The abundance, origin and phylogeny of plants: effects on natural enemies and implications for plant coexistence in grasslands

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„Imagination will often carry us to worlds that never were. But without it we go nowhere.“

— Carl Sagan (1934 – 1996)

"… a tangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, … so different from each other, and dependent upon each other in so complex a manner, ..."

Charles Robert Darwin (1809-1882) on species diversity and interactions.
From *On the Origin of Species* (1859)

Photos, from top to bottom and left to right: Experimental site in Central Germany, overlooking Seeburg; Hover fly (*Episyrphus balteatus*) on flower of field scabious (*Knautia arvensis*) at the experimental site; Rust fungus (*Uromyces striatus*) on leaves of honey clover (*Melilotus albus*) in a grassland of the Central United States, near St. Louis

Contents

SUMMARY

Understanding the diversity of species and the mechanisms responsible for their coexistence has always been an important goal of ecological research. However, we still lack a conclusive body of empirical studies on the quality and relative contribution of coexistence mechanisms in ecological communities. Chesson's theoretical framework (2000) identified two mechanisms that determine species coexistence on a local scale: *equalizing fitness differences* between species that drive the best-adapted species to exclude others, and *stabilizing niche differences* among species such as the partitioning of limiting resources and responses to natural enemies. The presence of stabilizing niche differences is characterized by a negative relationship between a plant's relative abundance within a community and its per capita rate of increase. Stable coexistence occurs when species' stabilizing niche differences overcome their relative fitness differences. While recent theoretical studies have focused on how interactions between plants and consumers influence equalizing and stabilizing mechanisms, empirical information on this relationship is lacking. Moreover, it is not clear how these antagonistic interactions are affected by other important factors such as plant phylogeny to impact species coexistence in the context of biological invasions and the release of plants from their natural enemies. With this thesis, I aim to contribute to a broader understanding of how trophic interactions influence species coexistence in *novel* communities that consist of native and exotic species. I address three central research questions:

1. How do the relative abundance, origin and phylogenetic affiliation of plant species influence interactions between plants and their natural enemies?
2. How do pathogens and insect herbivores mediate the coexistence of plant species in grassland ecosystems?
3. How are natural communities of fungal pathogens and insect herbivores altered by the application of pesticides?

To gain new insights into these processes, I applied a broad set of methods. Effects of plant species' relative abundance, origin and phylogeny on pathogen diversity and infection were investigated using an observational study conducted in grasslands of the central United States (chapter 2). Plant community diversity and productivity, plant species demography as well as insect and fungal community composition and diversity were recorded in a three-year field experiment in semi-natural grasslands of central Germany. In this experiment, I manipulated the relative abundance of plant species, their origin and the presence of two groups of natural enemies to study

the combined effect of these factors on coexistence mechanisms (chapter 3). High-throughput sequencing was used on soil samples of the same field experiment to study the effects of fungicide application, plant species identity and origin on soil fungal community diversity and composition (chapter 4). With respect to my research questions my thesis produced the following results:

1. Across all three chapters of my thesis, interactions between plants and their natural enemies were largely independent of plant species' relative abundances. Abundances of native and exotic species did not differ in their responses to natural enemies. Plant community phylogeny did not have an effect on patterns of pathogen infection.
2. Per capita rates of increase were negatively related to a plant species' relative abundance in native communities, which indicates the presence of stabilizing niche differences. However, this relationship was not linked to interactions between plants and their natural enemies. Exotic-dominated communities showed varying and off-setting responses to natural enemies, which points to the presence of equalizing fitness differences.
3. Fungicide application had no effect on α-diversity of soil fungal communities, but β-diversity and overall fungal OTU abundances were significantly reduced. These effects were primarily driven by changes in pathotrophic fungi. Insecticide application had negative effects on soil arthropod diversity and abundances, caused by declines in springtails.

The results of my thesis suggest that antagonistic interactions can be decoupled from plants' relative abundances, and other factors such as abiotic soil conditions and spatial heterogeneity, but not plant phylogeny, are more important for the outcome of these interactions. As such, my thesis suggests that resource competition among plants may be responsible for stabilizing niche differences. Exotic species are not released from their natural enemies, indicating that enemies accumulate over time since introduction and/or ubiquitous enemies are frequently co-introduced with their hosts. Coexistence between natives and exotics in novel communities may be promoted by trade-offs between competitive ability and susceptibility to pathogens and herbivores. My thesis also shows that pesticide application is an effective tool for excluding natural enemies in ecological experiments, but that pesticides can lead to homogenization of antagonist communities and to impacts on non-target organisms that are important for ecosystem functioning.

In conclusion, the findings of my thesis emphasize the varying importance of fungal pathogens and insect herbivores for the coexistence among native and exotic species in grassland ecosystems. The fact that coexistence of plants in novel communities was apparently affected by both stabilizing niche differences and equalizing fitness differences highlights the context-dependence of interactions between plants and their natural enemies. This thesis elucidates how future approaches of observational and experimental nature can address such context-dependencies. Finally, my thesis calls for a more coherent combination of modern coexistence theory and invasion ecology in order to understand mechanisms of species coexistence and ecosystem functions in novel communities.

ZUSAMMENFASSUNG

Schon immer ist es ein wichtiges Ziel ökologischer Forschung gewesen, Mechanismen der Koexistenz von Arten als Grundlage ihrer Diversität zu verstehen. Es fehlen jedoch noch immer aussagekräftige empirische Studien über die Qualität und den relativen Beitrag von Koexistenzmechanismen in ökologischen Gemeinschaften. Im theoretischen Rahmen von Chesson (2000) wurden zwei Mechanismen identifiziert, die die Koexistenz von Arten auf lokaler Ebene bestimmen: *ausgleichende Fitnessunterschiede* zwischen Arten, welche den Ausschluss von Arten durch besser angepasste Arten bedingen, und *stabilisierende Nischenunterschiede* zwischen Arten, wie z. B. die Partitionierung von begrenzenden Ressourcen und Reaktionen auf natürliche Feinde. Das Vorhandensein von stabilisierenden Nischenunterschieden ist durch eine negative Beziehung zwischen der relativen Häufigkeit einer Pflanze innerhalb einer Gemeinschaft und ihrer Pro-Kopf-Zuwachsrate gekennzeichnet. Eine stabile Koexistenz ist gegeben, wenn die stabilisierenden Nischenunterschiede der Arten ihre relativen Fitnessunterschiede überwiegen. Während sich neuere theoretische Studien darauf konzentriert haben, wie Interaktionen zwischen Pflanzen und Konsumenten ausgleichende und stabilisierende Mechanismen beeinflussen, fehlen empirische Informationen über diese Beziehung. Darüber hinaus ist nicht klar, wie diese antagonistischen Interaktionen von anderen wichtigen Faktoren wie der Phylogenie der Pflanzen im Kontext biologischer Invasionen und dem damit verbundenen Zurücklassen natürlicher Feinde beeinflusst werden. Mit dieser Arbeit möchte ich daher zu einem umfassenderen Verständnis der Frage beitragen, wie trophische Interaktionen die Koexistenz von Arten in *neuartigen* Gemeinschaften, die aus nativen und gebietsfremden Arten bestehen, beeinflussen. Ich gehe dabei drei zentralen Forschungsfragen nach:

1. Wie beeinflussen die relative Häufigkeit, Herkunft und phylogenetische Zugehörigkeit von Pflanzenarten Interaktionen zwischen Pflanzen und ihren natürlichen Feinden?
2. Auf welche Art und Weise vermitteln pflanzliche Pathogene und herbivore Insekten die Koexistenz zwischen Pflanzenarten in Grünlandökosystemen?
3. Wie werden natürliche Gemeinschaften von pathogenen Pilzen und herbivoren Insekten durch den Einsatz von Pestiziden beeinflusst?

Um neue Erkenntnisse über diese Prozesse zu gewinnen, habe ich ein breites Spektrum an Methoden angewandt. Die Auswirkungen der relativen Häufigkeit, Herkunft und Phylogenie von

Pflanzenarten auf die Pathogenvielfalt und -infektion wurden anhand einer Beobachtungsstudie in Grünlandgemeinschaften der zentralen Vereinigten Staaten untersucht. (Kapitel 2). Die Diversität und Produktivität von Pflanzengemeinschaften, die Demografie von Pflanzenarten sowie die Zusammensetzung und Vielfalt von Insekten- und Pilzgemeinschaften wurden in einem dreijährigen Feldexperiment in halb-natürlichem Grünland in Mitteldeutschland erfasst. In diesem Experiment habe ich die relative Häufigkeit von Pflanzenarten, ihre Herkunft und die Anwesenheit von zwei Gruppen natürlicher Feinde manipuliert, um die kombinierte Wirkung dieser Faktoren auf die Koexistenzmechanismen zu untersuchen (Kapitel 3). Mit Hilfe der Hochdurchsatz-Sequenzierung von Bodenproben aus demselben Feldexperiment wurden die Auswirkungen der Fungizidanwendung, der Pflanzenart und der Herkunft der Pflanzen auf die Vielfalt und Zusammensetzung der Pilzgemeinschaft im Boden untersucht. (Kapitel 4). In Bezug auf meine Forschungsfragen hat meine Doktorarbeit folgende Ergebnisse hervorgebracht:

1. Die Interaktionen zwischen Pflanzen und ihren natürlichen Feinden waren in allen Untersuchungen weitgehend unabhängig von der relativen Häufigkeit der Pflanzenarten. Die Häufigkeiten von nativen und gebietsfremden Pflanzenarten reagierten nicht unterschiedlich auf natürliche Feinde. Die Phylogenie von Pflanzengemeinschaften hatte keinen Einfluss auf die Infektionsmuster von Pathogenen.
2. Die Pro-Kopf-Zuwachsraten von Pflanzen in nativen Gemeinschaften standen in einem negativen Zusammenhang zur relativen Häufigkeit einer Pflanzenart, was auf die Anwesenheit von stabilisierenden Nischendifferenzen schließen lässt. Dieser Zusammenhang war jedoch nicht an die Interaktionen von Pflanzen mit ihren natürlichen Feinden geknüpft. Von gebietsfremden Arten dominierte Gemeinschaften zeigten unterschiedliche und gegensätzliche Reaktionen auf natürliche Feinde, was auf das Vorhandensein von ausgleichenden Fitnessunterschieden hindeutet.
3. Der Einsatz von Fungiziden hatte keine Auswirkungen auf die α-Diversität der Pilzgemeinschaften im Boden, aber die β-Diversität und die Gesamthäufigkeit von pilzlichen OTUs waren deutlich reduziert. Diese Auswirkungen waren in erster Linie auf Veränderungen bei pathogenen Pilzen zurückzuführen. Der Einsatz von Insektiziden hatte negative Auswirkungen auf die Vielfalt und Häufigkeit von Bodenarthropoden, was durch den Rückgang von Springschwänzen bedingt war.

Die Ergebnisse meiner Arbeit deuten darauf hin, dass antagonistische Interaktionen von der relativen Häufigkeit der Pflanzen entkoppelt werden können und dass andere Faktoren wie abiotische Bodenbedingungen und räumliche Heterogenität, nicht aber die Phylogenie der Pflanzen, bedeutsamer für das Ergebnis dieser Interaktionen sind. Meine Doktorarbeit weist daher darauf hin, dass der Ressourcenwettbewerb zwischen Pflanzen für die stabilisierenden Nischenunterschiede verantwortlich ist. Gebietsfremde Arten unterlagen keinem geringeren Druck durch natürliche Antagonisten, was darauf hindeutet, dass sich die natürlichen Feinde im Laufe der Zeit seit der Einführung auf den Pflanzen akkumulieren und/oder ubiquitäre Feinde häufig zusammen mit ihren Wirten eingeführt werden. Die Koexistenz zwischen nativen und gebietsfremden Arten in neuartigen Gemeinschaften wird möglicherweise durch eine gegenläufige Abhängigkeit zwischen der Konkurrenzfähigkeit und der Anfälligkeit für Pathogene und Herbivoren gefördert. Meine Arbeit zeigt auch, dass der Einsatz von Pestiziden ein wirksames Mittel ist, um natürliche Feinde in ökologischen Experimenten auszuschließen. Jedoch können Pestizide zu einer Homogenisierung der Antagonistengemeinschaften und zu Auswirkungen auf Nichtzielorganismen führen, die für das Funktionieren des Ökosystems wichtig sind.

Zusammenfassend lässt sich sagen, dass die Ergebnisse meiner Doktorarbeit die unterschiedliche Bedeutung von Pilzpathogenen und Insektenherbivoren für die Koexistenz zwischen nativen und gebietsfremden Arten in Grünlandökosystemen unterstreichen. Die Tatsache, dass die Koexistenz von Pflanzen in neuartigen Gemeinschaften offenbar sowohl durch stabilisierende Nischenunterschiede als auch durch ausgleichende Fitnessunterschiede beeinflusst wurde, verdeutlicht die Kontextabhängigkeit der Interaktionen zwischen Pflanzen und ihren natürlichen Feinden. Diese Arbeit zeigt auf, wie künftige Studien beobachtender und experimenteller Natur solche Kontextabhängigkeiten thematisieren können. Schließlich betont meine Doktorarbeit eine kohärentere Kombination aus moderner Koexistenztheorie und Invasionsökologie, um die Mechanismen der Koexistenz von Arten und Ökosystemfunktionen in neuartigen Gemeinschaften zu verstehen.

1

General introduction

1.1 Preface

The overwhelming diversity of species and communities in nature has inspired over a century of research into the processes underlying their emergence and the prerequisites for their conservation (Hutchinson 1959). Understanding how communities maintain diversity despite marked differences in their species' competitive ability and trophic interactions has been a major goal of ecological research ever since. However, we are short of a conclusive body of empirical work on the quality and relative contribution of species coexistence mechanisms in ecological communities (Siepielski and McPeek 2010, Bartomeus and Godoy 2018).

Classic theory predicts that species coexist because of differences in the competitors' niches (Volterra 1926, Lotka 1932). This niche differentiation causes intraspecific competition to be stronger than interspecific competition, in other words: species are limited more by themselves than by their competitors (Tilman 1982, Leibold 1995, Chesson 2000, Levine and HilleRisLambers 2009). For plants, these niche differences can occur due to differences in root depths, growth-limiting resources and interactions with specialist enemies (Cody 1991, Levine and HilleRisLambers 2009). Specialized plant antagonists are known to play an important role in maintaining plant species diversity (Janzen 1970, Connell 1971, Bartomeus and Godoy 2018). However, most empirical evidence comes from mammalian herbivores (Crawley 1989, Zobel et al. 1997, Roth et al. 2009, Borer et al. 2020), whereas other common groups of natural enemies, such as fungal pathogens and insect herbivores, have received less attention in studies investigating their role in realizing coexistence among plant species (Allan et al. 2010, Mordecai 2011), but see Borgström et al. 2016, Tamburini et al. 2018). This knowledge gap is particularly crucial considering the role of natural enemies in biological invasions (Liu and Stiling 2006). In the framework of the enemy release hypothesis, exotic species are released from their natural enemies, and gain advantages over competitors in their new range (Elton 1958, Williamson 1996, Keane and Crawley 2002). However, empirical evidence for this mechanism is inconclusive (Agrawal and Kotanen 2003, Levine et al. 2004, Chun et al. 2010, Meijer et al. 2016, Xu et al. 2021).

In my thesis, I investigate the role of (mostly fungal) pathogens and insect herbivores in shaping coexistence of plant species of native and exotic origin in grassland communities. Temperate grasslands are among the most species-rich plant communities (Wilson et al. 2012), prone to invasion (Mack et al. 2000, Mooney and Hobbs 2000), and thus, an excellent study system for this research topic. The results of my thesis particularly contribute to explain how plant species diversity is maintained in these vulnerable ecosystems. More generally, my thesis demonstrates how a detailed insight into plants' interactions with their natural enemies can enhance our

mechanistic understanding of how coexistence is realized in 'novel' plant communities, consisting of native species and introduced exotics.

In the following, I will introduce the major ecological theory underlying my thesis and the general concept surrounding the objectives of my studies.

1.2 Mechanisms of species coexistence

A large body of ecological research has focused on understanding coexistence mechanisms in the face of resource competition. According to the principle of competitive exclusion two species cannot coexist indefinitely, if they compete for a single limiting resource in a constant environment (Grinnell 1904, Gause 1934, Tilman 1982). The formulation of this principle served as a basis to explain the apparent contradiction with observations of so many species with similar ecological traits coexisting in the same community. This led ecologists to the first definition of the ecological niche: the sum of habitat preferences and behavioral adaptations that allow species to coexist (Grinnell 1924). After decades of research and debate, the niche concept was extended to a species-specific multi-dimensional hypervolume, with environmental conditions and resources as dimensions that are required for persistence (Hutchinson 1957). The first experimental validation of niche differences among species and their role in maintaining coexistence was provided by MacArthur (1958) and his study of New England Warblers, and was later complemented by further studies identifying patterns of species co-occurrence as niche differences (Diamond 1975, Ricklefs and Travis 1980).

The concepts of competitive exclusion (Tilman 1987, 1994) and niche differentiation (Leibold 1995, Chase and Leibold 2003) as two long-standing pillars of ecological theory were not adequately linked until Chesson (2000, 2018) developed his framework of coexistence theory. He introduced a mathematical toolbox that allowed to predict how stable coexistence among species is realized by two contrasting mechanisms. Following Chesson's framework, species can coexist because of the collective effects of equalizing mechanisms, that minimize relative fitness differences between competing species (Fig. 1.1a), and of stabilizing mechanisms that arise through differences in species' niches (Fig. 1.1b), e.g. by partitioning limiting resources and/or differential responses to host-specific natural enemies (Adler et al. 2010, HilleRisLambers et al. 2012, Godoy et al. 2020). Stable coexistence occurs when species' stabilizing niche differences overcome their relative fitness differences (Chesson 2000, 2018).

Niche differences, as well as relative fitness differences, are challenging to quantify and difficult to disentangle from each other, because both depend on the specific community composition and the entirety of environmental interactions (Adler et al. 2007, 2010). An often invoked approach to

assess if community dynamics are stabilized by niche differences, is to follow population sizes of co-occurring species over time and investigate whether the per capita rate of increase[1] of a particular species is determined by its relative abundance within a community (Clark and McLachlan 2003). The common thread of all niche differences is that they cause species to limit individuals of their own species more than they limit individuals of their competitors. Thus, niche differences stabilize coexistence by providing species with higher per capita rates of increase when they are rare compared to when they are common, creating 'rare species advantages' (Chesson 2000, Comita et al. 2010, Metz et al. 2010, HilleRisLambers et al. 2012). Hence, a strong indicator for stabilizing niche differences is a per capita rate of increase that is frequency-dependent in a negative way (Clark and McLachlan 2003, Adler et al. 2006, Levine and HilleRisLambers 2009). In the central experiment of my thesis, I quantify the presence of stabilizing niche differences by determining the fitness and per capita rates of increase of several plant species within the same community, and whether these depend on their respective frequencies.

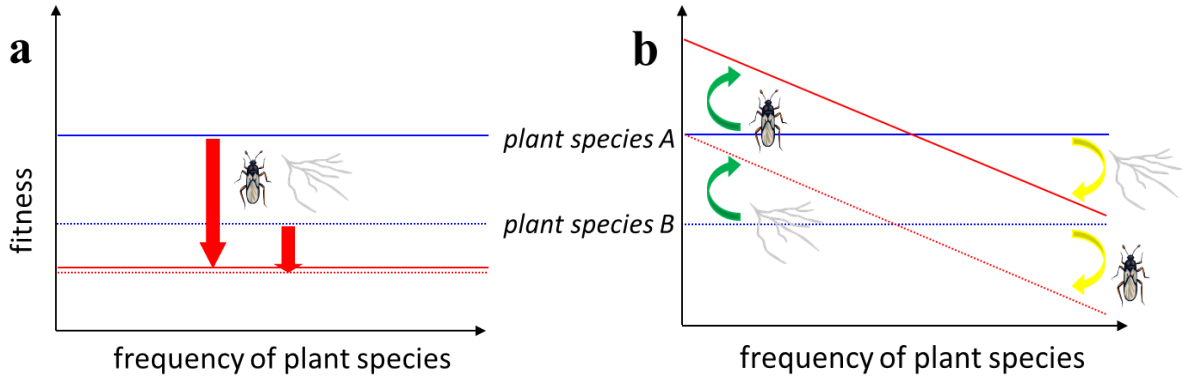

Figure 1.1 Chessons framework of coexistence theory proposes two contrasting, mutually not exclusive mechanisms for stable species coexistence: a) equalizing mechanisms that minimize fitness differences (red arrows) among plant species A (solid blue and red lines) and B (dotted blue and red lines), e.g. via a trade-off between a plant species' competitive ability and its defensive capabilities against natural enemies, and b) stabilizing mechanisms that originate from niche differences among plant species A and B, e.g. differential responses to host-specific natural enemies, and are characterized by giving species higher population growth rates (a fitness component) when rare (low frequency, green arrows) compared to when common (high frequency, yellow arrows). The resulting negative slope in this relationship shows the degree of stabilization. Blue lines indicate fitness without acting equalizing/stabilizing mechanisms, red lines indicate fitness with acting equalizing/stabilizing mechanisms. Natural enemies are here represented by an insect herbivore and hyphae from a pathogenic fungus. Substantially modified after Adler et al. (2007).

