	1	0	0
OTU_8	Atheliaceae	*Tylospora*	2055	33	3	3	3	4	6	7	3	4
OTU_109	Atheliaceae	*Tylospora*	248	6	0	0	1	1	0	2	2	0
OTU_2960	Atheliaceae	*Tylospora*	2	2	0	0	0	1	1	0	0	0
OTU_2897	Atheliaceae	*unidentified*	88	6	1	2	0	2	0	1	0	0
OTU_66	Bankeraceae	*Boletopsis*	8	2	1	0	0	0	0	0	1	0
OTU_2179	Bankeraceae	*Hydnellum*	2	1	0	0	0	0	1	0	0	0
OTU_1329	Bankeraceae	*Phellodon*	3	2	0	1	0	0	1	0	0	0
OTU_116	Bankeraceae	*Sarcodon*	214	7	1	1	0	0	2	3	0	0
OTU_1127	Bolbitiaceae	*Alnicola*	3	1	0	0	0	0	0	0	1	0
OTU_107	Bolbitiaceae	*Hebeloma*	341	4	0	1	0	1	0	1	0	1
OTU_144	Bolbitiaceae	*Hebeloma*	224	2	0	1	0	0	0	1	0	0
OTU_842	Bolbitiaceae	*Hebeloma*	34	2	0	1	0	1	0	0	0	0
OTU_300	Boletaceae	*Boletus*	53	8	2	0	1	0	3	0	2	0
OTU_356	Boletaceae	*Boletus*	121	8	1	0	0	1	1	1	3	1
OTU_771	Boletaceae	*Boletus*	5	1	0	1	0	0	0	0	0	0
OTU_833	Cantharellaceae	*unidentified*	8	3	0	0	0	0	0	2	1	0
OTU_1917	Cantharellaceae	*unidentified*	4	2	0	0	0	0	0	1	1	0
OTU_1234	Cantharellaceae	*unidentified*	4	1	0	0	0	0	0	1	0	0
OTU_2965	Ceratobasidiaceae	*Ceratobasidium*	3	2	0	0	0	1	0	0	1	0
OTU_1043	Ceratobasidiaceae	*Ceratobasidium*	1	1	0	0	0	1	0	0	0	0
OTU_206	Clavulinaceae	*Clavulina*	192	9	0	2	1	1	2	2	1	0
OTU_460	Clavulinaceae	*Clavulina*	27	6	2	0	0	0	1	2	0	1
OTU_449	Clavulinaceae	*Clavulina*	100	4	1	1	0	0	1	1	0	0
OTU_133	Cortinariaceae	*Cortinarius*	285	10	1	2	1	2	2	1	0	1
OTU_135	Cortinariaceae	*Cortinarius*	144	9	1	0	0	1	3	2	1	1
OTU_211	Cortinariaceae	*Cortinarius*	28	9	1	0	1	0	2	2	2	1
OTU_288	Cortinariaceae	*Cortinarius*	70	9	0	1	0	1	2	3	0	2
OTU_49	Cortinariaceae	*Cortinarius*	578	8	2	0	0	2	2	1	0	1
OTU_624	Cortinariaceae	*Cortinarius*	30	8	1	2	0	2	0	0	0	3

OTU_192	Cortinariaceae	*Cortinarius*	114	7	1	0	0	3	0	1	1	1
OTU_646	Cortinariaceae	*Cortinarius*	25	7	1	1	0	1	1	0	2	1
OTU_701	Cortinariaceae	*Cortinarius*	22	7	0	0	0	0	2	0	3	2
OTU_1238	Cortinariaceae	*Cortinarius*	14	6	1	0	1	1	0	0	3	0
OTU_397	Cortinariaceae	*Cortinarius*	49	6	0	0	0	2	0	1	3	0
OTU_1023	Cortinariaceae	*Cortinarius*	23	5	2	0	0	0	2	1	0	0
OTU_1606	Cortinariaceae	*Cortinarius*	45	4	0	0	0	1	0	0	1	2
OTU_198	Cortinariaceae	*Cortinarius*	16	4	2	1	0	0	0	0	1	0
OTU_228	Cortinariaceae	*Cortinarius*	68	4	2	0	0	0	0	0	2	0
OTU_42	Cortinariaceae	*Cortinarius*	96	4	0	0	1	1	1	0	1	0
OTU_468	Cortinariaceae	*Cortinarius*	28	4	0	0	0	1	0	1	0	2
OTU_1577	Cortinariaceae	*Cortinarius*	18	3	0	0	0	1	0	0	2	0
OTU_2392	Cortinariaceae	*Cortinarius*	10	3	1	1	0	0	0	1	0	0
OTU_261	Cortinariaceae	*Cortinarius*	52	3	0	1	0	0	0	1	1	0
OTU_2944	Cortinariaceae	*Cortinarius*	7	3	0	0	0	1	0	0	1	1