[1] The per capita rate of increase is the change of population size over some span of time per individual.

Over the past decades, effects of abiotic drivers of competition and coexistence among plants have been comprehensively studied (Raynaud and Leadley 2004, Tilman et al. 2006, Craine and Dybzinski 2013, Hendriks et al. 2015). The most common textbook example to explain niche differences in plants is species' abilities to utilize different limiting resources, illustrating the role of partitioning soil resources on maintaining species diversity (Tilman 1994). However, multitrophic interactions such as those between plants and their natural enemies have not received much attention until the second half of the 20th century (Janzen 1970, Connell 1971, Burkey 1994, Shurin 2001, Gilbert 2005, Bagchi et al. 2014, Bennett et al. 2017). This is surprising for two reasons (Bartomeus and Godoy 2018). First, interactions between organisms have been fundamental to ecology since its establishment as a scientific discipline in the late 19th century (Goodland 1975). Most notably, Darwin himself stressed that the 'struggle for existence', as he coined it, is not only between life and the abiotic environment but also includes interactions between species such as competition, predation, parasitism and mutualism (Darwin 1859). Second, there is an extensive body of theoretical work demonstrating that natural enemies shared by plant species living in the same community can indirectly modulate the competition for resources between those plant species (Cramer and May 1972, Caswell 1978, Holt 1984, Chase et al. 2002, Gross 2008, Koffel et al. 2021). Furthermore, the strength of these modulations can have the same limiting or promoting effects on the coexistence between species as does competition for soil resources (Chesson and Kuang 2008).

Developing a mechanistic understanding of how antagonistic interactions shape equalizing and stabilizing forces at the community level has become a research focus in the past decade (Mordecai 2013, Bartomeus and Godoy 2018, Lanuza et al. 2018). In the following section, I introduce the current body of knowledge on the importance of interactions between plants and their natural enemies for species coexistence, particularly in grasslands, and focus my attention on the two relevant and relatively understudied groups of natural enemies I considered in my thesis: fungal pathogens and insect herbivores.

1.3 Antagonistic interactions as controls of plant species coexistence

Ecologists recognize the importance of both competition and consumption in promoting the maintenance of diversity in a variety of taxa (Paine 1969), including terrestrial plants (Chesson and Kuang 2008). However, ecological research on antagonistic interactions in natural plant communities has primarily focused on few groups, such as vertebrate herbivores (Pimentel and Andow 1984). Other groups, such as fungal pathogens and insect herbivores have been primarily studied in agricultural systems (Crawley 1989, Mordecai 2011), resulting in a large body of literature

on the control and spread of crop diseases and pests (Henderson and Clements 1979, Blanton and Ewel 1985, Matson et al. 1997, Brown and Hovmøll 2002). Although the huge potential for pathogens and insect herbivores to regulate natural plant populations has increasingly been recognized (Van Der Heijden et al. 2008, Myers and Sarfraz 2017), these interactions are still overlooked in field studies. Part of my dissertation is a long-term field experiment in which I examine the influence of these two groups in order to fill this gap. The interactions between fungal pathogens and their host plants are highly complex, with differing modes of infection and fitness effects. Moreover, fungi can either be specialists or generalists with host ranges from one to several hundred plant species (Mordecai 2011, Bever et al. 2015). The most direct fitness effects of fungal pathogen infection are the modification of the hosts' growth, survival and reproduction, with the direction and strength of these effects varying widely (Jarosz and Davelos 1995, Klironomos 2003, Alexander 2010). When these fitness effects alter competition within and among plant species, fungi are able to mediate plant coexistence (Dinoor and Eshed 1984, Holt and Pickering 1985, Alexander and Holt 1998). Plant-fungal interactions may stabilize coexistence if the pathogens' effect on plant population growth rate increases with plant abundance.

This effect can be due to increases in the prevalence of a pathogen as its preferred host becomes more abundant (Mordecai 2011). A famous example for this stabilizing mechanism is the Janzen-Connell hypothesis, which postulates that tree seedling survival in tropical forests increases with distance from the parent tree due to release from host-specific enemies (Janzen 1970, Connell 1971). Empirical evidence for the hypothesis has been provided frequently (Augspurger 1983, Gilbert et al. 1994, Bagchi et al. 2010) and it has been extended to other plant communities, including grasslands (Petermann et al. 2008). A closely related stabilizing mechanism are negative feedbacks between plants and specialist pathogens that accumulate in the soil of abundant species, thereby enabling and facilitating rare species to invade soil inoculated by these abundant species (Mills and Bever 1998, Bever 2003).

In contrast, the effect of pathogens on plant population growth rate may also be destabilizing when pathogens preferentially harm rare species and as a result create positive feedbacks between a hosts' abundance and its population growth (Dobson 2004, Power and Mitchell 2004). This happens, for example, during spillover events in which a non-susceptible host sustains a large reservoir of pathogen inoculum that has a detrimental effect on other, more susceptible, plant community members but not on its non-susceptible main host (Anacker et al. 2008). An ensuing decline of the other community members' population growth rates can lead to an increase in abundance of the non-susceptible species (Rizzo and Garbelotto 2003, Cobb et al. 2010) and, thus, may promote single-species dominance (Mordecai 2011).

The majority of past studies investigating plant-pathogen interactions focused on the effects of soil microbes (Mangan et al. 2010, Schnitzer et al. 2011, Bagchi et al. 2014) while the contribution of other pathogen communities, e.g. foliar pathogens, received less attention (but see Allan et al. (2010)). Furthermore, many experiments were conducted in tropical forests or grasslands, with a focus on North American grasslands (Parker et al. 2015, Bennett and Cahill 2016, Chung and Rudgers 2016, Spear and Mordecai 2018). In my thesis I consider not only soil fungal pathogens but the effects of the whole pathogen community, including aboveground pathogens, on plant population and communities in North American and central European grasslands. Additionally, in chapter 4 of my thesis, I highlight how the composition of whole soil fungal communities, not only pathogens, is affected by changes in plant community composition.

Similar to fungal pathogens, the most direct effect of insect herbivores on plant fitness is through their consumption of plant tissue (Crawley 1989, Cahill and Coupe 2003, Myers and Sarfraz 2017). Few studies have shown that insect herbivores can have strong frequency-dependent effects on plant populations (Fagan et al. 2005, Bagchi et al. 2014) and maintain plant diversity (Carson and Root 2000, Stein et al. 2010). There is some evidence that preferential feeding of insects on dominant species can increase plant-species turnover (Fraser and Grime 1998, Schädler et al. 2004) and for the presence of trade-offs between a plant's growth and its defensive capabilities (Kempel et al. 2015, 2020). Recent experiments in tropical forests (Bagchi et al. 2014) and temperate grasslands (Kempel et al. 2015) have shown that invertebrate herbivores may also act by equalizing fitness differences among plant species.

However, most experimental work on insect herbivory has focused on simple pairwise plant-plant-interactions (Borgström et al. 2016, Tamburini et al. 2018). Theory and models based on these experiments are of limited reliability for studying the importance of multispecies dynamics in natural communities, because they are not able to predict how mechanisms of coexistence work (Dormann and Roxburgh 2005). Therefore, it is essential to investigate plant-enemy interactions in multispecies plant communities with realistic diversity levels (Dinnage 2013, Moretti et al. 2013). Furthermore, above- and belowground herbivores may differ in their effects on grassland communities (Stein et al. 2010), which makes it important to study the combined effect of both groups of herbivores. In the third chapter of my thesis, I consider these issues by investigating above- and belowground plant-insect interactions in manipulated grassland communities whose diversity levels were chosen to mirror the diversity of semi-natural grasslands in central Germany.

In general, the stabilizing effects of insect herbivores required for stable coexistence have mainly been attributed to specialists, rather than generalists, because they create rare species advantages via frequency-dependent predation. However, generalist herbivores can also have a stabilizing

effect on plant communities if they preferentially feed on the current most abundant species in a community (Murdoch 1969, Chase et al. 2002). My thesis does not aim to differentiate between the effects of generalist and specialist herbivores (or pathogens, respectively), due to the fact that it is difficult to specifically manipulate the density of either group in field experiments (Kempel et al. 2015). Nonetheless, the joint effect of both groups is often invoked to explain the success of exotic species, whose effects on coexistence among plants I cover in the next section.

1.4 Exotic species, the enemy release hypothesis and their impact on species coexistence

The 'Anthropocene' marks the commencement and ever-increasing speed of significant human impact on earths' abiotic and biotic environment (Steffen et al. 2011, Lewis and Maslin 2015). One of its defining features is the gradual loss of biogeographical barriers caused by global anthropogenic dispersal of organisms into new regions, where they can naturalize, establish and spread (Elton 1958, Mack et al. 2000, Richardson et al. 2000b, Davis 2006) and cause ecological, socioeconomic and public health damage (Pimentel et al. 2000, 2005, Simberloff et al. 2013, van Kleunen et al. 2015, Dawson et al. 2017). Understanding the mechanisms responsible for invasion processes is based on two complementary concepts. The first concept, invasiveness, focuses on traits that allow particular species to become invasive, whereas the second concept, invasibility, focuses on the characteristics of the recipient communities that make them prone to being invaded (Godoy 2019). Both concepts have been linked by the naturalization-invasion continuum, which links invasion processes with a sequence of abiotic and biotic barriers that an exotic species must overcome to become established and invasive (Richardson and Pyšek 2006). This concept is useful to illuminate why some exotic species are able to sustain viable populations outside their native ranges, and even become dominant at local scales.

When applied to biological invasions, modern coexistence theory predicts that the successful establishment of exotic species should either be promoted by niche differences, or by a higher fitness on exotic compared to native residents. If niche differences dominate, interactions with native species would be low, whereas if fitness differences dominate, the exotic species can competitively exclude the native ones (Fig. 1.2). One frequently invoked fitness difference contributing to the invasiveness of numerous species is the release from natural enemies (Wolfe et al. 2004, Zou et al. 2008, Norghauer et al. 2011). The enemy release hypothesis (ERH, Williamson 1996) postulates that biotic interactions are weaker in the exotic compared to the native ranges of plants because the invading plants are introduced without their pathogens, parasites and/or herbivores (Mitchell et al. 2006). In theory, this resulting decrease in regulation by natural enemies leads to an increase in fitness, and thus abundance and distribution, in the exotic range.

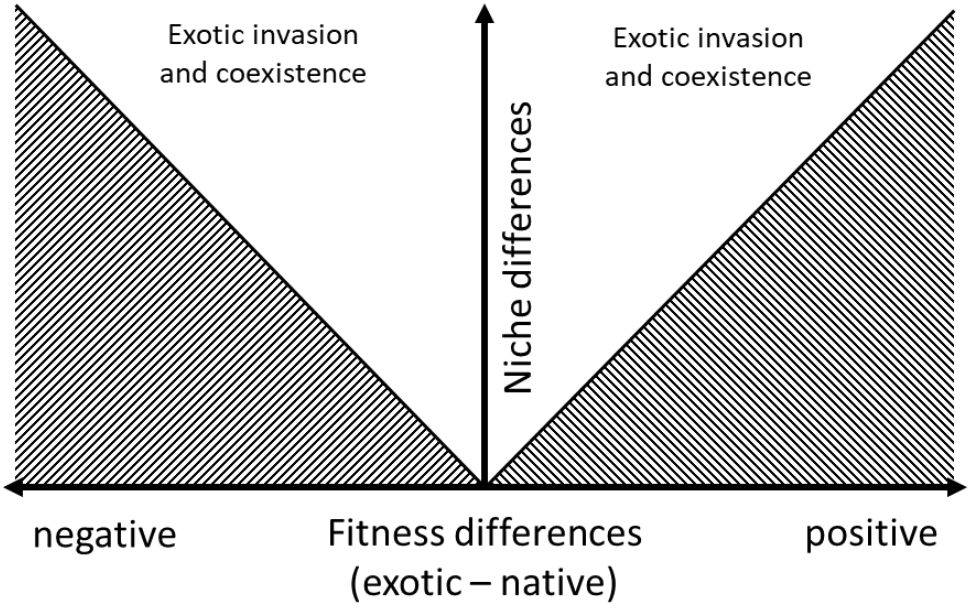

Figure 1.2 The effects of fitness (*x*-axis) and niche differences (*y*-axis) on biological invasions. Large niche differences increase the probability that exotic species coexist stably with the native community either with fitness advantages for native species (left-handed free space) or fitness advantages for the exotic species (right-handed free space). Large fitness differences lead to either competitive exclusion of the exotic invader (left-hand hatched area, fitness advantage of native species outweighs niche differences) or the native community (right-hand hatched area, fitness advantage of exotic species outweighs niche differences). Substantially modified after MacDougall et al. (2009).

Upon introduction of a species, the ERH infers that 1) specialist enemies of the exotic species are absent in its new range, 2) specialist enemies of native relatives rarely switch hosts and 3) generalist enemies in the new range will have small effects on the exotic species (Keane and Crawley 2002). Instead of stabilizing coexistence, enemy release gives exotic plants a fitness advantage, driving competitive exclusion of native residents, regardless of the exotic plants' commonness or rarity (Fig. 1.2, MacDougall et al. 2009, Granda et al. 2015, Korell et al. 2019). If, on the other hand, the effect of natural enemies on exotic plants depends on the plants' relative abundance in the community, indicating the presence of stabilizing niche differences, then it is likely that native residents and exotic species can stably coexist (MacDougall et al. 2009). This stabilizing effect highlights that interspecific competition can be an even more important factor for the establishment of plants than the direct effects of natural enemies (Keane and Crawley 2002, Hierro et al. 2005).

Although the idea behind the ERH makes intuitive sense and is one of the most cited explanations for the success of invasive species worldwide (Maron and Vila 2001, Liu and Stiling 2006, Xu et

al. 2021), empirical evidence is less conclusive. For example, Colautti et al. (2004) reviewed 25 studies testing the ERH, out of which 15 found support for enemy release, six found no support and five showed results contradicting the ERH (one study showed results both in favor and against enemy release). Meijer et al. (2016) quantitatively reviewed 68 datasets and found general support for the ERH in systems of plants and insect herbivores, but high variability across studies, possibly due to the large variety of study systems considered. For plants and their pathogens it has been shown that exotic plants in the US host significantly less pathogen species and suffer less damage from pathogen attack than in their native range (Mitchell and Power 2003). A range of experimental studies also showed the presence of weaker negative feedbacks between exotic plants in North America and their soil microbes, compared to native plants (Klironomos 2002) and to populations in their native range (Reinhart et al. 2003, MacKay and Kotanen 2008). However, geographic spread of exotic plant species from North America in Europe was not related to a release from their pathogens species (van Kleunen and Fischer 2009). In contrast, when restricted to fungi, there even was a negative association between the geographic spread of an exotic species and their release from fungal pathogens species (van Kleunen and Fischer 2009). Thus, it is clear that there is no simple relationship between enemy release and the success of exotic plant species (Meijer et al. 2016), and that the effects of enemy release are highly context-dependent (Maron et al. 2014, Myers and Sarfraz 2017).

An important context for the ERH is the phylogenetic affiliation of the invading species. Closely related plant species share similar traits (Blomberg et al. 2003, Chazdon et al. 2003, Swenson and Enquist 2007), such as those that determine morphological and chemical resistance against natural enemies (Farrell 2001, Agrawal 2007, Pearse and Hipp 2009, Rønsted et al. 2012). When an insect herbivore or fungal pathogen has the necessary combination of traits to successfully attack a particular plant species by surmounting or avoiding the plants' defenses, it is also likely to successfully attack a closely related plant species with similar, phylogenetically conserved defense traits (Gilbert and Webb 2007, Gilbert et al. 2015). Furthermore, phylogenetic signals also translate to the host ranges of most plant pests and pathogens, i.e. the probability that two plant species share a common enemy declines with phylogenetic distance between them (Gilbert and Webb 2007, Vacher et al. 2008, De Vienne et al. 2009, Gilbert et al. 2012). It is a long-standing hypothesis that phylogenetically distinct species may have an advantage when colonizing new habitats, because they will not share specialist enemies with the resident communities (Darwin 1859, Cadotte et al. 2018). However, the hypothesis is contested because, as Darwin himself noted, traits that are shared among close relatives may also preadapt the plants to their new environment (Darwin 1859, Duncan and Williams 2002). Another important context is the time passed since

introduction of the exotic species. There is evidence that natural enemies accumulate on exotic species and, therefore, a potential release from enemies is transitory (Brändle et al. 2008). This is especially significant for pathogens, which often have short reproduction cycles and the potential for rapid adaptation (Flory and Clay 2013). The relationship between a plant's origin, phylogenetic affiliation and attack by natural enemies is complex. In the second chapter of my thesis I investigate this otherwise rarely studied interplay (but see Parker et al. 2015) of plant species' relative abundances, origins and phylogeny with plant pathogen diversity and infection in grasslands of the central United States.

1.5 Approaches to study the effects of antagonistic interactions on plant communities

Both observational studies and manipulative experiments on the influence of antagonistic interactions on plant species' coexistence and invasion processes are necessary to answer research questions and test scientific hypotheses, and can be used complementarily to achieve this goal (Hector et al. 2007) for the following reasons. First, observational studies provide more realistic settings than experiments, because even the most carefully controlled experiment cannot capture the whole range of variation seen in nature (Shaffer and Johnson 2008). Second, only controlled, randomized and replicated experiments can, on the other hand, determine causal relationships, because results from observational studies could be due to a multitude of possible factors. I therefore chose to complement an observational study capturing the effects of plant species' relative abundance, origin and phylogeny on pathogen diversity and infection (chapter 2) with an experimental approach, measuring plant community and species responses to the manipulation of natural enemies, frequency and origin of plant species (chapter 3).

Experimentally studying the effects of natural enemies on plant community dynamics requires manipulation of these groups in the study system, which can be accomplished in mesocosms (Korell et al. 2016a), or field experiments (Carson and Root 1999). Among the various approaches for manipulating insect herbivores, like adding herbivores or increasing their activity by excluding higher-level consumers (Schmitz et al. 2000), exclusion by using cages (Engelkes et al. 2016) or predators (Schmitz and Suttle 2001), I chose to use insecticides in my field experiment (chapter 3). Because of their small size and mobility, physically excluding insect herbivores is difficult to accomplish without also altering other herbivores, light levels or microclimate (Siemann et al. 2004). Physical exclusion of belowground herbivores is even more problematic. Hence, chemical exclusion via insecticide is one of the most valuable methods to study effects of above- and belowground insect herbivores on plant populations and communities in terrestrial ecosystems, if

one is aware of the limitations of this method, like potential nutrient input, phytotoxic and other effects on non-target organisms (Siemann et al. 2004).

To my knowledge, effective exclusion of fungi from study systems is only possible by applying fungicides. However, fungicide application can have similar non-target effects as insecticide application, like phytotoxicity, unintended fertilization and toxic effects on invertebrate or vertebrate feeding (Allan et al. 2010). In addition, fungal species that are phylogenetically similar but belong to different ecological guilds, show the same responses to a particular fungicidal agent. For example, effects of fungicide application on plant communities are often attributed to the suppression of mutualists, like mycorrhizal fungi, that physiologically respond in the same way to fungicide application as closely related fungal pathogens (Bever et al. 2015, Hage-Ahmed et al. 2019). Fungicide application can also cause shifts in fungal community composition if pathogens respond differentially to the fungicidal agent (Vargas 2004, Parker et al. 2015). On the other hand, chemically reducing fungal pathogens has the great benefit to study the effect of natural pathogen levels in an otherwise undisturbed plant community (Paul et al. 1989, Alexander and Holt 1998). Therefore, manipulations of this kind are essential to link results from artificial few-species plant-pathogen assemblages with observational insights from natural communities (Allan et al. 2010), and is probably the only practical method of investigating the direct effects of fungal pathogens and other fungal guilds in natural communities (Paul et al. 1989).

Fungicides are developed for managing agricultural ecosystems, and the effects of fungicide application on fungal communities in natural ecosystems remain comparatively understudied (Köhler and Triebskorn 2013, Zubrod et al. 2019). Only very few studies so far used advanced molecular methods, such as high-throughput sequencing, to investigate the effects of fungicide application on the dynamics of soil fungal communities (Shi et al. 2019). This is surprising, given the importance of some fungal microbiomes for plant growth and health in both agricultural and natural ecosystems (Newton et al. 2010), and the role of fungal pathogens in biological invasions (Dawson and Schrama 2016, see chapter 1.4, and chapters 2 and 3). Thus, I chose a high throughput sequencing approach to study the effects of fungicide application on the diversity and composition of soil fungal communities associated with natural plant communities, and to test for effects of plant species origin and identity of the most abundant plant species on soil fungi, in chapter 4.

For insecticides, the effects on target and non-target organisms have been comparatively well documented (Nicholls and Altieri 2013, Mulé et al. 2017, Zaller and Brühl 2019). However, I also tested the effects of the insecticidal agent I used to ensure efficacy of insecticide application in my specific experimental setting (see Appendix 7.2.4).

1.6 Principal objectives and concept of this thesis

This general introduction is followed by three distinct studies. While all three studies in the following chapters focus on the ecological outcome of interactions between plants and their natural enemies and how these results contribute to a comprehensive understanding of species coexistence in novel communities, each one addresses specific goals (see Fig. 1.3 for an outline of this thesis). Specifically, my thesis addresses three central research questions:

1. How do the relative abundance, origin and phylogenetic affiliation of plant species influence interactions between plants and their natural enemies?
2. How do pathogens and insect herbivores mediate the coexistence of plant species in grassland ecosystems?
3. How are natural communities of fungal pathogens and insect herbivores altered by the application of pesticides?

In **chapter 2**, I present the results of an observational study conducted in three natural grasslands in the central United States, investigating, together with my co-authors, the effects of plant species' relative abundance, their origin and phylogenetic relationships on patterns of pathogen infection and diversity. By combining correlational and database-founded approaches we study a wide range of pathogen taxa as well as a high diversity of plant species of native and exotic origin in natural, unmanipulated communities. This study is unique in that it considers relative abundance, origin and phylogenetic affiliation of plants simultaneously to gain insights into plant-pathogen patterns (with a focus on foliar fungi) on a community level. Specifically, I phrased the following hypotheses:

- 2.1: Infection of pathogens increases with the relative abundance of their host plant species.
- 2.2: Exotic plant species show less pathogen diversity and infection compared to native plant species and pathogens restricted to North America infect exotic plants to a lesser extent than native plants.
- 2.3: Conservation of defense traits leads to a phylogenetic signal in the levels of pathogen infection of plant species.

Complementing the observational data from natural grasslands presented in chapter 2, in **chapter 3**, I report the outcomes of a three-year field experiment, situated in central Germany. Along with

my co-authors, I established native and exotic-dominated communities, manipulated the frequencies of native and exotic plant species within these communities and reduced the effects of fungal pathogens and insect herbivores by applying pesticides. Community-level measurements were recorded to show plant community responses to these manipulations. In addition, we collected fitness data of life cycle transitions of four selected species to investigate how individual species are affected by manipulations of frequency and natural enemy regime. This experimental approach is one of only few to explicitly quantify the role of antagonistic interactions for the maintenance of species diversity and, to our knowledge, the only rigorous experimental test of the importance of these interactions for the coexistence of plant species in the context of biological invasions. Specifically, I phrased the following hypotheses:

- 3.1: Exotic-dominated communities are more productive, but less diverse, compared to native communities.
- 3.2: With reduction of natural enemies, productivity of native communities increases, but diversity decreases. Exotic-dominated communities are less affected by these manipulations.
- 3.3: Per capita rates of increase (and other fitness components) are lower at high frequency compared to low frequency.
- 3.4: With reduction of natural enemies, differences in per capita rates of increase (and other fitness components) are smaller. For exotic species, this effect is less pronounced.

In **chapter 4**, I study the effects of fungicide application on non-target soil fungal communities. Using a fungal ITS rDNA pyrotaq sequencing approach, I investigate together with my co-authors, how fungicides influence the diversity and composition of fungal communities in the soil of native and exotic plant communities of the same field experiment as in chapter 3, complementing the knowledge gained from the manipulation of aboveground interactions with insights from the dynamics of manipulated belowground interactions. I particularly highlight differences among fungal guilds in their responses to these manipulations. This study is one of the first to not only focus on a particular fungal guild but to also target the entire soil fungal community, by using a high-throughput sequencing approach, to investigate the effects of fungicide application in an ecological context. Specifically, I phrased the following questions:

- 4.1: Fungicide application will decrease soil fungal diversity and abundance and alter the composition of non-target soil fungal communities.

- 4.2: Exotic plants accumulate less pathotrophs in their soil community compared to native plants.
- 4.3: Soil community composition will differ dependent on the identity of the most abundant plant species.

I synthesize the results of my studies in a general discussion, **chapter 5**, and demonstrate how they fit into the past and current knowledge body of coexistence and invasion ecology and highlight perspectives for future experimental approaches.

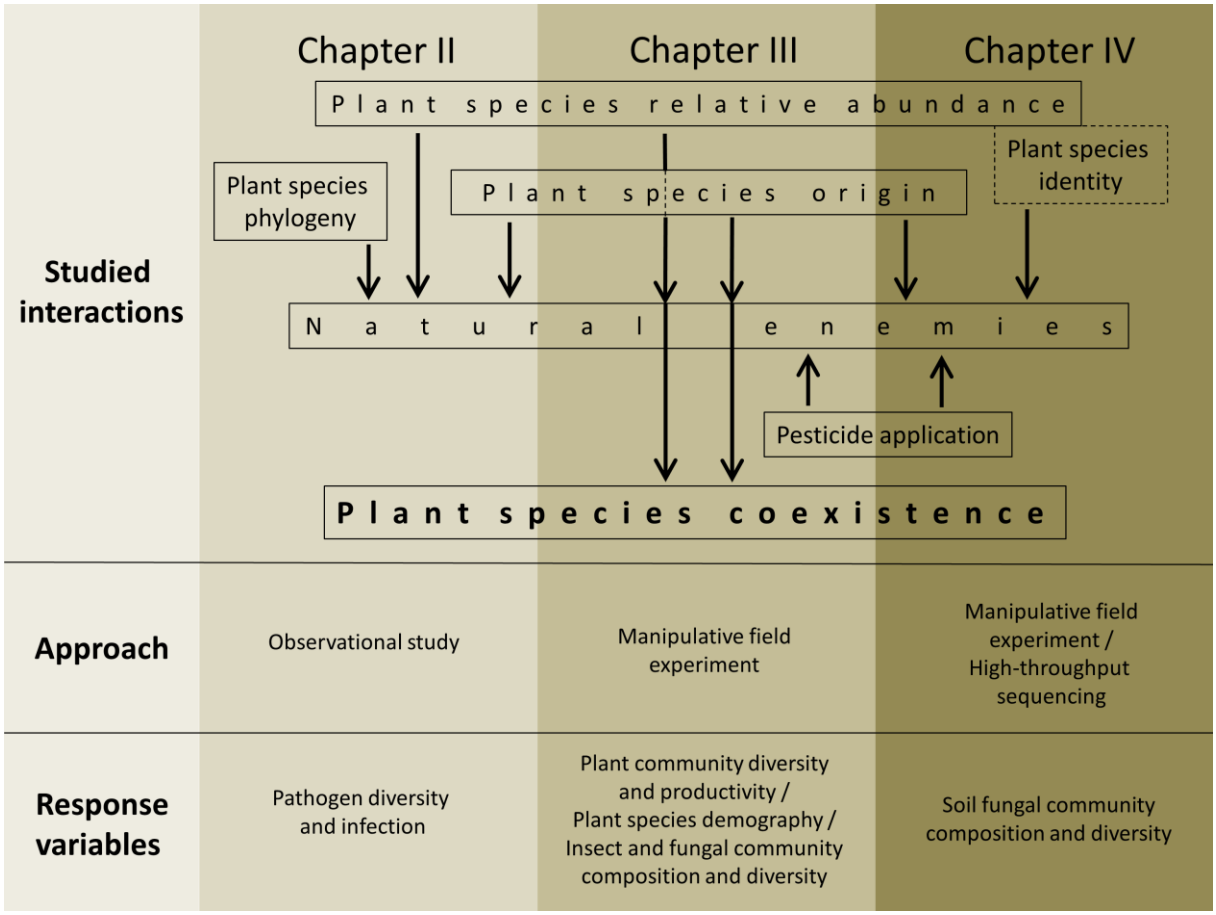

Figure 1.3 Outline of this PhD-Thesis. The chart depicts all observed and manipulated factors influencing natural enemies and plant species coexistence, the approaches chosen to study these effects and the measured response variables. In chapter 3 I studied, among others, effects of plant species relative abundance and origin on plant species coexistence, which are mediated by two groups of natural enemies,

indicated by the two continuous arrows. In chapter 4 I studied, among others, the identity effect of the most abundant plant species within a respective community, on natural enemies.

2

Predictors of plant pathogen infection

This chapter has been published in *Ecology and Evolution 10: 5506–5516* as: Schmidt R, Auge H, Deising HB, Hensen I, Mangan SA, Schädler M, Stein C, Knight TM (2020): Abundance, origin, and phylogeny of plants do not predict community-level patterns of pathogen diversity and infection. DOI: 10.1002/ece3.6292

2.1 Abstract

Pathogens have the potential to shape plant community structure, and thus it is important to understand the factors that determine pathogen diversity and infection in communities. The abundance, origin and evolutionary relationships of plant hosts are all known to influence pathogen patterns, and are typically studied separately. We present an observational study that examined the influence of all three factors and their interactions on the diversity of and infection of several broad taxonomic groups of foliar, floral and stem pathogens across three sites in a temperate grassland in the central United States. Despite that pathogens are known to respond positively to increases in their host abundances in other systems, we found no relationship between host abundance and either pathogen diversity or infection. Native and exotic plants did not differ in their infection levels, but exotic plants hosted a more generalist pathogen community compared to native plants. There was no phylogenetic signal across plants in pathogen diversity or infection. The lack of evidence for a role of abundance, origin and evolutionary relationships in shaping patterns of pathogens in our study might be explained by the high generalization and global distributions of our focal pathogen community, as well as the high diversity of our plant host community. In general, the community-level patterns of above-ground pathogen infections have received less attention than below-ground pathogens, and our results suggest that their patterns might not be explained by the same drivers.

Keywords

Enemy release hypothesis, exotic species, host abundance, phylogenetic community context, plant-fungal interactions, temperate grasslands

2.2 Introduction

Pathogens can dramatically influence plant fitness and are thereby assumed to mediate patterns of community assembly, such as plant species coexistence, plant invasions and dominance (Alexander 2010, Bever et al. 2015). To better understand these processes, we need to identify the factors that determine pathogen diversity and infection in plant communities. While most of the literature on host-pathogen patterns has focused on one or a few host plants and their interactions (e.g. Krupinsky et al. 2002), there is a growing literature examining community-level patterns of pathogen diversity and infection (Mommer et al. 2018, Vaz et al. 2018, Massoni et al. 2019, see also review by Mishra et al. 2020). Three main factors are commonly invoked to explain patterns of pathogen diversity and infection across plant species in a community (Mordecai 2011): (1) host plant abundance (Connell 1971, Janzen 1970, Parker et al. 2015), (2) host plant origin (Klironomos 2002, Mitchell and Power 2003), and (3) plant defense traits (Holt and Dobson 2006, Viola et al. 2010).

Pathogens can respond positively to increases in abundance of their host, and subsequently decrease host population growth rates, thereby providing an advantage to rare host species yet to be heavily infected (Jarosz and Davelos 1995, Gilbert 2005). This negative frequency-dependence caused by pathogens has been hypothesized to be one of the dominant stabilizing mechanisms that maintain plant species coexistence (Bever 2003, Chesson 2000, Connell 1971, Janzen 1970, Mangan et al. 2010). Indeed, both the incidence and severity of pathogen infection have been shown to be positively related to host abundance in natural communities (Burdon and Chilvers 1982, Parker et al. 2015), but see Spear and Mordecai (2018). If multiple potential pathogens for a given host species exist, the probability of co-infection by more than one species of pathogen could increase with host abundance (Burdon and Chilvers 1982, Seabloom et al. 2015). However, empirical studies testing for a relationship between plant abundance and pathogen diversity are scarce (Mundt and Browning 1985, Moore and Borer 2012) or test the relationship indirectly by modifying diversity and relative abundances of plant species (Mommer et al. 2018). Furthermore, interactions between co-infecting pathogens and differences across studies in the degree of specialization of pathogens make it difficult to synthesize results across studies (Ostfeld et al. 2005, Seabloom et al. 2015, see review by Benítez et al. 2013).

Exotic plant species are expected to exhibit lower pathogen species diversity and disease severity than native species because they left behind many of their native pathogens when introduced into a new area (Elton 1958, Williamson 1996, Keane and Crawley 2002). The Enemy Release Hypothesis further posits that low pathogen infection allows exotic plants to have increased fitness and a competitive advantage against enemy-regulated native species, thereby contributing to

invader establishment and spread (Hierro et al. 2005). Results from broad scale studies support the Enemy Release Hypothesis by showing that plants are attacked by more pathogen species in their native compared to their naturalized ranges (Mitchell and Power 2003). However, pathogen species are known to accumulate on exotic plants over time (Stricker et al. 2016, Flory et al. 2018), and some studies have even shown more pronounced pathogen attacks on exotic plants than on their native relatives (Colautti et al. 2004).

According to the Enemy Release Hypothesis, reduced pathogen attack on naturalized plants should result from the absence of specialists pathogens (Keane and Crawley 2002). The source of infection for naturalized plants should be pathogens that are generalized in their host range and/or those that have cosmopolitan geographic distributions and are thus present in both the native and naturalized range of plants (Parker and Gilbert 2004, Prospero and Cleary 2017). Indeed, exotic plant species have been shown to be released from pathogens with a restricted geographical distribution (van Kleunen and Fischer 2009). A so far untested prediction is whether pathogens with a restricted geographical distribution, regardless of their host specificity, infect exotic hosts to a lower extent than native hosts.

Plant morphological and chemical traits, including those determining resistance against natural enemies, are often phylogenetically conserved (Farrell 2001), and thus poorly defended plants that have high diversity of pathogens should cluster together on a phylogeny creating phylogenetic signal in the diversity of pathogens found on plants (Clark and Clegg 2017). Closely related plants are likely to be attacked by the same pathogen species (Gilbert and Webb 2007), and thus evolutionarily distinct plant species in a community might be attacked by fewer pathogen species. Indeed, it has long been hypothesized that evolutionary distinct species might have an advantage when colonizing new habitats because they will not share enemies with the resident community (Darwin's Naturalization Hypothesis, Darwin 1859, Cadotte et al. 2018). Therefore, the relationship between pathogen infection and host abundance or origin may be masked by the phylogenetic affiliations of species within a community (Parker et al. 2015).

Interactions between phylogeny, abundance and origin should determine the diversity and infection of pathogens observed on plant species in a community. For example, it is possible that native but not exotic plants suffer from high pathogen diversity and infection levels when they occur at high densities, if the Enemy Release Hypothesis results in low pathogen diversity and infection for exotic plants regardless of their abundance (Keane and Crawley 2002). We are not aware of any studies that have simultaneously considered how all three factors influence patterns of pathogen diversity, incidence and severity on plant species in a community. Additionally, aboveground pathogens have rarely been considered as drivers of grassland plant community

structure and composition in grasslands (Borer et al. 2007, Allan et al. 2010, Parker and Gilbert 2018, Spear and Mordecai 2018). Here, we report the results of an observational study conducted on 51 host species on three sites in a temperate grassland in the central United States to test the following predictions for patterns of foliar, stem and floral pathogen infection:

1. Effects of host abundance: If pathogens can respond positively to increases in the abundance of their host, pathogen infection should increase with the abundance of host species.
2. Effects of native vs. exotic host origin: If plant species leave specialized enemies behind when introduced to a new range, exotic plant species should display less pathogen diversity and infection compared to native species. Furthermore, pathogens restricted to North America, being either specialists or generalists, might infect exotic invaders to a lower extent than native hosts.
3. Effects of phylogeny: If defense traits are phylogenetically conserved, we expect a phylogenetic signal in the diversity of pathogen groups and levels of infection on plant species.

Our focal pathogens include a wide range of taxa, including downy mildews, rusts, fungal leaf spot diseases, bacteria and viruses. These taxa differ broadly in their traits and thus might also differ in their community-level patterns. Thus, we considered how 'pathogen group' interacted with 'host abundance' and 'host origin', to determine patterns of pathogen infection.

2.3 Methods

Study area

The field sites were located at Washington University's Tyson Research Center (38°31'N, 90°33'W), located near St. Louis, Missouri, USA. The climate is temperate with 914 mm annual precipitation. The cherty, stony, and well-drained soils of the Gasconade-Clarksville-Menfro Association dominating this area were formed in chert free limestone residuum, cherty limestone residuum and deep loess (Benham 1982). These soils rest on bedrock primarily composed of Mississippian limestone (*Burlington-Keokuk formation)* and Devonian sandstones (Bushberg sandstone). The vegetation in this region mainly consists of Oak-Hickory forest, interspersed with patches of old fields, which are marked by the dominance of several species of perennial grasses and tall-growing Asteraceae (https://tyson.wustl.edu/).

Study design

For our study, we selected three sites separated from each other by at least 1.2 km. At each site, we documented plant community composition by collecting information on the identity and percent cover of all plant species in 20 1m^2 plots placed in a grid design that spanned the site (plots separated by 3-10 m, depending on site size). From this species pool we sampled pathogens on all vascular plant species that contained at least 10 individuals at a site and at our time of sampling (July 2015, N=51 total plant species of which 40 are native and 11 exotic). A threshold of 10 individuals allowed adequate sample size for statistical power while also allowing relatively rare species to be included in our observations. Plant species abundance ranged from 0.02 to 11.4 percent cover on a particular site (see Appendices 7.1.1 – 7.1.3). Exotic species showed the same typical lognormal distribution in abundance as native species did, with a few very abundant species and many species with less than one percent cover.

Plant individuals were randomly chosen by blindly throwing a pencil in the surrounding vegetation and subsequently picking the individual of the respective species closest to the fallen pencil. We sampled 10-15 individuals per plant species at each site. For each individual, pathogen infection apparent on the surface of stems, leaves and flowers were assessed separately using a percentage-based nine-level rating scheme, according to Oberforster (2001), ranging from 1 = 0% infection to 9 ≥ 70% infection. Additionally, we estimated pathogen infection for all above-ground parts for each individual, using the same rating scheme. As floral infections were rare (N=2 infected individuals of *Festuca subverticillata*) and the results for foliar and stem surface infection rates were similar to those based on all above-ground parts, we only present results for all aboveground parts. We assigned all pathogens observed on individual plants to broad pathogen groups following Rottstock et al. (2014): fungal leaf spot diseases, powdery mildews, rusts and downy mildews. While the 'fungal leaf spot disease' group is not a taxonomically defined group, species in this group produce morphologically similar disease symptoms, which allows for accurate attribution of infection signs. We recorded rust and downy mildew infection as presence of sporulation structures, powdery mildews as presence of mycelium, and fungal leaf spot diseases as presence of necrotic leaf lesions (Rottstock et al. 2014). We chose this as our measure of pathogen infection, because loss of photosynthetic tissue is a direct indicator of pathogen impact on host productivity and directly comparable across host species (Parker and Gilbert 2007, Parker et al. 2015). Although being a major pathogen group, we did not find any smut fungi on our plants. However, we also found a considerable amount of infection signs not relatable to the mentioned fungal, or fungal-like, pathogen groups, but which we could attribute to be caused by pathogenic bacteria or plant viruses (henceforth called 'bacterial and viral diseases'). While infection patterns may differ broadly

between bacteria and viruses and among groups of fungal pathogens (García-Guzmán and Heil 2014, Rodriguez-Moreno et al. 2018) our goal was to cover infection patterns over a broad range of pathogen groups, and disease of bacterial and viral origin constituted a considerable proportion of the visible infection signs in our community. Distinct morphological and life-history characteristics across these pathogen groups allow pathogen groups for each host plant to be documented in the field.