OTU_329	Cortinariaceae	*Cortinarius*	57	3	1	0	0	0	1	0	1	0
OTU_1142	Cortinariaceae	*Cortinarius*	12	2	0	0	0	0	0	0	2	0
OTU_2097	Cortinariaceae	*Cortinarius*	9	2	0	1	0	0	1	0	0	0
OTU_2593	Cortinariaceae	*Cortinarius*	3	2	0	0	0	1	0	0	0	1
OTU_369	Cortinariaceae	*Cortinarius*	39	2	0	0	0	0	0	0	1	1
OTU_407	Cortinariaceae	*Cortinarius*	21	2	0	1	0	0	0	1	0	0
OTU_485	Cortinariaceae	*Cortinarius*	16	2	1	0	0	0	0	1	0	0
OTU_496	Cortinariaceae	*Cortinarius*	25	2	1	0	0	0	0	0	0	1
OTU_762	Cortinariaceae	*Cortinarius*	48	2	0	0	0	1	1	0	0	0
OTU_915	Cortinariaceae	*Cortinarius*	7	2	0	0	0	0	0	0	1	1
OTU_918	Cortinariaceae	*Cortinarius*	10	2	0	1	0	0	0	1	0	0
OTU_989	Cortinariaceae	*Cortinarius*	9	2	0	1	0	0	0	0	1	0
OTU_1348	Cortinariaceae	*Cortinarius*	2	1	0	0	0	0	0	0	1	0
OTU_167	Cortinariaceae	*Cortinarius*	234	1	0	0	0	1	0	0	0	0
OTU_1904	Cortinariaceae	*Cortinarius*	1	1	1	0	0	0	0	0	0	0
OTU_705	Cortinariaceae	*Cortinarius*	1	1	0	0	1	0	0	0	0	0

OTU_1186	Cortinariaceae	*Inocybe*	26	2	0	1	0	0	0	1	0	0
OTU_1875	Cortinariaceae	*Inocybe*	2	2	0	0	0	0	0	2	0	0
OTU_318	Entolomataceae	*Entoloma*	15	8	0	0	0	0	3	2	1	2
OTU_635	Entolomataceae	*Entoloma*	9	5	1	1	0	1	1	0	1	0
OTU_1677	Entolomataceae	*Entoloma*	2	2	1	0	0	0	0	1	0	0
OTU_869	Entolomataceae	*Entoloma*	6	2	0	0	1	0	0	0	0	1
OTU_1141	Gomphidiaceae	*Chroogomphus*	7	3	0	0	1	0	0	0	2	0
OTU_1922	Gomphidiaceae	*Gomphidius*	3	1	0	0	0	0	1	0	0	0
OTU_362	Hydnaceae	*Hydnum*	39	2	0	0	1	0	0	0	1	0
OTU_158	Hygrophoraceae	*Hygrophorus*	164	4	1	1	0	0	1	1	0	0
OTU_219	Hygrophoraceae	*Hygrophorus*	177	2	1	0	0	0	0	1	0	0
OTU_1872	Hysterangiaceae	*Hysterangium*	8	5	0	1	0	0	0	3	1	0
OTU_1067	Hysterangiaceae	*Hysterangium*	3	2	1	0	0	0	0	1	0	0
OTU_607	Paxillaceae	*Alpova*	18	5	0	0	0	1	0	1	2	1
OTU_1425	Rhizopogonaceae	*Rhizopogon*	5	3	0	0	0	0	0	0	3	0
OTU_2057	Russulaceae	*Lactarius*	2006	20	2	3	1	1	6	4	2	1
OTU_1813	Russulaceae	*Lactarius*	865	18	2	2	1	1	5	5	1	1
OTU_1418	Russulaceae	*Lactarius*	340	16	2	2	0	0	5	4	1	2
OTU_1757	Russulaceae	*Lactarius*	123	12	1	1	1	1	3	3	1	1
OTU_2058	Russulaceae	*Lactarius*	45	6	0	1	0	0	2	2	0	1
OTU_2133	Russulaceae	*Lactarius*	18	4	0	1	0	0	1	2	0	0
OTU_366	Russulaceae	*Lactarius*	31	3	1	0	0	0	2	0	0	0
OTU_2188	Russulaceae	*Lactarius*	2	2	0	1	0	0	0	1	0	0
OTU_2686	Russulaceae	*Lactarius*	4	2	0	0	0	0	1	1	0	0
OTU_595	Russulaceae	*Lactarius*	8	1	0	0	0	0	0	1	0	0
OTU_124	Russulaceae	*Russula*	404	15	0	4	1	1	2	4	2	1
OTU_22	Russulaceae	*Russula*	1257	15	2	3	1	2	3	3	1	0
OTU_25	Russulaceae	*Russula*	1319	14	2	2	1	2	3	4	0	0