Most pathogens can not be directly identified to species in the field. However, to attain an entire pathogen species list for each host plant species across all sites, we took one sample of each occuring sign of pathogen infection for each plant species and site, which we dried and pressed to preserve them for further determination via digital microscopy (VHX-2000, Keyence Corp., Osaka, Japan). Digital microscopic imaging extends conventional microscopy by combining the power of optical imaging, electronic detection and computerized analysis (Chen et al. 2011), thereby providing the opportunity to efficiently conduct pathogen identification on a single device. Taxonomy and determination of pathogens to species followed Farr et al. (1989), Brandenburger (1985), Klenke and Scholler (2015) and MycoBank, the online database of the International Mycological Association (Crous et al. 2004).

Pathogen diversity and infection

At each site, we quantified pathogen diversity with two metrics: the total number of pathogen groups for each host species ('pathogen groups per species') and the mean absolute number of pathogen groups per plant individual ('number of pathogen groups per plant individual'). For each site and pathogen group, we quantified pathogen infection with three metrics: the percentage of infected individuals per plant species ('incidence'), the mean percentage of infected plant tissue per plant species ('severity') and the product of both ('overall infection'). Moreover, we calculated the incidence, severity and overall infection across all pathogen groups to estimate total pathogen load for each host species ('total incidence', 'total severity' and 'total overall infection', see Table 2.1, also see Rottstock et al. (2014)).

Plant phylogeny

We used a dated molecular phylogeny of vascular plants (Zanne et al. 2014) to create a phylogeny for our 51 focal plant species (Appendix 7.1.5). Our focal species that were missing from this tree were bound into the phylogeny at the genus level using the function congeneric.merge in the pez package of R (Pearse et al. 2015).

Pathogen specialization and distribution

For each pathogen identified to species level, we calculated the mean number of host genera and families, as a measure of host range, by extracting the number of known host genera and families from the USDA Fungus-Host distribution database (https://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm). We restricted our metric to these higher taxonomic levels, since the number of known host species is unavailable for some of our pathogen species. We used the same database to extract the geographic distribution for each pathogen.

Statistical analysis

We used Blombergs K (Blomberg et al. 2003) to test for phylogenetic signal of pathogen diversity, incidence, severity and overall infection across plant species. For plant species occurring on multiple sites, values of pathogen diversity and infection were averaged across sites. Blombergs K was computed along with an associated p-value by comparing the real association between each pathogen response variable and phylogeny to a null distribution obtained by random permutations of the data. P-values less than 0.05 indicate non-random patterns (i.e. phylogenetic signal). We found no evidence of a phylogenetic signal in any pathogen response variable (see 2.3 Results), and thus did not consider phylogeny in any other statistical analyses.

Statistical analysis on the effects of host abundance and origin on pathogen diversity and infection were performed using generalized linear mixed models in SAS® Release 9.4 (procedure GLIMMIX). We analyzed the total number of pathogen groups using a model with Poisson error distribution and a logarithmic link-function. The dataset for the number of pathogen groups per individual was square root-transformed (Ahrens et al. 1990) and analyzed using models with gaussian error distribution and identity link-function. For the response variables incidence and total incidence we used models with binomial error distribution and a logit link-function. The percentage data for severity, total severity, overall infection and total overall infection was logit-transformed (Warton and Hui 2011) and analyzed using models with Gaussian error distribution and identity link-function. Plant species occurring on more than one site were treated separately for each site. In each analysis, 'site' (N=3 sites) was considered as a random effect and 'host abundance' (N=18 species for the Perilla site, N=23 species for the Carduus site and N=20 species for each site) and 'host origin' (N=2 categories), as well as their interaction, were treated as fixed effects. We considered 'pathogen group', as well as its interactions with 'host abundance' and 'host origin', as a fixed factor in the analyses examining incidence, severity and overall infection.

To test for differences in pathogen specialization between exotic and native hosts we used a Mann-Whitney-U-test with host origin ('exotic' and 'native') as predictor variable and the mean number of host genera (and families, respectively) for each pathogen species as response variable

(procedure NPAR1WAY). To compare the geographic distribution of pathogens ('restricted to North America' vs. 'distributed on more than one continent') between exotic and native host plants, we performed a Chi-square-test for independence (procedure FREQ).

Table 2.1 Definition of response variables used in text.

Response variables	Description
Pathogen groups per plant species	total number of pathogen groups per plant species and site
Number of pathogen groups per individual	Mean absolute number of pathogen groups per plant individual
Pathogen incidence	percentage of infected individuals per plant species, site and pathogen group
Pathogen severity	mean percentage of infected plant tissue per plant species, site and pathogen group
Overall infection	product of pathogen incidence and pathogen severity per plant species, site and pathogen group
Total incidence	incidence across all pathogen groups per plant species and site
Total severity	sum of severity across all pathogen groups per plant species and site
Total overall infection	sum of overall infection across pathogen groups per plant species and site

2.4 Results

Pathogen community

Of the 51 sampled plant species across three sites, 46 showed visible signs of pathogen infection (Appendices 7.1.1 – 7.1.3). In total, we found one downy mildew, three powdery mildew, seven rust and 27 fungal leaf spot diseases, among them three species not yet reported on the respective host in the United States. From these 38 pathogen infections, we could identify 24 to species, which included 15 fungal leaf spot diseases (mainly from the genera *Cercospora* and *Septoria*), seven rusts and two powdery mildews (Appendix 7.1.4). Most (23) pathogen species visibly infected only one out of 51 host species, potentially reflecting high local host specificity (Appendix 7.1.4). An additional 36 cases of pathogen infection remained unidentified and were attributed to bacterial and viral diseases (Appendices 7.1.1 – 7.1.3).

Effects of host phylogeny on pathogen diversity and infection

Our 51 host species represent a broad range of angiosperms, including both monocots and dicots (Appendix 7.1.5). There was high variation across these plants in their pathogen incidence (ranging

from 0% to 100%), severity (ranging from 0.1% to 14.3%) and overall infection (ranging from 0% to 12.6%, data not shown). Despite this, we found no evidence of a phylogenetic signal in any pathogen response variable (Table 2.2).

Effects of host abundance on pathogen diversity and infection

Host abundance did not influence pathogen diversity nor any of the three metrics of pathogen infection (Table 2.3 and Fig. 2.1). The incidence, severity and overall infection differed across pathogen groups, but this effect was only significant for overall infection (Fig. 2.1 and Table 2.3). There was also no effect of host abundance on total incidence, total severity or total overall infection (Table 2.3).

Effects of host origin on pathogen diversity and infection

The origin of plant species did not significantly influence any metric of pathogen diversity or infection (Table 2.3). Exotic host species showed similar levels of diversity (1.31 vs. 1.19 pathogen groups per species in exotic vs. native hosts), number of pathogen groups per individual (0.87 vs. 0.78 pathogen groups per plant individual), incidence (18% vs. 16%), severity (3.9% vs. 2.7%) and, consequently, overall infection (0.7% vs. 0.5%) compared to native host species.

Effects of host origin on pathogen specialization and distribution

The pathogen species found on exotic plant species had a greater reported host range (mean number of host genera) compared to pathogens infecting native hosts (Fig. 2.2). This relationship was marginally significant when the mean number of reported host families was considered (24 vs. 11.4 reported host families, $p = 0.0561$). The geographic distribution of pathogens (pathogens restricted to North America vs. pathogens distributed on more than one continent) did not significantly differ between exotic and native hosts (Table 2.4).

Table 2.2 Results of the phylogenetic analyses measuring the strength of phylogenetic signal of pathogen diversity, incidence, severity and overall infection among the plant species found on our three study sites. For incidence, severity and overall infection, pathogen groups were analyzed separately. Powdery mildews (3 plant species) and Downy mildews (1 plant species) were not considered because of low sample size.

Pathogen group	Response variable	N	Blomberg's K	P-value
All	Pathogen groups per species	51	0.075	0.321
Bacterial and viral diseases	Incidence	51	0.114	0.124
	Severity	30	0.142	0.121
	Overall infection	51	0.092	0.374
Fungal leaf spot diseases	Incidence	51	0.025	0.824
	Severity	27	0.247	0.422
	Overall infection	51	0.035	0.774
Rusts	Incidence	51	0.188	0.165
	Severity	8	0.688	0.441
	Overall infection	51	0.136	0.348

Table 2.3 Summary of results of generalized linear mixed models for the effects of host abundance, origin and pathogen group, as well as their interactions, on pathogen diversity, pathogen infection and overall infection levels. Study site was included as a random effect, and did not have statistically significant effects on any response variable. Please note that downy and powdery mildews were excluded from all analyses considering 'pathogen group', due to low sample size. Hyphens ("-") indicate analyzes that were not performed, see methods section for further details. For description of terms see Table 2.1.

	Host abundance [A]	*Host origin [O]*	*Pathogen goup [PG]*	*A x O*	*A x PG*	*O x PG*	*A x O x PG*
Pathogen diversity			F-values				
D.f.	1, 55	1, 55	-	1, 55	-	-	-
Pathogen groups per species	0.12	1.95	-	2.19	-	-	-
Number of pathogen groups per individual	0.22	0.80	-	0.22	-	-	-
Pathogen infection			F-values				
D.f.	1, 169	1, 169	2, 169	1, 169	2, 169	2, 169	2, 169
Incidence	0.11	0.09	1.95	0.15	1.08	0.12	0.10
D.f.	1, 58	1, 58	2, 58	1, 58	2, 58	1, 58	1, 58
Severity	0.21	0.87	2.10	1.19	0.24	0.18	0.18
D.f.	1, 169	1, 169	2, 169	1, 169	2, 169	2, 169	2, 169
Overall infection	0.40	1.51	5.56**	2.15	0.49	0.22	0.04
Total infection			F-values				
D.f.	1, 55	1, 55	-	1, 55	-	-	-
Total incidence	0.28	0.81	-	0.05	-	-	-
Total severity	0.15	0.82	-	2.86	-	-	-
Total overall infection	0.11	2.00	-	2.37	-	-	-

* P<0.05, ** P<0.01, *** P<0.001

Table 2.4 Total number of pathogen species of large geographic distribution (more than one continent, according to the USDA Fungus-Host distribution database) and species whose distribution is restricted to North America (NA), on native and exotic hosts. A Chi-square test showed that pathogen distribution and host origin are distributed independently: $\chi^2 = 0.33$ with $p = 0.57$.

	Pathogen species with a large geographic distribution	Pathogen species with a distribution restricted to NA	
Pathogen species on native hosts	13	4	17
Pathogen species on exotic hosts	6	1	7
	19	5	

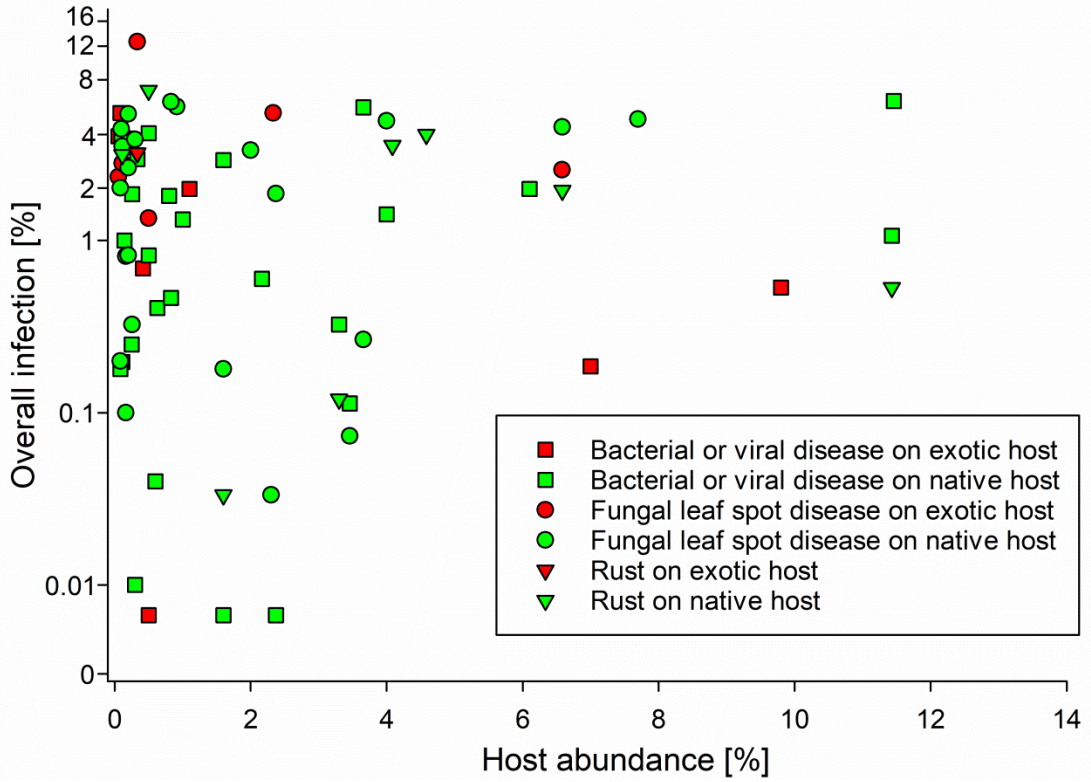

Figure 2.1 Effect of host abundance (percent cover) on overall infection by different pathogen groups on native and exotic hosts. Each symbol represents a host species with its corresponding abundance and mean overall infection levels across all infected individuals and sites. Please note downy and powdery mildews were excluded due to low sample size.

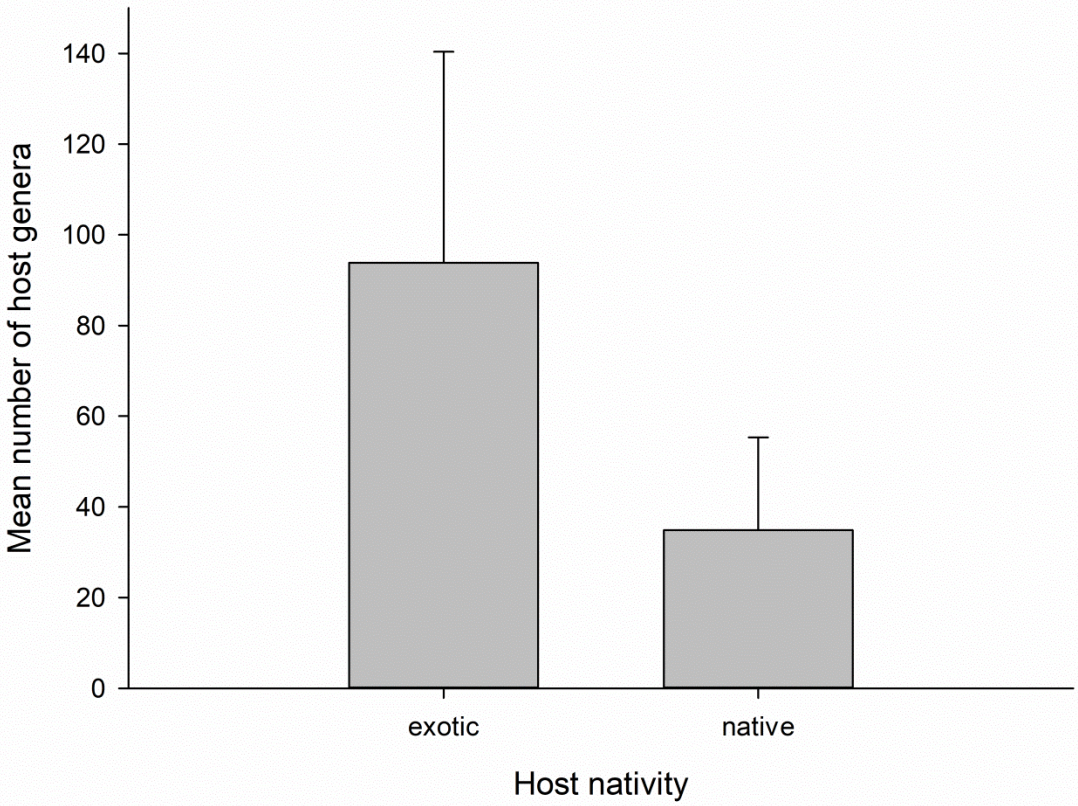

Figure 2.2 Mean number of host genera (and standard error) for pathogen species infecting exotic (n = 7) and native (n = 17) host plants on our three study sites,. A Mann-Whitney U test showed that pathogen species found on exotic hosts have a greater reported host range than pathogen species on native hosts: Z = 2.194 with p = 0.028 (two-tailed).

2.5 Discussion

At our study sites in grasslands in the central United States, we observed frequent infection of almost all sampled plant species by pathogens (>90%). Yet these observed infection levels were not related to either host abundance, origin or phylogeny. Pathogens are expected and often observed to have higher infection on more abundant hosts, creating a stabilizing mechanism that allows rare plants to persist in diverse plant communities (Alexander 2010, Mangan et al. 2010, Mordecai 2011, Bever et al. 2015). However, it has also been shown that diverse natural communities favor multi-host pathogens, thereby decoupling the frequency of pathogen infection from the abundance of individual host species (May 1991, Spear et al. 2015). It is not yet fully understood how the degree of pathogen specialization and its role in promoting species coexistence varies among different pathogen groups (Mordecai 2011). While most of our pathogen species were only observed on a single plant species, these pathogen species are known to attack multiple genera of plants. Our results suggest these multi-host pathogens are able to infect both rare and common plants, and observations might depend on the abundance of the pathogen species in the community (many of which are likely to be rare) and on pathogen transmission rates.

Overall infection levels differed across pathogen groups, which is unsurprising, given differences in pathogen life cycles and transmission mechanisms (Deacon 2006). Rusts, which showed the lowest overall infection levels of all pathogen groups, are often specialists and dependent on an alternate host for sexual development (Leung and Kot 2015). Fungal leaf spot diseases, which showed the highest infection levels of all pathogen groups, usually have no host shifts during their life cycle and are mostly generalists with broad host ranges (Deacon 2006). Among bacteria and viruses there are species with host shifts and species without host shifts. Similarly, the degree of specialization also varies widely across this broad groups of plant pathogens, which demonstrated intermediate levels of overall infection.

We find that many of our pathogen species that are known to attack many genera of hosts are only observed to infect a small proportion of their potential hosts in our plant community. In particular, fungal leaf spot diseases infected only few of their possible hosts in our focal communities (see Appendix 7.1.4). Several mechanisms might explain this. First, many of the pathogen species may have low transmission rates (Gilbert and Levine, 2017). Second, the dilution effect, a reduction in disease levels observed when host species diversity is high and when hosts vary in their susceptibility to a pathogen (Ostfeld and Keesing 2000, Civitello et al. 2015), can cause patterns of high local host specificity of pathogens (see also Rottstock et al. 2014). Temperate grasslands are among the most species-rich plant communities (Wilson et al. 2012) and for soil-borne (Maron et al. 2011, Schnitzer et al. 2011) and foliar (Mitchell et al. 2002) pathogens it has been shown that an increase in host species diversity reduces disease levels, due to a lower realized host density. Finally, if pathogen species abundance distributions in this community have a long tail of rare pathogen species, our sample size would need to be much larger in order to observe many of these pathogen species on multiple individual plants in the community, and thus document their more generalized host specificity patterns (Rottstock et al. 2014).

Exotic plants in our study did not show lower diversity or infection levels, and instead seem susceptible to a broad range of pathogens. This finding differs from other studies showing that plants escape foliar pathogens in their naturalized range (Mitchell and Power 2003) or that introduced plants experience reduced attack by fungal and viral pathogens, compared to native plants (Agrawal et al. 2005). However, these studies focused mainly on obligate, biotrophic fungi, which display a high host-specificity and are hence rather unlikely to be co-introduced with their host (Parker and Gilbert 2004). In contrast, the pathogen community infecting exotic hosts in our study were mostly generalist necrotrophic and hemibiotrophic species that are globally distributed and are found in a broad range of ecosystems, see also Parker and Gilbert (2007). We find that

pathogens infecting exotic hosts had broader host ranges than those infecting native hosts, in accordance with our expectation, see also Blaisdell and Roy (2014).