OTU_11	Russulaceae	*Russula*	2156	9	1	3	0	1	2	2	0	0
OTU_2038	Russulaceae	*Russula*	5	3	0	1	0	0	1	0	1	0
OTU_861	Russulaceae	*Russula*	10	3	1	0	0	1	1	0	0	0

OTU_1716	Russulaceae	*Russula*	3	2	0	1	0	0	0	1	0	0
OTU_1957	Russulaceae	*Russula*	6	2	0	0	0	0	0	1	1	0
OTU_3080	Russulaceae	*Russula*	4	2	1	0	0	0	1	0	0	0
OTU_431	Russulaceae	*Russula*	49	2	1	0	0	0	1	0	0	0
OTU_620	Russulaceae	*Russula*	7	2	0	0	0	0	0	2	0	0
OTU_98	Russulaceae	*Russula*	386	2	1	0	0	0	1	0	0	0
OTU_55	Russulaceae	*unidentified*	596	19	2	1	3	3	3	4	1	2
OTU_536	Sclerodermataceae	*Scleroderma*	34	4	0	1	0	2	0	0	0	1
OTU_104	Sebacinaceae	*unidentified*	297	21	1	1	5	2	2	3	4	3
OTU_2997	Sebacinaceae	*unidentified*	84	8	1	0	2	0	1	2	2	0
OTU_39	Sebacinaceae	*unidentified*	543	8	0	2	1	1	0	1	2	1
OTU_393	Sebacinaceae	*unidentified*	124	8	1	1	1	1	1	2	1	0
OTU_327	Sebacinaceae	*unidentified*	81	7	0	0	0	1	0	2	2	2
OTU_395	Sebacinaceae	*unidentified*	19	6	0	1	0	1	1	1	1	1
OTU_1061	Sebacinaceae	*unidentified*	55	5	0	1	0	1	0	1	1	1
OTU_2817	Sebacinaceae	*unidentified*	10	5	1	0	1	0	1	0	2	0
OTU_585	Sebacinaceae	*unidentified*	19	4	0	1	0	0	0	1	1	1
OTU_685	Sebacinaceae	*unidentified*	7	4	0	0	0	0	0	2	2	0
OTU_1563	Sebacinaceae	*unidentified*	5	3	0	0	3	0	0	0	0	0
OTU_186	Sebacinaceae	*unidentified*	157	3	0	0	1	0	0	0	1	1
OTU_1808	Sebacinaceae	*unidentified*	23	2	1	0	0	0	0	1	0	0
OTU_402	Sebacinaceae	*unidentified*	46	2	0	0	0	1	0	0	1	0
OTU_1006	Sebacinaceae	*unidentified*	1	1	0	0	0	1	0	0	0	0
OTU_1303	Sebacinaceae	*unidentified*	1	1	0	1	0	0	0	0	0	0
OTU_20	Sistotremataceae	*Sistotrema*	1012	24	5	4	0	2	6	5	1	1
OTU_24	Sistotremataceae	*Sistotrema*	1085	7	0	1	1	2	0	0	1	2
OTU_1373	Sistotremataceae	*Sistotrema*	4	3	0	0	0	0	2	1	0	0
OTU_305	Sistotremataceae	*Sistotrema*	5	1	0	0	0	0	1	0	0	0
OTU_215	Suillaceae	*Suillus*	453	18	1	1	1	5	2	2	2	4
OTU_234	Suillaceae	*Suillus*	82	7	1	0	1	2	1	1	0	1
OTU_363	Suillaceae	*Suillus*	106	7	0	0	2	2	0	1	0	2

OTU_2853	Suillaceae	*Suillus*	10	5	0	0	2	2	0	1	0	0
OTU_87	Suillaceae	*Suillus*	325	4	0	0	0	1	0	1	2	0
OTU_2819	Suillaceae	*Suillus*	9	3	0	0	0	1	0	0	2	0
OTU_1985	Suillaceae	*Suillus*	3	2	0	0	1	0	0	1	0	0
OTU_321	Thelephoraceae	*Pseudotomentella*	42	3	0	0	0	1	1	1	0	0
OTU_409	Thelephoraceae	*Pseudotomentella*	4	2	0	0	0	1	0	0	0	1
OTU_1692	Thelephoraceae	*Pseudotomentella*	1	1	0	1	0	0	0	0	0	0
OTU_73	Thelephoraceae	*Thelephora*	525	8	0	1	2	1	0	0	3	1
OTU_344	Thelephoraceae	*Tomentella*	33	4	0	2	0	0	0	2	0	0
OTU_423	Thelephoraceae	*Tomentella*	27	4	0	0	1	0	2	0	1	0
OTU_9	Thelephoraceae	*Tomentella*	2215	3	1	0	0	0	1	0	1	0