The lack of differences between native and exotic host species in our study is a robust result, as we have good statistical power (see Table 2.2), and several exotic and native plants were found to have high diversity and infection of pathogens (e.g., exotic *Melilotus albus* had the highest level of pathogen severity of all sampled plant species). This indicates one or more of the following mechanisms: (1) hosts are introduced with their pathogens to new continents, (2) enemy release is transitory, and natural enemies accumulate with time since introduction (Brändle et al. 2008, Mordecai 2011) and/or (3) that native pathogens effectively infect exotic plants (Elton 1958). Our study was not designed to distinguish between these mechanisms but, as many of the pathogens in our system have a global distribution (see Table 2.4), our results support the first and/or the third option. We also found one case of a native pathogen, only reported from North America, infecting an exotic species (*Phyllachora lespedezae* on *Kummerowia stipulacea*). An increasing time since first introduction (ca. 300 years in the case of *Melilotus albus*), as well as multiple introduction events (Dutech et al. 2010), might additionally contribute to a high pathogen load. We note that few of our exotic plant species are considered invasive (only *Lespedeza cuneata* and *Melilotus albus*, according to the Missouri Department of Conservation, (https://nature.mdc.mo.gov/status/invasive), and it is possible that communities with more invasive exotic plants might show more support for the enemy release hypothesis (Mitchell and Power 2003). Finally, anthropogenic disturbance might override the importance of enemy release (Colautti et al. 2004, Elton 1958, Hierro et al. 2006, Lozon and MacIsaac 1997, St. Clair et al. 2016) for exotic plants in our system. Grazing by deer (*Odocoileus virginianus*), and management using mowing and prescribed fires are common at our study sites and may create niche opportunities for exotic plants.

Our statistical analysis approach tested whether abundance and origin influenced pathogen infection using data pooled into broad groups (i.e., our pathogen groups). This approach gives us more power to test for the relationship than if we had used species-level identifications of pathogens, which we mostly have for all pathogen groups except viruses and bacteria. For our test of host abundance, specialization is the precondition that by which there is a relationship between host abundance and infection (Mordecai 2011, Spear and Mordecai 2018). If host abundance plays a role in infection for many specialized pathogens, then there would be greater ability to detect this in an analysis that combines many specialized pathogen species together in a single analysis. Likewise, if natives species are infected by more specialized pathogens than exotics species, we expect to be better able to detect a significant effect of origin on pathogen infection in an analysis that considers pathogen groups.

We found no phylogenetic signal across plants in the diversity and infection of pathogens. This is surprising, given the role of phylogenetic signal found in other community-level studies (e.g. Parker et al. 2015). However, there is evidence in other systems that both pathogen virulence and susceptibility to pathogens and herbivores are more dependent on local selective conditions than evolutionary history (Agrawal and Fishbein 2006, Haak et al. 2014, Rudgers et al. 2004, Thrall et al. 2002). For example, heterogenous landscapes (such as the matrix of forest and grassland habitats in our system) can favor rapid local adaption of both host and pathogen populations (Forester et al. 2016), creating unique patterns of host susceptibility and pathogen virulence (Thrall et al. 2002) that weaken phylogenetic signals across hosts in pathogen infection levels (Strauss et al. 2002, Haak et al. 2014).

In conclusion, our study suggests that neither host abundance nor origin or evolutionary relationship play an important role in explaining general patterns of pathogen infection in our studied grassland communities. Our broad methodology provides a guide that could be applied to other ecosystems, so that the generality of this result and/or its context dependence (e.g. we consider relatively disturbed temperate grasslands) could be synthesized in the future. Our study could be expanded by considering experiments that manipulate host abundance, by replicating observations across years to examine temporal variabilitity of results (see Rottstock et al. 2014), and by designing observational and experimental studies to test amongst underlying mechanisms (Hector et al. 2007). Studies such as this one, which consider multiple factors and multiple pathogen groups, are critical to understanding the role of pathogens in mediating community dynamics.

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3

Effects of natural enemies on plant coexistence

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3.1 Abstract

Species' responses to antagonists, such as fungal pathogens and insect herbivores, are part of a species' niche and are thought to contribute to species coexistence. In theory, coexistence is supposed to be maintained by the effects of equalizing mechanisms that reduce fitness differences among species, and stabilizing mechanisms that are the result of niche differences. The presence of stabilizing niche differences is hallmarked by negative frequency-dependent per capita rates of increase. However, rigorous experimental testing of how insect herbivores and fungal pathogens contribute to negative frequency dependence among co-occurring plant species, in particular in the context of biological invasions, is scarce. We present an experimental study in a grassland in central Germany, in which we established native and exotic-dominated communities on 1 m^2-plots, and reduced above- and belowground fungal pathogens and insect herbivores by applying pesticides. We manipulated the frequencies of six native and six exotic focal species, in order to assemble one dominant and five rare species per community. We quantified productivity and diversity for each community, and recorded data on per capita rates of increase and other fitness components for two native and two exotic focal species over three years. We found that native and exotic-dominated communities did not differ in their productivity and diversity. Reduction of fungi and insects increased productivity but the effect was similar for both species origins, suggesting that effects of enemy release are transitory or not existent. Negative frequency-dependence was common in our species, but could not be linked to interactions with antagonists. Although focal species responded to the reduction of antagonists, these effects were only present at low frequency, suggesting a greater importance of intraspecific competition, e.g. for resources, in explaining the observed stabilizing niche differences. Offsetting responses of the two exotic focal species to both antagonist groups highlight the significance of equalizing mechanisms among native and exotic species at the community level. Our study demonstrates the importance of both equalizing and stabilizing mechanisms in the context of biological invasions and differential contributions of antagonistic interactions to these mechanisms.

Keywords: Enemy release hypothesis, equalizing and stabilizing mechanisms, exotic species, negative frequency-dependence, productivity, species diversity

3.2 Introduction

Responses to antagonists are part of a species' niche and can dramatically affect community structure and dynamics (Chesson 2000, HilleRisLambers et al. 2012). Experimental work suggests that interactions of plants with antagonists, like fungal pathogens and insect herbivores, can play an important role in maintaining diversity of plant communities (Mordecai 2011, Bever et al. 2015, Myers and Sarfraz 2017). However, despite the large number of experiments demonstrating the importance of antagonistic interactions for the maintenance of species diversity, surprisingly few studies explicitly quantify the underlying mechanisms (Levine and HilleRisLambers 2009, Siepielski and McPeek 2010).

Contemporary niche-based theory predicts that coexistence in communities, and hence species diversity, are maintained by the joint effects of 'equalizing' and 'stabilizing' mechanisms (Chesson, 2000). While equalizing mechanisms minimize relative fitness differences between competing species, e.g. due to differences in the ability to tolerate herbivory, stabilizing mechanisms act through differences in species' niches (Adler et al. 2007). Stable coexistence occurs when niche differences among species are large enough to overcome relative fitness differences, thereby promoting diversity during community assembly by preventing competitive exclusion of species by superior competitors (Bartomeus and Godoy 2018). The demographic signature of these stabilizing niche differences is negative frequency-dependent population growth: when the per capita population growth of a species is determined by its frequency within a community, it attains higher growth rates when it is rare compared to when it is common, a so-called 'rare-species-advantage' (HilleRisLambers et al., 2012). Per capita population growth declines with increasing frequency of a species within a community because negative intraspecific interactions are enhanced relative to interspecific interactions; in other words, species limit themselves more than their competitors when they are abundant (Chesson 2000). Niche differences driving this negative frequency-dependent population growth arise from differences between species in their effect on, and response to, limiting abiotic and biotic factors, including resources and antagonists such as fungal pathogens and insect herbivores (HilleRisLambers et al. 2012).

Fungal pathogens can severely affect the performance of their hosts and thereby contribute to mechanisms that influence the coexistence of their host species (Mordecai 2011). Negative frequency-dependent disease dynamics have been shown to be a major driver for the maintenance of species diversity in various ecosystems, i.e. acting as stabilizing mechanism (Janzen 1970, Connell 1971, Klironomos 2002). Due to common host-specificity, the negative effect of fungal pathogens on per capita population growth rates increases with host species frequency, rather than with overall plant density (Mordecai 2011). Highly diverse plant communities can not only

promote a higher diversity of fungal pathogens, but also lower infection rates per plant, because host-specific pathogen transmission is low in diverse stands with individual species occurring at low frequency (Schnitzer et al. 2011, Rottstock et al. 2014). However, there are also examples for destabilizing effects of pathogens on community structure, such as accelerated plant species turnover and spillover effects mediated by pathogens (van der Putten et al. 1993, Meentemeyer et al. 2004). Furthermore, studies investigating plant-pathogen interactions focus mainly on the effects of soil biota (Bever 2003, Mangan et al. 2010, Schnitzer et al. 2011), whereas the effects of the whole pathogen community, including aboveground pathogens, have received less attention so far (Allan et al. 2010).

Similarly, effects of insect herbivores on plant community dynamics have been studied less frequently than, for example, effects of vertebrate herbivores (Allan et al. 2010), although the biomass of invertebrate herbivores may be up to ten-fold higher than that of vertebrate grazers in temperate terrestrial ecosystems (Pimentel and Andow 1984). Besides the direct effect of insect herbivory on plant fitness via consumption of plant tissue (Crawley 1989, Cahill and Coupe 2003), insect herbivores have been shown to exert strong frequency-dependent effects on plant populations (Fagan et al. 2005, Bagchi et al. 2014) and maintain plant diversity (Carson and Root 2000, Stein et al. 2010). Further studies also show an acceleration of plant species turnover facilitated by preferential feeding of insect herbivores on dominant plant species (Fraser and Grime 1998, Schädler et al. 2004). Furthermore, it is argued that these effects are highly context-dependent and not generalizable across communities (Maron and Crone 2006, Maron et al. 2014, Myers and Sarfraz 2017). However, across a multi-site experiment Kempel et al. (2015) found that especially those plant species benefitted from insect exclusion that are preferred by generalist herbivores, indicating that a growth-defense trade-off drives plant community composition.

In the context of biodiversity loss, the introduction of exotic species can represent a major alteration of the biotic environment, with effects on species diversity and ecosystem functioning on a global scale (van Kleunen et al. 2015, Pyšek et al. 2017). Exotic species often reach high productivity (Korell et al. 2016a) and frequency in their new range, for which one possible explanation is the release from specialist antagonists upon introduction, and a lower relative impact of generalist antagonists, according to the enemy release hypothesis (ERH; Maron and Vila 2001, Keane and Crawley 2002). The ERH further predicts that exotic species, being released from their antagonists, experience an increase in fitness and a competitive advantage against antagonist-regulated native species, resulting in increased dominance in invaded communities (Hierro et al. 2005). Results from broad-scale studies support the ERH by showing that exotic plant species are less infected by fungal and viral pathogens than native plants (Mitchell and Power 2003). Similarly,

meta-analyses have revealed that exotic plants suffer less from insect herbivores than native congeners (Liu and Stiling 2006), and that insect diversity and abundance are higher on native plants than on exotic plants in the same community (Meijer et al. 2016).

However, the effect of enemy release on the success of exotic species remains equivocal, as several studies and meta-analyses found no effect or even results opposing the ERH, i.e. more antagonists and/or an increased attack by antagonists on exotic plants (Agrawal and Kotanen, 2003, Colautti et al., 2004, Levine et al., 2004, Chun et al., 2010). Further, most of these studies did not consider the frequency of host plants and rigorous experimental evidence for the ERH, for example from experiments involving the removal of pathogens and herbivores, is rare (Allan et al. 2010, Myers and Sarfraz 2017). We are unaware of any study considering the importance of antagonistic interactions on the mechanisms of plant species coexistence in the context of exotic species.

Here we report the results of a field experiment conducted in a grassland in central Germany, in which we established plant communities with different frequencies of native and exotic species, and then used pesticides to reduce above- and belowground fungal pathogens and herbivorous insects. The experiment was designed to show the combined effect of two important antagonist groups (i.e. aboveground as well as belowground), in contrast to other studies, which excluded either soil-borne pathogens (e.g. Schnitzer et al. 2011) or leaf pathogens (Allan et al. 2010). We addressed the following hypotheses considering the community level and the species level:

Community level

1. According to the ERH, exotic-dominated communities should be more productive than native communities when these antagonists are present, because exotic plant species are less attacked by pathogens and insects than native species. However, owing to a negative frequency-dependent response of native plant species to antagonists, native communities should have more species and higher evenness than exotic-dominated communities (see Fig. 3.1).

2. When antagonists are reduced, productivity of native communities should increase, but their species richness and evenness should decrease because the stabilizing effect of antagonists is no longer present. These effects should be smaller in exotic-dominated communities, because otherwise suppressed native competitors benefit more from reduction of antagonists than exotic species (see Fig. 3.1).

Species level

3. Because species limit themselves more than their competitors when they are abundant, as a result of niche differences among species, per capita rate of increase (and other fitness components) of species should be lower (but total biomass of individual species should be larger) at high frequency compared to low frequency (see Fig. 3.2). However, total biomass of species should be higher when sown at high frequency as more individuals (albeit of lower fitness) become established.

4. Because antagonists contribute to the negative frequency-dependence, differences in fitness and in species biomass between high and low frequency should be smaller when antagonists are reduced, mitigating the 'rare-species-advantage'. This effect of antagonist reduction should be less distinct for exotic species compared to native species as they are less attacked by insects and pathogens (see Fig. 3.2).

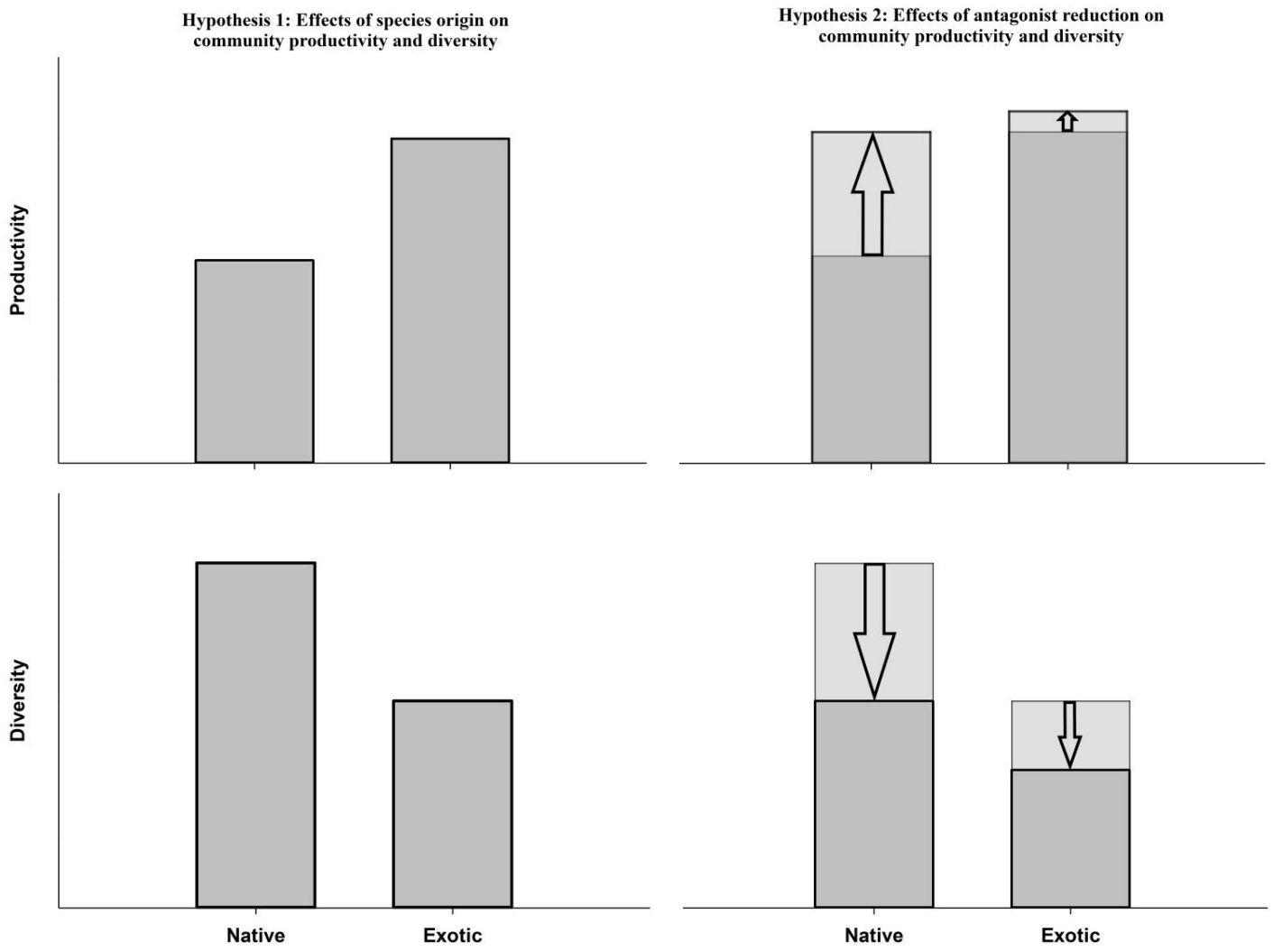

Figure 3.1 Conceptual diagram for our expectations regarding the effects of species origin (hypothesis 1) and antagonist reduction (hypothesis 2) on experimental grassland communities. Arrows indicate the increase in productivity and the decrease in diversity, respectively, that we expect when antagonists are reduced.

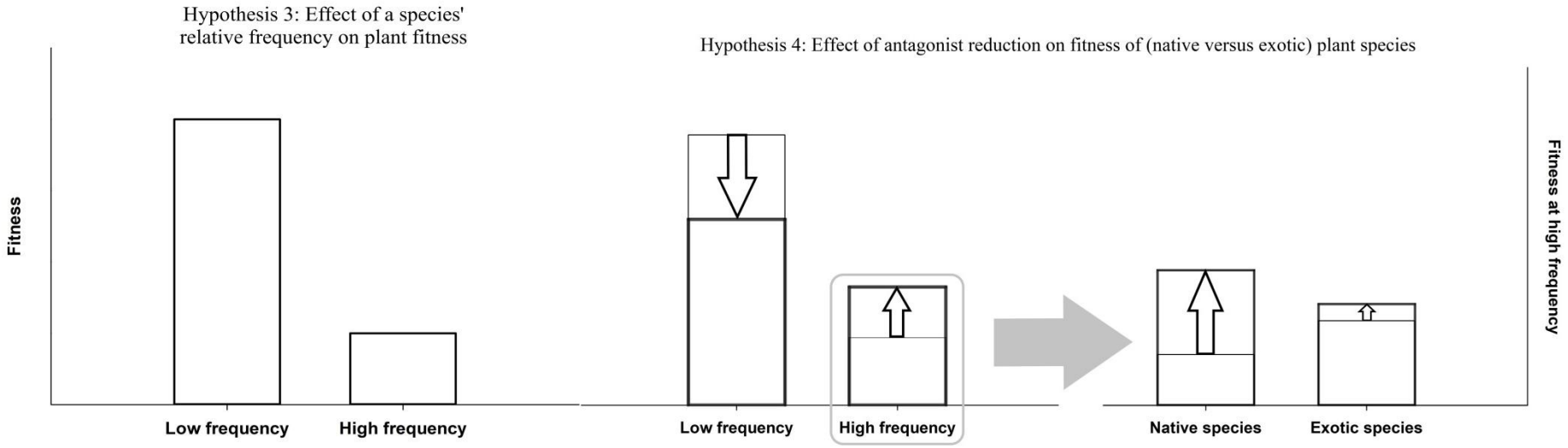

Figure 3.2 Conceptual diagram for our expectations regarding the effects of a species' relative frequency in the seed mixture (hypothesis 3) and antagonist reduction (hypothesis 4) on fitness of individual plant species. Hypothesis 3 depicts what we, throughout the manuscript, refer to as negative frequency-dependence, a higher plant fitness at low frequency compared to high frequency. Hypothesis 4 shows that, by reducing antagonists, the "rare-species-advantage" is mitigated, i.e. common species benefit most (because they are more frequently attacked by antagonists) and gain a competitive advantage over rare species, potentially destabilizing the community. We also hypothesized divergent outcomes of antagonist reduction on fitness of native versus exotic species at high frequency, as shown on the right and indicated by the filled arrow. Empty arrows indicate the increase and decrease in fitness, respectively, that we expect when antagonists are reduced.

3.3 Methods

Study site

The field experiment was located approximately 20 km west of the city of Halle in central Germany (State of Saxony-Anhalt, 51°29'N 11°49'E, 119 m above sea level). The area is characterized by a sub-continental climate with an average annual precipitation of 488 mm and an average annual temperature of 9.0 °C (German Meteorological Office, DWD). The former arable field site was used as an occasionally mulched grassland since 1992. The soil is a shallow rendzina with marl and characterized by high amounts of clay (18%), carbonate (>5%), magnesium (>1%), calcium (>6%) and relatively low amounts of organic carbon (<2%) and nitrogen (<0.2%, Schubert 2010). The original vegetation consisted of a mix of semi-dry grassland and ruderal species, interspersed by shrubs and small trees. This vegetation was removed by plowing the field in fall 2013, six weeks before sowing, in order to create similar starting conditions for the addition of native and exotic (henceforth termed 'species origin') seed mixtures. The field site was fenced as protection against naturally occurring wild boars (*Sus scrofa*), and had an area of ca. 3000 m².

Experimental design

We selected six native focal species (*Arrhenatherum elatius*, *Lotus corniculatus*, *Medicago falcata*, *Tragopogon pratensis*, *Hypericum perforatum*, *Falcaria vulgaris*) for being typical species of semi-dry to moist central German grasslands and six exotic focal species (*Lolium multiflorum*, *Onobrychis viciifolia*, *Medicago* x *varia*, *Dianthus giganteus*, *Pimpinella peregrina*, *Bunias orientalis*), for being naturalized exotic species in grasslands of Germany, with the exception of *P. peregrina*, that is considered to be a casual close to naturalization (floraweb.de, Bundesamt für Naturschutz 2019). Most of these species are perennials, except for *T. pratensis*, which is either biennal or perennial, and *L. multiflorum*, which is either annual, biennial or perennial (floraweb.de, Bundesamt für Naturschutz, 2019). Focal species were selected in such a way that each species origin contained one grass, two legumes and three non-legume forbs, thereby reflecting the proportions of functional groups in natural grasslands. Furthermore, species selection was also limited by the rarity of exotic grasses in Central Europe (Floraweb.de, Bundesamt für Naturschutz 2019). In addition to our twelve focal species, we used ten native species, typically occurring on semi-dry grassland sites in that region, as 'matrix species'. Naturally occurring plant species also invaded the plots and contributed to the 'community matrix' of species, the intention of which was to form a species network rich in interspecific interactions (May 1972, Novak et al. 2016) and to provide a vegetation as natural as possible, to achieve a reasonable species density on the plots and to create similar starting conditions for all communities, after removing existing vegetation (see above).

Seeds of focal and matrix species were either self-collected or purchased from local seed traders from regional (within a distance of <100 km around the experimental site), wild-growing populations (one to four sampled populations). Seeds of all legume species where scarified with fine sandpaper prior to sowing. See Appendix 7.2.1 for detailed information on all focal and matrix species.

On the experimental site, we established 240 plots of 1 m^2 size, arranged in five blocks of 48 plots, in November 2013. Plots were spaced at a distance of 1.5 m from each other. Distance between blocks varied between 1.5 m and 5 m. Within each block, 24 randomly selected plots were sown with a seed mixture containing the six native focal species, and 24 plots were sown with a seed mixture containing the six exotic focal species. The relative frequency of a given species in the seed mixture (henceforth called 'relative frequency') was varied across plots: each of the focal species was sown at high frequency on four of these plots, while the remaining five focal species were sown at low frequency on the same plots. Hence, a high frequency plot for a particular focal species (henceforth termed 'dominant species') at the same time represented a low frequency plot for the other five focal species (henceforth termed 'rare species'), and vice versa. We sowed a seed mixture of ten species on each plot, containing the six focal species (being either native or exotic) and four additional native matrix species. The particular combination of matrix species was constant among plots within a block, but varied among the five blocks in order to represent all matrix species as equally as possible across all blocks, and in such a way that each combination consisted of one grass species and three forb species (see Appendix 7.2.1). Total seed density was held constant at 4 g m^{-2} on each plot, with focal species being sown at 2.4 g m^{-2} for dominant species (i.e. 60% of the total seed mixture) and 0.04 g m^{-2} for each rare species (i.e. 1% of the seed mixture). Each of the matrix species was sown at a density of 0.35 g m^{-2}. To ensure an equal distribution of the seeds across the plot, 50 g of quartz sand was added to each seed mixture prior to sowing. Seed mixtures were filled in 50 ml Falcon® tubes and dispensed manually across the whole plot, as evenly as possible. Sowing took place in December 2013. Plots were not weeded.

Within each block, the four high frequency plots of each focal species were randomly assigned to four pesticide application treatments: no application of pesticides, fungicide application only, insecticide application only, application of fungicide and insecticide. The resulting 48 treatment combinations (2 species origins × 6 dominant species (nested within species origin) × 4 pesticide application treatments) were replicated five times according to the number of blocks. See Appendix 7.2.2 for a schematic overview of the treatment structure.

Pesticide treatments

In order to control a broad spectrum of the two groups of antagonists, we used the commercially available fungicides Acrobat® Plus WG [90 g/kg Dimethomorph (systemic mode of action, MOA) and 600 g/kg Mancozeb (contact MOA), BASF SE, Ludwigshafen, Germany] and a combination of Champion® [233 g/l Boscalid (translaminar and systemic MOA) and 67 g/l Epoxiconazol (systemic MOA), BASF SE, Ludwigshafen, Germany] and Diamant® [114 g/l Pyraclostrobin (F500®; translaminar MOA) and 43 g/l Epoxiconazol (systemic MOA) and 214 g/l Fenpropimorph (systemic MOA), BASF SE, Ludwigshafen, Germany] and the commercially available broad-spectrum insecticide Confidor® WG70 [700 g/kg Imidacloprid (systemic and contact MOA), Bayer CropScience AG, Monheim, Germany]. We are aware that our application of several agents with fungicidal properties probably also affected mycorrhizal and saprotrophic fungi. To our knowledge, however, there is no way to target a broad spectrum of pathogenic fungi without, at the same time, also affecting plant-associated fungal symbionts and other beneficial fungi. The fungicides were applied alternately for the same number of times per vegetation period. Both types of pesticides were applied every three weeks for three (2014-2016) vegetation periods, starting in April and ending in September, in amounts according to the instruction manual. For reasons of practicability, each plot received the respective amount of the pesticide stock solution, diluted with tap water at a final volume of one liter. Control plots received the same volume of tap water. For applying pesticides and water we spread the liquid as evenly as possible across the plot using a watering can. This procedure assured that all aboveground plant parts were covered with the pesticide solution. Due to the systemic mode of action of all used pesticide formulations and as the pesticide solutions were absorbed by the soil, we ensured to reduce above- and belowground antagonists (see results and Appendices 7.2.3 and 7.2.4).

Measurement of community productivity and diversity

We recorded species richness and identity, as well as the number of individuals of all vascular plant species on a central 0.25m² square on each plot in May 2014. For statistical analysis, individual numbers were extrapolated to the whole 1 m² plot. Due to a severe drought in the spring of that year (Zink et al. 2016), emergence of seedlings was substantially delayed. Information on species richness, and identity and percent cover of all occurring plant species was collected in May and June of 2015 and 2016 for each plot. We harvested aboveground biomass on each plot in September 2014 and in July and September of 2015 and 2016. These two mowing events reflect the standard management practice in extensively used grasslands in Central Europe, as these habitats are semi-natural and need to be maintained by such management (Ellenberg and Leuschner 2010). A majority of the species in these grasslands start flowering in May and are able

to produce seeds before the first mowing event and again between the first and second mowing event (Ellenberg and Leuschner 2010). Harvesting was conducted using two 0.1 m² metal frames randomly placed on each plot. Biomass harvested from one of these frames per plot was separated by species during the July harvest in 2015 (see next section). For analysis, data was extrapolated to the whole 1 m² plot. Biomass samples were bagged, dried at 60°C for 48 hours and subsequently weighed. We calculated Pielou's evenness $J = \sum(P_i \times \ln P_i)/\ln S$, where P_i is the proportional cover of species i, and S is the species richness of the particular plot, considering all species present on a plot (focal, matrix and other species). Individuals of native focal species occurring on plots with exotic-dominated communities were not considered as focal species for that particular plot, and vice versa.

Measurement of species demography

To investigate whether the frequency of species affected per capita rates of increase (and other fitness components), and how antagonists might contribute to this effect, we recorded demographic measurements for the selected focal species according to the following criteria. First, the species should be common enough on our plots to minimize zero-inflation of resulting data, and should cover a reasonable range of relative frequencies to allow adequate comparisons among treatments. Second, flowering phenology and morphology of species should allow adequate assessment of fecundity (or proxies of it) in the time period between the two mowing events (July and September). Third, the sample of species should comprise both native and exotic focal species, and functional affiliation of species should not differ according to species origin. Based on these criteria, we selected two native non-legume forbs, *H. perforatum* and *T. pratensis*, and two exotic non-legume forbs, *D. giganteus* and *P. peregrina*, for demographic measurements. However, data sets of two of these species (and most of the other species, see results) were still zero-inflated due to moderate seed germination especially on plots with low frequency.

We documented demographic information on these four species between the July and September harvests in 2015, according to their phenology. For each species we recorded the number of vegetative and flowering individuals on five replicate plots of each frequency × fungicide × insecticide combination (i.e. one replicate per block). As each species was sown at low frequency on five different plots (each with another dominant species) of each fungicide application × insecticide application combination in each block, we used plots with the same dominant species within a block but randomly chose a different dominant species among blocks. Additionally, we collected mature seeds of *T. pratensis* and *P. peregrina* in order to calculate fecundity for these species. Seeds were bagged, dried at 60°C for 48 hours and subsequently weighed, to compute the number

of seeds per flowering individual. However, it was not possible to collect seeds from *H. perforatum* and *D. giganteus* at the same time because of later seed maturation in these species. Instead, we used the number of inflorescences per individual as a proxy for fecundity for both species.

To estimate plant fitness and its components, we calculated establishment success, proportion of flowering individuals and fecundity of flowering individuals in the second year of the experiment, and the resulting per capita rate of increase for these species. For this purpose, we first determined the establishment success per plot as the total number of individuals present in the second year after sowing relative to the number of sown seeds. Proportion of flowering individuals per plot was then calculated as the number of flowering individuals relative to the total number of individuals present in the second year after sowing. Fecundity was defined as the number of seeds produced per flowering individual for *T. pratensis* and *P. peregrina* and as the number of inflorescences per flowering individual for *H. perforatum* and *D. giganteus* in the second year after sowing. As a proxy for the per capita rate of increase we used the total seed production per plot relative to the number of sown seeds for *T. pratensis* and *P. peregrina*, and the total number of inflorescences per plot relative to the number of seeds sown for *H. perforatum* and *D. giganteus*.

As an additional component of plant fitness, we calculated germination success for ten of our twelve species, as the total number of individuals present in the first year after sowing relative to the number of sown seeds. Due to poor germination, we were unable to calculate the germination success for *O. viciifolia* and *B. orientalis*. We also analyzed biomass for nine of our twelve species. Due to excessive zero values, we did not analyze individual biomass data for *F. vulgaris*, *O. viciifolia* and *B. orientalis*. Furthermore, we related the per capita rate of increase, as estimated by the number of seeds or the number of inflorescences, to the realised abundance after two years, as estimated by the number of individuals in that year, for the selected four focal species. We did this because the accumulation of fungal pathogens and insect herbivores may depend less upon the numbers of seeds initially sown than upon the density of individuals that established.

Thus, we were able to subdivide the whole life-cycle (from seed to seed) of focal species into three independent transitions: (1) establishment: transition from seed to established individual two years after sowing (which integrates seed germination and seedling survival), (2) flowering: transition from established individual to flowering individual, and (3) seed production of flowering individuals. This enabled us to investigate which life-cycle transition is most responsive to experimental treatments, and whether this is consistent across the focal species. Note that for statistical analyses (except for species biomass), variables were modelled using absolute values as dependent variables, and considering the number of sown seeds (for establishment success and per capita rate of increase), the total number of individuals (for proportion of flowering

individuals) or the number of flowering individuals (for fecundity) as offset variables (see 'Statistical analysis').

Statistical analysis

All statistical analysis were performed using SAS® Release 9.4. We analyzed the effects of species origin and pesticide application on community productivity, species diversity and evenness using repeated measures generalized linear mixed models (procedure GLIMMIX) with a first-order autoregressive covariance structure. For community biomass data, we applied a model with lognormal distribution and identity link function, to account for the distribution of residuals and to convert multiplicative into additive effects (Rees and Brown 1992, Schädler et al. 2007), while species richness data were analyzed using a model with Poisson distribution and log link function. Due to the negative skew of residuals, a reflected square-root transformation (SAS Institute 2015) was used for evenness data, before applying a model with Gaussian distribution and identity link function. For all community level analysis, 'species origin', 'fungicide application' and 'insecticide application', and 'date of harvest', as well as their interactions, were considered fixed factors, whereas 'block' and 'dominant species' (nested within 'species origin'), as well as its interactions with 'fungicide application' and 'insecticide application' and 'date of harvest', were considered random factors.

When analysing effects of relative frequency and pesticide application on demography of individual species, we had to consider two important aspects of data distribution. First, some species showed considerable spatial clumping of individuals among plots within the same treatment combination, corresponding to a negative binomial distribution of residuals. Second, we had to account for zero-inflation, because in a moderate number of plots, some of the species sown at low frequency did not establish at all, due to generally low germination percentage. We therefore used the procedure GENMOD to apply generalized linear models with zero inflation. We analyzed establishment success, proportion of flowering individuals, per capita rates of increase and per capita rates of increase relative to the realised abundance after two years for all four species, fecundity for *T. pratensis* and *P. peregrina* and germination success for nine out of ten species (except for *D. giganteus*) using a model with negative binomial error distribution and log link function. Due to a considerable number of plots where no individuals were present or no seeds were produced, we applied the same model and added a zero-inflation component for analysing the establishment success and per capita rates of increase of *T. pratensis* and *P. peregrina*, for analysing the germination success of *L. corniculatus*, *M. falcata*, *T. pratensis*, *H. perforatum*, *L. multiflorum* and *P. peregrina* and for analysing the per capita rates of increase relative to the realised abundance after two years for *P.*

peregrina. Fecundity of *H. perforatum* and *D. giganteus* were analyzed using models with Poisson error distribution and log link function because they provided a better fit than the model with negative binomial error distribution. We applied the same model and added a zero-inflation component for analysing the germination success of *D. giganteus*, due to a considerable number of plots in which no individuals were present. We added an overdispersion parameter if necessary. For each of these models we used offset variables: the logarithm of the number of sown seeds per plot for establishment success, for per capita rates of increase and for germination success; the logarithm of the number of individuals per plot for proportion of flowering individuals and for the per capita rate of increase relative to the realised abundance after two years; and the logarithm of the number of flowering individuals per plot for fecundity. We applied a model with lognormal distribution and identity link function to analyze the effects of relative frequency and pesticide application on biomass of nine of our focal species. To reduce excess zeros, we averaged biomass data for each species across the five low frequency plots per pesticide treatment and block, resulting in 40 observations for each species in total. Due to an imbalanced data structure we used type 3 sum of squares for F-tests and χ^2-tests in all models (Shaw and Mitchell-Olds 1993). For each analysis on species level, all independent variables, including the block, had to be considered fixed effects, because the GENMOD procedure does not allow random effects, in contrast to the GLIMMIX procedure which in turn does not consider zero inflation. In a few cases where all plots of a certain treatment combination produced zero values we had to skip either higher-order interactions or the block effect to allow model convergence. We repeated analyses of data sets that were not zero-inflated with procedure GLIMMIX considering block as random, but found no qualitative difference between the results of the two types of models. In order to test the effects of insecticide or fungicide application at each level of relative frequency separately, we decomposed the respective pesticide x frequency interactions into "simple main effects" (Woodward and Bonett 1991).

Please note that we do not provide a statistical test for the second part of hypothesis 4 (exotic species should be less affected by a reduction of antagonists at high frequency, compared to native species) because of the low power (n = 2 species per origin), but consider a simple vote-counting most appropriate.

3.4 Results

General information on seedling emergence

In the first year after sowing (2014), our native focal species emerged at an average of 34.4 individuals per plot as a dominant species and 6.9 individuals as a rare species (see Appendix

7.2.12). Exotic species showed an average of 76.7 individuals per plot when sown as a dominant species compared to 1.8 individuals per plot when sown as rare species. Although exotic species reached higher individual numbers in 2014 compared to native species (10107 individuals vs. 8277 individuals), the proportion of focal species relative to the total individual number per plot was lower for exotic plots (22.9 % vs. 29.7 %, see Appendix 7.2.13), due to a lower total number of plant individuals on these plots (26,793 individuals on exotic vs. 33,518 individuals on native plots). The native species with the highest number of individuals per plot was *M. falcata* with an average of 72.0 individuals as a dominant species, while the exotic *P. peregrina* reached an average of 223.7 individuals as a dominant species (Appendix 7.2.12). Proportional biomass of focal species, relative to total biomass per plot, in the second year after sowing (2015) was higher for natives, compared to exotics (48.8 % vs. 23.9 %, see Appendix 7.2.13), in contrast to no difference in total community productivity between species origins (see 'Community-level responses – hypothesis 1'). Matrix species emerged with an average of 17.0 individuals per plot, summed up for all four matrix species on a particular plot (data not shown).

The near absence of germination in *B. orientalis* in our experiment was surprising (see Appendices 7.2.12 and 7.2.13), given the species' adaptation to anthropogenic disturbances (Steinlein et al. 1996, Dietz et al. 1999). However, this may be linked to the lack of molehills at our study site (personal observation), which have been shown to play a crucial role for the species' success in colonizing new sites (Kiełtyk and Mirek 2015). Excluding this species, and high frequency plots of this species, from analysis, did not yield results that differed qualitatively from the results shown here.

Evaluation of fungicide and insecticide efficacy

We quantified diversity and infection of foliar fungal pathogens on two selected focal species, native *A. elatius* and exotic *M.* x *varia*, in October 2016, after three years of fungicide application. Overall infection was significantly lower when fungicide was applied for both species, when considering each pathogen group separately and across all pathogen groups. For detailed information on pathogen diversity and infection see Appendix 7.2.3A-D. Similarly, we quantified diversity and abundance of soil arthropods on additional 1 m² plots, which were established in March 2015 at the same site as the main experiment. The insecticide treatment reduced the total abundance of all arthropods by more than 50% on plots treated with insecticide compared to control plots, indicating an effective application of insecticide. For detailed information on insect diversity and abundance see Appendix 7.2.4A-C.

Community-level responses - hypothesis 1

We hypothesized that exotic-dominated communities should be more productive and less diverse, compared to native communities (Fig. 3.1), but did not find such consistent effect. Community productivity varied significantly across all dates of harvest, ranging from 32.3 g m^{-2} per plot in September 2016 to 340.0 g m^{-2} per plot in July 2016 (Table 3.1 and Appendix 7.2.5). Across all dates of harvest native communities on average produced 196.9 g m^{-2} aboveground biomass compared to 215.0 g m^{-2} in exotic-dominated communities, but this difference was not statistically significant (Table 3.1). The interaction of date of harvest and species origin had a significant effect on community productivity, but only in September 2016, when exotic-dominated communities yielded on average 1.5 times as much aboveground biomass than native communities (Table 3.1 and Appendix 7.2.5). Although species richness and evenness varied significantly among years (Appendix 7.2.6), we did not find any effect of species origin on these two metrics of community diversity (average species richness: 18.0 on native vs. 19.0 on exotic plots; average evenness: 0.517 on native vs. 0.515 exotic plots, Table 3.1).

Community-level responses - hypothesis 2

We furthermore hypothesized that pesticide application would lead to higher productivity and lower diversity of communities, a response that would be less distinct in exotic-dominated communities (Fig. 3.1), and found that both, fungicide and insecticide application, had a positive effect on aboveground productivity, increasing biomass by 11.6% and 14.1%, on average and across all dates of harvest, respectively (Fig. 3.3 and Table 3.1). Neither pesticide application influenced community diversity (Table 3.1): species richness on plots with fungicide application reached 18.5 species on average, compared to 18.7 species on plots with no reduction of fungi, while plots treated with insecticide harbored on average 18.7 species, compared to 18.5 species on plots with no reduction of insects. On plots treated with fungicide, evenness amounted to 0.515 on average, compared to 0.517 on plots with no fungicide application. Evenness on plots treated with insecticide was 0.514 on average and 0.518 on control plots. The interaction of fungicide and insecticide application had no effect on community productivity and diversity (Table 3.1). As mentioned above, native and exotic communities did not differ consistently in productivity and diversity.

Species-level responses - hypothesis 3

We hypothesized per capita rates of increase and other fitness components to be lower at high frequency compared to low frequency (Fig. 3.2), and found this relationship for three out of the four species for which we quantified the respective life-cycle transitions. With exception of the exotic *D. giganteus*, all species showed significantly higher establishment success at low frequency, compared to high frequency (Appendices 7.2.7A and 7.2.7B).

Relative frequency had a highly significant effect on the proportion of flowering individuals of the native *H. perforatum*: when sown at low frequency, the proportion of flowering individuals was more than twice as high compared to high frequency plots (Appendices 7.2.8A and 7.2.8B). Proportion of flowering individuals of other species was not affected by relative frequency. Fecundity showed no effect of relative frequency in any of the four species.