OTU_1743	Thelephoraceae	*Tomentella*	2	2	1	0	0	0	1	0	0	0
OTU_2461	Thelephoraceae	*Tomentella*	7	2	1	0	0	0	1	0	0	0
OTU_824	Thelephoraceae	*Tomentella*	18	2	0	0	0	0	0	1	0	1
OTU_1989	Thelephoraceae	*Tomentella*	1	1	0	0	0	0	0	1	0	0
OTU_199	Thelephoraceae	*Tomentellopsis*	22	7	3	0	0	1	1	1	1	0
OTU_2190	Thelephoraceae	*Tomentellopsis*	84	5	3	0	0	1	1	0	0	0
OTU_2720	Thelephoraceae	*Tomentellopsis*	4	2	1	0	0	1	0	0	0	0
OTU_2574	Thelephoraceae	*Tomentellopsis*	1	1	0	1	0	0	0	0	0	0
OTU_169	Tricholomataceae	*Tricholoma*	158	3	0	1	0	0	0	2	0	0
OTU_1263	Tricholomataceae	*Tricholoma*	3	2	0	0	0	0	1	1	0	0
OTU_195	Tricholomataceae	*Tricholoma*	26	2	0	0	0	1	0	0	0	1
OTU_88	Tricholomataceae	*Tricholoma*	36	2	0	1	0	0	1	0	0	0
Basidiomycota - Tremellomycetes												
OTU_272	Exidiaceae	*Sebacina*	134	12	3	2	0	0	3	4	0	0
OTU_36	Exidiaceae	*Sebacina*	570	4	0	1	0	0	1	2	0	0
OTU_1923	Exidiaceae	*Sebacina*	52	2	1	0	0	0	1	0	0	0
OTU_601	Exidiaceae	*Sebacina*	17	2	0	1	0	0	0	1	0	0
Ascomycota - Dothideomycetes												
OTU_146	Incertae_sedis	*Cenococcum*	135	33	3	5	6	4	4	3	3	5
OTU_41	Incertae_sedis	*Cenococcum*	770	31	4	2	3	2	3	6	5	6

OTU_152	Incertae_sedis	*Cenococcum*	320	28	6	4	4	2	5	3	3	1
OTU_130	Incertae_sedis	*Cenococcum*	317	22	3	4	2	2	4	4	2	1
OTU_1669	Incertae_sedis	*Cenococcum*	70	11	2	3	1	0	1	2	2	0
OTU_278	Incertae_sedis	*Cenococcum*	39	11	3	0	1	2	3	0	1	1
OTU_2177	Incertae_sedis	*Cenococcum*	15	7	3	0	0	1	2	1	0	0
OTU_774	Incertae_sedis	*Cenococcum*	16	6	2	1	0	1	1	1	0	0
OTU_1585	Incertae_sedis	*Cenococcum*	10	3	0	2	0	1	0	0	0	0
OTU_1708	Incertae_sedis	*Cenococcum*	3	3	1	1	1	0	0	0	0	0
Ascomycota - Leotiomycetes												
OTU_416	Incertae_sedis	*Cadophora*	53	12	1	1	0	1	3	4	1	1
OTU_1800	Incertae_sedis	*Cadophora*	6	6	0	3	0	0	1	1	1	0
OTU_1233	Incertae_sedis	*Cadophora*	17	4	1	2	0	0	1	0	0	0
OTU_2627	Incertae_sedis	*Cadophora*	22	4	0	1	0	0	1	1	0	1
OTU_560	Incertae_sedis	*Cadophora*	11	4	0	0	0	1	0	1	1	1
OTU_504	Incertae_sedis	*Cadophora*	42	3	0	1	0	1	0	0	0	1
OTU_1494	Incertae_sedis	*Cadophora*	2	1	0	0	0	0	0	0	0	1
Ascomycota - Pezizomycetes												
OTU_99	Helvellaceae	*Helvella*	32	2	0	0	1	0	0	0	1	0
OTU_1000	Discinaceae	*Hydnotrya*	16	3	0	1	1	0	0	0	1	0
OTU_240	Discinaceae	*Hydnotrya*	62	3	0	0	1	0	0	0	2	0
OTU_791	Pezizaceae	*Peziza*	11	3	0	0	0	0	0	1	1	1
OTU_2209	Pezizaceae	*Peziza*	2	2	0	1	1	0	0	0	0	0
OTU_341	Tuberaceae	*Tuber*	18	4	0	1	1	1	0	0	1	0
OTU_532	Tuberaceae	*Tuber*	14	4	0	1	1	0	0	1	1	0
Ascomycota - Sordariomycetes												
OTU_2557	Chaetosphaeriaceae	*Chloridium*	4	4	1	0	0	0	0	1	2	0