The resulting estimates of per capita rates of increase were significantly higher for all species, except for *D. giganteus*, when sown at low frequency (Fig. 3.4 and Table 3.2), indicating a negative frequency-dependence (as shown in Fig. 3.2).

When relating the per capita rate of increase to the realised abundance after two years, relative frequency had a significant effect on *P. peregrina* (Appendix 7.2.11): At low frequency, rate of increase was higher compared to when sown at high frequency. In contrast, the per capita rates of increase relative to the realised abundance after two years was significantly higher for *H. perforatum* when sown at high frequency, compared to low frequency (Appendix 7.2.11).

In addition to fitness estimates of these four target species, we analyzed germination success (in the first year after sowing) and biomass (in the second year after sowing) of all target species except for those with too few non-zero values. Relative frequency had a significant effect on germination success of 9 out of 10 studied species (Appendices 7.2.10A - 7.2.10E), with all of these species showing higher germination success at low frequency, compared to high frequency. Only *M.* x *varia* showed no differences in germination success between high and low frequency plots (Appendices 7.2.10C and 7.2.10E).

Except for native *H. perforatum* and exotic *M.* x *varia* a high frequency led to significantly higher aboveground biomass compared to low frequency (Tables 3.3 and 3.4 and Figures 3.5 and 3.6). *A. elatius* was by far the most productive focal species, yielding on average 132.9 g/m² on high frequency plots and 83.3 g/m² at low frequency, while *T. pratensis* was, out of the nine analyzed species, the least productive one, reaching on average 2.7 g/m² on high frequency plots and 0.2 g/m² at low frequency (Figures 3.5 and 3.6).

In all cases with zero-inflated data (for establishment success, per capita rate of increase, germination success and per capita rate of increase relative to realised abundance), excess zeros (i.e. excessive number of plots with no individuals present) were explained by relative frequency

with significantly more zeros at low frequency compared to high frequency (Table 3.2, Appendices 7.2.7 and 7.2.10).

Species-level responses - hypothesis 4

We originally hypothesized that pesticide application would result in less pronounced negative frequency-dependence (Fig. 3.2), but did not find such a consistent effect across our studied species. Fungicide application had a positive effect on establishment of the native *T. pratensis*, but only at low frequency (significant relative frequency × fungicide application interaction). We found no effect of insecticide application on the establishment of any species.

Insecticide application resulted in a significantly lower proportion of flowering individuals in exotic *D. giganteus* at low frequency. Exotic *P. peregrin*a showed significantly higher proportions of flowering individuals when fungicide and insecticide were applied. However, these effects were only present at low frequency (significant relative frequency × fungicide application interaction and significant relative frequency × insecticide application interaction, Appendices 7.2.8A and 7.2.8B). Additionally, the significant interactive effect of relative frequency, fungicide and insecticide application led to lowest proportion of flowering individuals at low frequency on control plots, and to highest proportion of flowering individuals at low frequency when both, fungicide and insecticide, were applied.

Fungicide application led to a significantly higher number of inflorescences (measure of fecundity) in *H. perforatum*, at high frequency (significant relative frequency × fungicide application interaction, Appendices 7.2.9A and 7.2.9B). In contrast, application of fungicide resulted in a higher number of inflorescences in *D. giganteus*, but only on low frequency plots (significant relative frequency × fungicide application interaction, Appendices 7.2.9A and 7.2.9B). Insecticide application had no effect on the fecundity of any species.

While *T. pratensis* showed a higher rate of increase on plots treated with insecticide, compared to control plots, *D. giganteus* reached the highest rate of increase on control plots. For both species, these effects were specifically pronounced on plots with a low frequency (significant relative frequency × insecticide application interaction, Fig. 3.4 and Table 3.2). We found significantly lower per capita rates of increase in *P. peregrina* when fungicide was applied. Insecticide application had the same effect on per capita rate of increase of this species. These effects were especially pronounced at low frequency (significant relative frequency × insecticide application interaction, Fig. 3.4 and Table 3.2).

Per capita rate of increase relative to the realised abundance was significantly affected by insecticide application in only one species (Appendix 7.2.11): *D. giganteus*' per capita rates of increase were

significantly higher on plots not treated with insecticide. However, this effect was only present, and very pronounced, on low frequency plots. Additionally, per capita rates of increase relative to realised abundance was highest on fungicide-treated plots and lowest on plots treated with both, fungicide and insecticide (significant fungicide × insecticide interaction, Appendix 7.2.11). This effect was especially pronounced on low frequency plots (significant relative frequency × fungicide × insecticide interaction, Appendix 7.2.11).

Native *T. pratensis* showed much higher germination success when fungicide was applied (Appendix 7.2.10B), but this effect was only present at low frequency (significant relative frequency × fungicide application interaction, Appendix 7.2.10D). Insecticide application resulted in higher germination success of *P. peregrina*, but only at high frequency (significant relative frequency × insecticide application interaction, Appendix 7.2.10E). *D. giganteus* reached highest germination success on control plots, while on plots treated with fungicide, insecticide or both pesticides germination success was significantly reduced (significant fungicide × insecticide application interaction, Appendix 7.2.10E). Germination success of the other analyzed species was not affected by either fungicide or insecticide application (Appendices 7.2.10A - 7.2.10E).

Fungicide application significantly increased aboveground biomass of three species (Tables 3.3 and 3.4). While this effect was only present at low frequencies in *A. elatius* (Fig. 3.5), fungicide application increased biomass in *L. multiflorum* only at high frequencies (Fig. 3.6). In the exotic *P. peregrina* fungicide application had an overall effect on biomass that was specifically pronounced at low frequencies, increasing biomass more than tenfold (Table 3.4 and Fig. 3.6). In contrast, insecticide application had no effect on aboveground biomass of any species (Tables 3.3 and 3.4 and Figures 3.5 and 3.6). The three-way interaction of relative frequency, fungicide and insecticide application significantly affected biomass of *M. falcata* and *P. peregrina* (Tables 3.3 and 3.4).

In contrast to our hypothesis, exotic species did not differ consistently in their response to antagonists from native species: one out of our two exotic focal species (*P. peregrina*) showed a significant decrease in per capita rate of increase (and other fitness components) in response to both, reduction of fungi and insects at low frequency and no increase at high frequency, while the other exotic species (*D. giganteus*) showed a much lower per capita rate of increase (and other fitness components) when fungi were reduced. Similarly, one out of our two native focal species (*T. pratensis*), showed a significant decrease in per capita rate of increase (and other fitness components) in response to reduction of fungi at low frequency and no increase at high frequency, while the other native species (*H. perforatum*) showed almost no responses to antagonist reduction at all.

Table 3.1 Results of repeated measurement generalized linear mixed models on the effects of species origin (*hypothesis 1*), fungicide application (*hypothesis 2*), insecticide application (*hypothesis 2*), and date of harvest, as well as their interactions on biomass, species richness and evenness. Random effects (block, dominant species nested within origin, as well as its interactions with fungicide application, insecticide application and date of harvest) were tested using Wald Z statistics. Abbreviations for factors are given in brackets. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Source	***Biomass***		***Species richness***		***Evenness***	
	d.f.	*F* values	*d.f.*	*F* values	*d.f.*	*F* values
Species origin [O]	1, 10	0.78	1, 10	4.32	1, 10	0.01
Fungicide application [F]	1, 10	13.14**	1, 10	0.24	1, 10	0.08
Insecticide application [I]	1, 10	7.19*	1, 10	0.26	1, 10	0.18
O x F	1, 10	0.15	1, 10	2.71	1, 10	3.14
O x I	1, 10	0.23	1, 10	1.08	1, 10	0.11
F x I	1, 10	0.43	1, 10	0.29	1, 10	1.18
O x F x I	1, 10	0.39	1, 10	0.34	1, 10	2.50
Date of harvest [D]	4, 40	766.73***	2, 20	16.56***	1, 10	8.30*
D x O	4, 40	3.06*	2, 20	2.27	1, 10	0.90
D x F	4, 40	0.21	2, 20	2.46	1, 10	0.07
D x I	4, 40	2.60	2, 20	0.75	1, 10	0.46
D x O x F	4, 40	0.33	2, 20	0.60	1, 10	1.48
D x O x I	4, 40	0.53	2, 20	0.09	1, 10	0.31
D x F x I	4, 40	0.51	2, 20	1.56	1, 10	4.75
D x O x F x I	4, 40	0.35	2, 20	0.05	1, 10	1.03
	Variance estimates		Variance estimates		Variance estimates	
Block	0.0249		0.0081		0.0004	
Dominant species (O) [DS]	0.0091		0.0006		0.0017	
F x DS (O)	0.0000		0.0000		0.0000	
I x DS (O)	0.0000		0.0000		0.0000	
F x I x DS (O)	0.0022		0.0000		0.0000	
D x DS (O)	0.0043		0.0008		0.0007	
D x F x DS (O)	0.0000		0.0000		0.0000	
D x I x DS (O)	0.0021		0.0000		0.0000	
D x F x I x DS (O)	0.0000		0.0000		0.0001	
Autoregression	0.3029***		0.4807***		0.3091***	
Residual	0.2395***		0.6853***		0.0064***	

Table 3.2 Results of generalized linear models on the effects of a species' relative frequency in the seed mixture *(hypothesis 3)*, fungicide application *(hypothesis 4)*, and insecticide application *(hypothesis 4)*, as well as their interactions, and the effect of block on the estimates of per capita rate of increase, as estimated by the number of seeds, or the number of inflorescences, produced after two years relative to the number of seeds sown, of two native (*Hypericum perforatum* and *Tragopogon pratensis*) and two exotic (*Dianthus giganteus* and *Pimpinella peregrina*) focal species. Note that the three-way interaction had to be excluded for *P. peregrina* because of zero values for all replicates of a certain treatment combination, and that zero inflation was only present in the datasets of *T. pratensis* and *P. peregrina*. See method section for further information on model structure. Abbreviations for factors are given in brackets. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Source		***H. perforatum***	***T. pratensis***	***D. giganteus***	***P. peregrina***
	d.f.		χ^2 values		
Relative frequency [RF]	1	58.67***	35.38***	0.03	34.10***
Relative frequency – Zero inflation	1	-	21.24***	-	24.55***
Fungicide application [F]	1	0.35	0.19	0.90	8.70**
Insecticide application [I]	1	0.09	6.24*	7.83**	5.57*
RF x F	1	0.16	0.37	0.30	9.17**
RF x I	1	0.17	5.25*	7.21**	7.84**
F x I	1	0.93	0.00	1.35	1.26
RF x F x I	1	0.13	0.75	1.33	-
Block	4	6.20	12.15*	9.95*	3.77

Table 3.3 Results of linear models on the effects of a species' relative frequency in the seed mixture *(hypothesis 3)*, fungicide application *(hypothesis 4)*, and insecticide application *(hypothesis 4)*, as well as their interactions, and the effect of block on aboveground biomass two years after sowing of five native focal species. Note that one species (*F. vulgaris*) had to be omitted because of many zero values for biomass. See method section for further information on model structure. Abbreviations for factors are given in brackets. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Source		***A. elatius***	***L. corniculatus***	***M. falcata***	***T. pratensis***	***H. perforatum***
	d.f.			X^2 values		
Relative frequency [RF]	1	7.96 **	17.89 ***	22.34 ***	13.65 ***	0.64
Fungicide application [F]	1	0.94	1.92	0.48	1.33	0.78
Insecticide application [I]	1	0.02	1.61	0.67	0.01	0.37
RF x F	1	3.85 *	0.89	0.03	0.56	0.42
RF x I	1	0.52	1.13	1.29	0.66	0.99
F x I	1	2.96	0.08	0.08	0.89	0.01
RF x F x I	1	0.02	1.41	5.77 *	0.02	0.19
Block	4	34.26 ***	4.65	0.92	4.41	1.88

Table 3.4 Results of linear models on the effects of a species' relative frequency in the seed mixture *(hypothesis 3)*, fungicide application *(hypothesis 4)*, and insecticide application *(hypothesis 4)* as well as their interactions, and the effect of block on aboveground biomass two years after sowing for four exotic focal species. Note that two species (*O. viciifolia, B. orientalis*) had to be omitted because of the many zero values for biomass. See method section for further information on model structure. Abbreviations for factors are given in brackets. (* $P < 0.05$, *** $P < 0.001$)

Source		***L. multiflorum***	***M.* x *varia***	***D. giganteus***	***P. peregrina***
	d.f.		X^2 values		
Relative frequency [RF]	1	17.32 ***	1.99	34.56 ***	31.26 ***
Fungicide application [F]	1	3.48	1.71	0.46	5.12 *
Insecticide application [I]	1	0.12	0.45	0.01	0.01
RF x F	1	6.26 *	0.11	0.00	6.55 *
RF x I	1	0.02	0.00	0.29	3.77
F x I	1	0.82	0.28	0.47	0.00
RF x F x I	1	0.30	0.08	0.00	6.40 *
Block	4	5.47	6.86	9.07	4.08

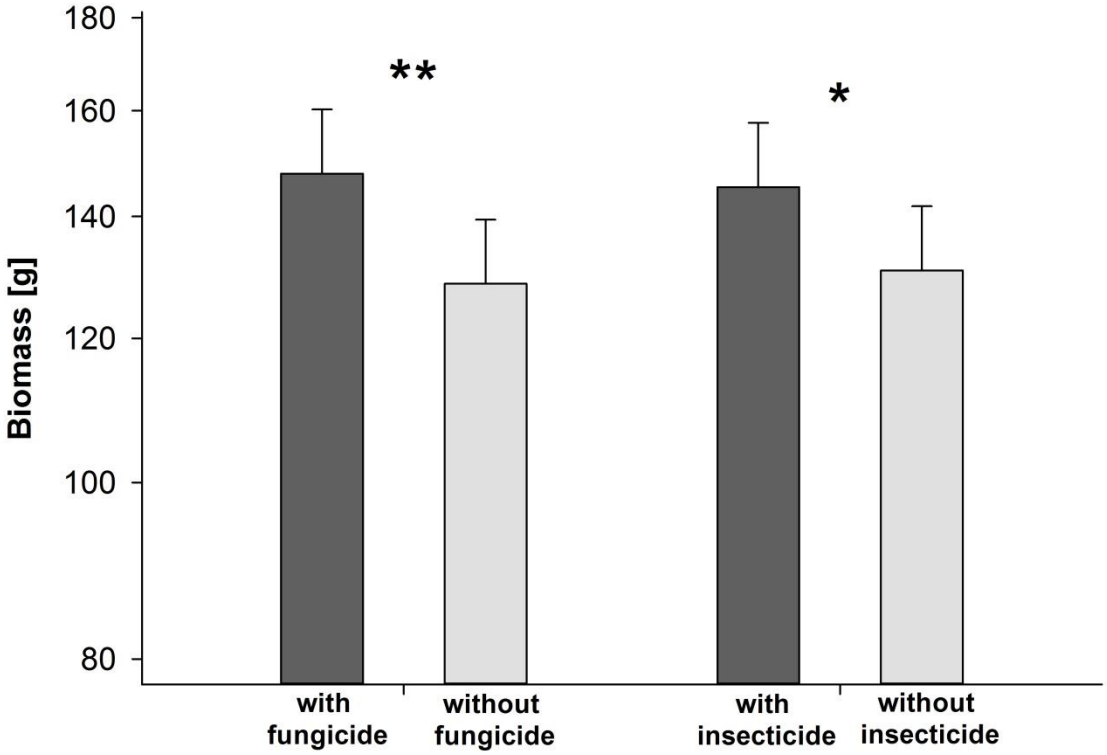

Figure 3.3 Aboveground biomasses of experimental plant communities treated with fungicide and insecticide and the respective controls (least square means and standard errors of the mean across native and exotic species, and across all five harvest dates). Differential shading visualizes the respective pesticide treatment (dark) and the control treatment (bright). The interaction effect of fungicide and insecticide application is not shown, because it was not significant (see Table 3.1). Asterisks indicate significant differences in productivity among the two species origins (* $P < 0.05$, ** $P < 0.01$). Note the logarithmic scale of the y-axis.

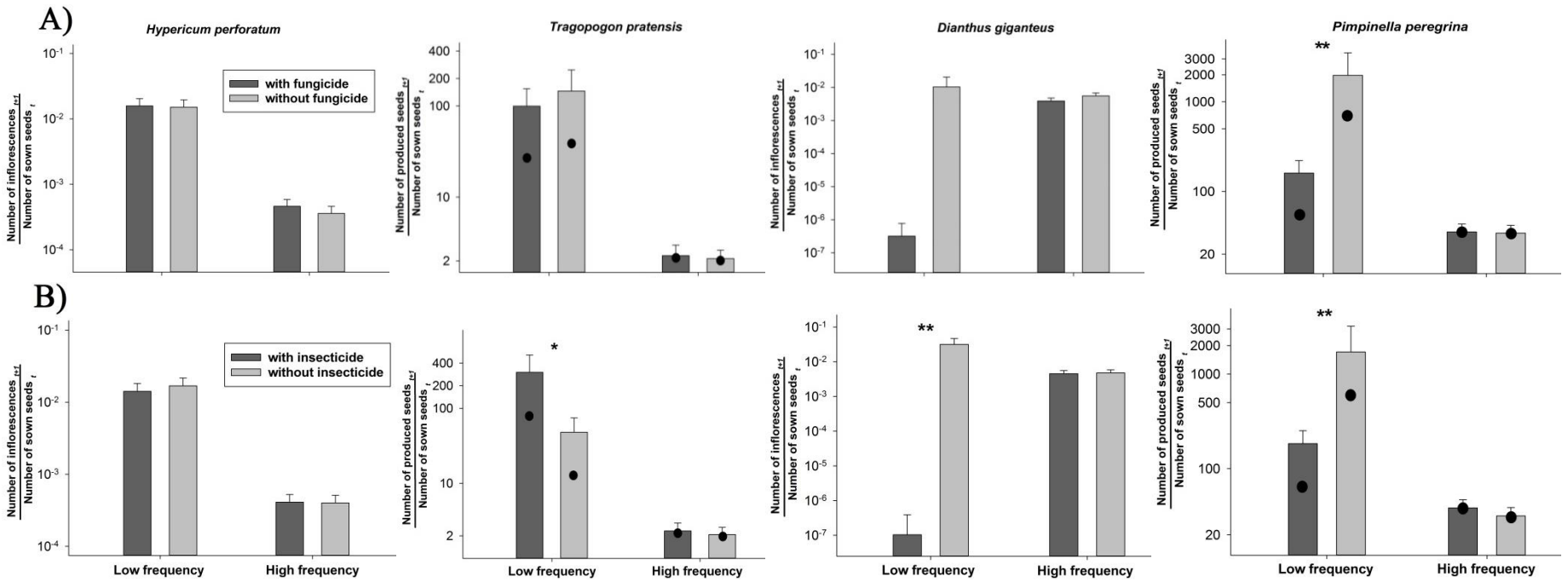

Figure 3.4 Per capita rates of increase (least square means and standard errors of the mean), as estimated by the number of seeds, or the number of inflorescences, produced after two years relative to the number of sown seeds, for two native (*Hypericum perforatum* and *Tragopogon pratensis*) and two exotic (*Dianthus giganteus* and *Pimpinella peregrina*) focal species on low and high frequency plots treated with A) fungicide and B) insecticide and the respective controls. Note that for *T. pratensis* and *P. peregrina*, columns and error bars represent means and standard errors adjusted for excessive zeros. Black dots indicate mean values including excessive zeros (i.e. plots with zero values exceeding the number of plots expected by the underlying distribution, see methods for detailed information). The interaction effect of fungicide and insecticide application is not shown, because it was not significant (see Table 3.2). Asterisks indicate significant effects of insecticide or fungicide application on per capita rate of increase at the respective level of relative frequency (* $P < 0.05$, ** $P < 0.01$). Note the logarithmic scale of the y-axis.

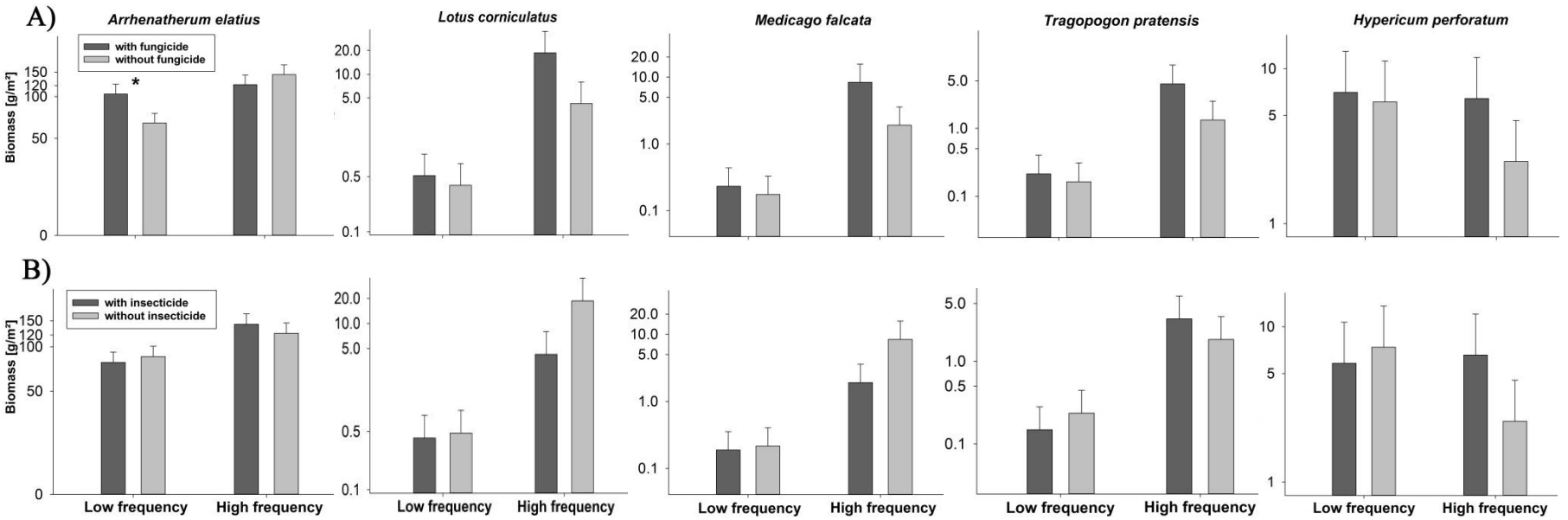

Figure 3.5. Aboveground biomasses (least square means and standard errors of the mean) of five native focal species on low and high frequency plots treated with A) fungicide and B) insecticide and the respective controls. Note that one species (*F. vulgaris*) had to be omitted because of many zero values for biomass. Although there is one significant three-way interaction (*M. falcata*: RF × F × I, see Table 3.3), it is not shown because of consistency. Asterisks indicate significant effects of insecticide or fungicide application on aboveground biomass at the respective level of relative frequency (* $P < 0.05$). Note the logarithmic scale of the y-axis.

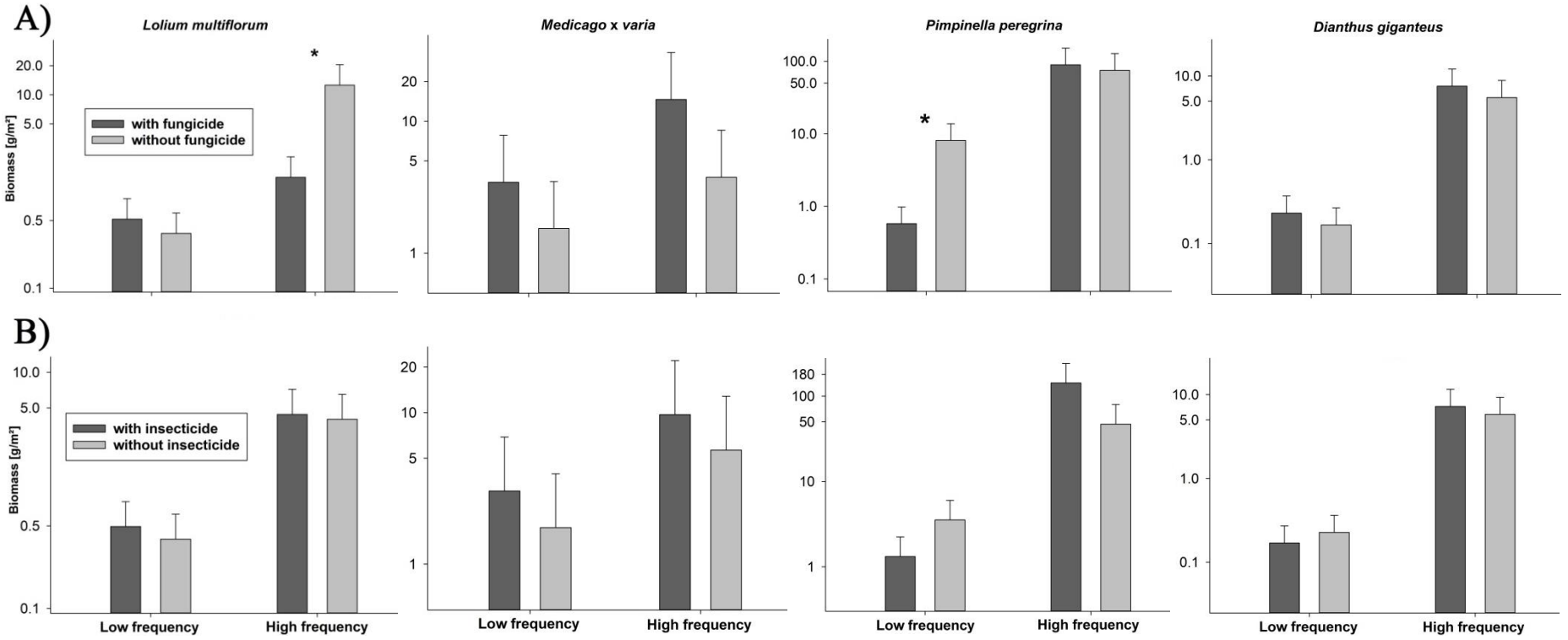

Figure 3.6. Aboveground biomasses (least square means and standard errors of the mean) of four exotic focal species on low and high frequency plots treated with A) fungicide and B) insecticide and the respective controls. Note that two species (*O. viciifolia*, *B. orientalis*) had to be omitted because of many zero values for biomass. Although there is one significant three-way interaction (*P. peregrina*: RF × F × I, see Table 3.4), it is not shown because of consistency. Asterisks indicate significant effects of insecticide or fungicide application on aboveground biomass at the respective level of relative frequency (* $P < 0.05$). Note the logarithmic scale of the y-axis.

3.5 Discussion

Our field experiment revealed no differences in productivity and diversity among native and exotic-dominated communities (in contrast to our hypothesis 1, see Fig. 3.1). We found a positive effect of fungicide and insecticide application on community productivity, but there were no differences between the responses of native and exotic-dominated communities (as opposed to our hypothesis 2, see Fig. 3.1). These results indicate that, on the community level, the establishment of the exotic species used in this study was not favored by a release from fungal pathogens and insect herbivores. Similar responses of native- and exotic-dominated communities have been shown to point to the importance of equalizing mechanisms, e.g. a competition-defense trade-off, in generating species coexistence in grasslands, by reducing fitness differences among species (Chesson 2000, Gross et al. 2014).

Antagonists reduce community biomass, but no evidence for enemy release of exotic species

Our findings differ from the results of other studies showing that exotic plants are released from antagonists in their new range (e.g., Mitchell and Power 2003, Agrawal et al. 2005). However, effects of enemy release are often transitory and antagonists accumulate with time since introduction of the species (Brändle et al. 2008, Mordecai 2011). Our results strongly support these studies, as most of our exotic species were introduced at least more than 200 years ago, with the exception of *D. giganteus*, which has been documented in Germany only since the late 20th century (Sonnberger and Schuhwerk 2005). In addition to a long time since introduction, multiple introductory events can lead to diminishing effects of enemy release, because each event makes co-introductions of antagonists from the native range of the exotic species more likely (Dutech et al. 2010). Two of the fungal pathogens species (*C. medicaginis* and *S. meliloti*) we found on *M.* x *varia*, for example, have not been recorded in Germany so far, nor on its parent species *M. sativa* and *M. falcata*, see Appendix 7.2.3C (Klenke and Scholler 2015, Farr and Rossman 2019).

We are also aware that only two of our exotic species (*D. giganteus* and *B. orientalis*) are considered potentially invasive (see Appendix 7.2.1; Neobiota.de, Bundesamt für Naturschutz 2019), and it is possible that studies, conducted with communities harboring more invasive exotic species, might yield results that show more support for the ERH (Mitchell and Power 2003). Finally, release from antagonists has been shown to play little role in explaining the success of exotic species in a considerable proportion of studies in which it has been tested (Gilbert and Parker 2006, Myers and Sarfraz 2017). Other factors, such as differences among natives and exotics in fitness components not related to response to antagonists, are known to determine the outcome of

invasion success and establishment, even when exotic species experience similar or higher impairment from pathogens and herbivores (Schutzenhofer et al. 2009, Stricker and Stiling 2014).

Negative frequency-dependence indicates niche differences, but is not linked to antagonistic interactions

Three of the four species (*H. perforatum*, *T. pratensis* and *P. peregrina*) we selected for recording demographic measurements displayed a negative frequency-dependence in their per capita rate of increase, i.e. they had a higher rate of increase when rare compared to when common within a given species assemblage. This finding is in accordance with our hypothesis 3 (Fig. 3.2) and indicates that the niche differences among our species have a stabilizing effect on the community (HilleRisLambers et al. 2012). The same three species showed a negative frequency-dependence in their establishment success, suggesting that this major bottleneck of life history plays a crucial role in the context of frequency-dependent population growth (Jongejans et al. 2006, Jorritsma-Wienk et al. 2007). Proportion of flowering individuals only displayed a negative frequency-dependent relationship in response to different relative frequencies in one species, the native *H. perforatum*, while fecundity showed no general frequency-dependent responses in any species. These results suggest that these fitness components are of subordinate importance in their contribution to population growth of the studied species, and ultimately, for species coexistence in our communities, as has been recently shown in other invaded grasslands (Spear and Mordecai 2018). The absence of a general frequency-dependent response in the exotic *D. giganteus* might reflect that, in theory, species coexistence can partially be decoupled from niche differences driving negative frequency-dependence, when temporal variability of environmental factors is high (Cordonnier et al. 2006, Yi and Dean 2013). For example, the disturbance regime in our study system may provide sufficient temporal (and spatial) fluctuation that species show differential fitness responses to relative frequency or to antagonist attack (Cordonnier et al. 2006), and thus, could be considered as a driver of an operating storage effect in our communities (Chesson and Warner, 1981, Chesson, 1994).

Although negative frequency-dependence was present in our species, it could not be attributed to the effects of fungal pathogens and insect herbivores (hypothesis 4, see Fig. 3.2). In particular, all observed effects of both groups of antagonists on fitness (as in the native *T. pratensis*, for example), with one exception, were present at low frequency and disappeared at high frequency. The almost complete absence of antagonistic effects at high frequency might be explained by increasing intraspecific competition, suggesting that other factors, such as competition for resources, rather than responses to antagonists explain niche differences that result in the observed negative frequency-dependence (McKane et al. 2002, Adler et al. 2007). However, as soil conditions at our

study site were relatively poor, the contribution of antagonistic interactions to niche differentiation and ultimately species coexistence could be different in more fertile soils. For example, Griffin et al. (2016) showed that the impact of foliar bacteria on seedling performance is crucially dependent on soil concentrations of sodium, nitrogen and potassium. High nutrient availability can also lead to communities with a large niche overlap among plant species (Price et al. 2014), thereby potentially increasing the impact of antagonistic interactions on community members.

No difference between exotic and native species in their response to antagonists

In contrast to our hypothesis, exotic plant species did not show a consistently different response to antagonists compared to native species. The negative effect of insecticide application on per capita rate of increase in the exotic *D. giganteus*, only occurring at low frequency, might be the result of destabilizing positive feedbacks between this species and the local insect herbivores (Turchin 1989, von Holle and Simberloff 1999, Hambäck and Englund 2005). These feedbacks may lead to a disproportionate favoring of conspecific individuals over heterospecific individuals by increasing fitness differences among species. This could happen either directly, e.g. via allelopathy, or indirectly, e.g. by promoting antagonists that have more negative effects on other community members than on the species itself, and has been frequently shown to occur in exotic plant invasions (van der Putten et al. 2013, Crandall and Knight 2015). However, this potentially destabilizing effect was not present at the community level, as evenness of exotic-dominated communities did not differ from that of native communities. This result also contrasts with our hypothesis 4 (Fig. 3.2), that predicts a smaller effect of antagonist reduction on fitness at high frequency in exotic species, as we found no differences in per capita rates of increase at high frequency among insecticide-treated and control plots. In contrast, fungicide application influenced the fecundity, a different fitness component, of *D. giganteus* in a negative frequency-dependent manner, suggesting that *D. giganteus* is more affected by fungal pathogens than by insect herbivores. Known fungal pathogens on *D. giganteus* are distributed, at least, across the northern hemisphere (USDA Fungus-Host distribution database, Farr and Rossman 2019) and co-introduction of ubiquitous and generalist pathogens in exotic plant invasions is rather common (Parker and Gilbert 2007, Blaisdell and Roy 2014). These opposing responses to fungal pathogens and insect herbivores in a positive and negative frequency-dependent manner highlight the importance of different antagonist groups in their contribution to introductions of exotic species (Lombardero et al. 2012, Kirichenko et al. 2013).

The reduction in per capita rate of increase of the exotic *P. peregrina* at low frequency and when antagonists were reduced, suggests that the competitive advantage through enemy release only

operates when this species is rare and supports our hypothesis 4, as it shows no significant effect of antagonist reduction at high frequency (see Fig. 3.2, Hierro et al. 2005). The contrasting responses of proportion of flowering individuals in this species (higher when antagonists were reduced) did not translate into the same patterns in per capita rate of increase, which indicates that differential contribution of fitness components to population growth is common (Jorritsma-Wienk et al. 2007, also see above). Furthermore, the presence of more close relatives of *P. peregrina* in our community (three members of the same family, among them one congeneric species), compared to the other exotic species, *D. giganteus* (one member of the same family), may confound the relationship between a species' frequency and the levels of pathogen infection and insect herbivory it experiences (Darwin 1859, Parker et al. 2015, Cadotte et al. 2018). To summarize, we did not find consistent differences between exotic species and responses of native species in terms of their responses to antagonists. This was not only true for fitness of those species that were investigated in detail, but also for biomass responses of all target species considered in our experiment.

Conclusion

Our study shows that native and exotic-dominated communities may not differ in their response to fungal pathogens and insect herbivores, and thus, that enemy release is unimportant for the establishment of exotic species at the community level in our study system. Instead, we suggest that our results indicate that either effects of enemy release are transitory (Brändle et al., 2008) or that there is no enemy release at all (Parker and Gilbert 2007). Although we find that negative frequency-dependence is common in our species, suggesting a stabilizing effect of niche differences on species coexistence, it was rarely linked to antagonistic interactions. Furthermore, effects of both antagonist groups were only present at low plant frequencies, which may highlight the importance of competition for resources in our system (McKane et al. 2002, Adler et al. 2007). For both exotic species, i.e. *D. giganteus* and *P. peregrina*, our findings show contrasting responses to both groups of antagonists, which implies that these species are differentially affected by fungal pathogens and insect herbivores. As a result, these varying effects at species level may offset each other and explain that there is no effect at the community level, pointing to the potential importance of equalizing mechanisms in our system (Chesson 2000, Gross et al. 2014). Furthermore, as our results suggest that both, equalizing and stabilizing mechanisms, are present in our study system, both mechanisms may in parallel promote species coexistence in native and exotic-dominated communities (Kandlikar et al. 2019).

By reducing both above- and belowground antagonists we were able to show the complete effect of two important antagonist groups, in contrast to studies which exclude only parts of antagonist

groups (Allan et al. 2010). Our experimental approach is, to our knowledge, one of the few experiments rigorously testing to which extent release from natural enemies contributes to invasion success of exotic species. Nonetheless, our study could be expanded by considering more invasive exotic species and other important plant antagonists in grasslands (e.g. molluscs, vertebrates). Finally, future studies should consider species coexistence in the context of multiple drivers of global change, which will have a large impact on community dynamics in grasslands (Valladares et al. 2015).

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4

Impacts of fungicide application and plant species on soil fungi

This chapter is in preparation for publication as: Schmidt R, Auge H, Deising HB, Hensen I, Lentendu G, Schädler M, Wirsel S, Wubet T: Impact of fungicide application, plant species origin and identity on soil fungi community composition.

4.1 Abstract

Fungicide application has effects on non-target organisms and communities that are often not well understood. In ecological experiments, these compounds are employed to study the importance of fungi for the dynamics of natural ecosystems, e.g. how they interact with invasions of exotic species and how plant community composition influences fungal communities. We conducted a study in experimental grasslands in central Germany composed of native and exotic plant communities. In these grasslands, the impact of fungicide application, plant species origin and identity of the most abundant plant species in the community on the diversity and composition of soil fungi was analyzed by an ITS rDNA pyrotaq sequencing approach. Fungicide application had a negative effect on the β-diversity of soil fungi while α-diversity remained unchanged, showing that fungal species turnover between plots was reduced and the same taxa survived on all plots. We found that α-diversity and β-diversity of soil fungal communities were not affected by plant species origin. The identity of the most abundant plant species affected fungal community composition in few cases. Decreases in β-diversity of soil fungi by fungicide application and identity effects of plant species on fungal community composition were mainly driven by changes in abundances of pathotrophic fungi while saprotrophic fungi showed a weaker, but still significant, response. Our study shows that plant species origin does not influence the composition of soil fungal communities, possibly due to co-introduction of fungi with their hosts and/or transience of enemy release. The rarity of dissimilarities between soil fungal communities of different plant species may be due to phylogenetic relatedness of individual plant community members. In general, our results support previous findings that fungicides are able to affect soil fungal communities of natural plant communities and that these effects should be taken into account when environmental impacts of fungicide use are assessed or when fungal exclusion experiments are conducted.

Keywords: Fungicide application, enemy release hypothesis, soil fungi communities, exotic species, plant species identity, plant species relative abundance

4.2 Introduction

Conventional agriculture depends on the use of synthetic pesticides like broad spectrum fungicides (henceforth simply referred to as 'fungicides') that are applied to control fungal diseases of crops. However, fungicide application has been shown to exert harmful non-target effects on human and environmental health (Heusinkveld et al. 2013, Pirozzi et al. 2016, Santísima-Trinidad et al. 2018) and is controversially discussed (Deising et al. 2017, Meemken and Qaim 2018). Environmental exposure to fungicides may result from poor adherence of users to environmental standards, wind drift, the persistence of many fungicidal agents and their toxicity to a broad range of organisms (Zubrod et al. 2019, Halbach et al. 2021). Specifically, non-target organisms in agricultural soil communities have been found to experience DNA damage, reductions in enzyme activity, growth, survival and reproduction due to exposure to fungicides (Carniel et al. 2019, Ma et al. 2019). Moreover, how these chemical compounds, developed to control fungal diseases in agricultural crops, influence soil fungal communities in the rhizosphere of natural ecosystems remains understudied (Köhler and Triebskorn 2013, Zubrod et al. 2019). Studies conducted so far found increased stress indices in microbial communities under fungicide application (Sulowicz et al. 2016). Only few studies have so far used high-throughput sequencing to quantify effects on the structure and diversity of soil fungal communities. They found that α-diversity of soil fungal communities in natural plant communities responded stronger to fungicide application than those of agricultural soil (Shi et al. 2019). Soil fungi are crucial for various ecosystem functions, like decomposition and nutrient cycling, engagement in symbiotic interactions such as mycorrhiza and as causal agents of plant diseases (Deacon 2006). Thus, it is imperative to understand how fungicides affect soil fungi of natural plant communities and how these effects influence biotic interactions and ecosystem properties.

Apart from their designated use in agriculture, commercial fungicides are often used in ecological experiments: Excluding fungi from experimental plots using pesticides enables ecologists to study the importance of fungi for dynamics of natural ecosystems (Parker and Gilbert 2007, Allan et al. 2010, Bagchi et al. 2014, Schmidt et al. 2020b). Such experiments have shown that soil fungi can maintain plant diversity and contribute to the positive diversity-productivity relationship in grasslands (Maron et al. 2011, Schnitzer et al. 2011, Mommer et al. 2018, Semchenko et al. 2018). Furthermore, feedbacks between soil fungi and plants are known to contribute to the negative frequency-dependent population growth that is necessary to generate stable coexistence (*sensu* Chesson 2000) in plant communities (Janzen 1970, Connell 1971, Mangan et al. 2010, Chung and Rudgers 2016, Stein and Mangan 2020). Interactions between plants and soil fungi have also been

in the focus of experiments studying the contribution of soil microbes, most frequently pathotrophs, as potential drivers of plant invasions (Bever et al. 2015, Dawson and Schrama 2016). Understanding the role of soil fungi in plant invasions has made remarkable progress in the past two decades (Kulmatiski et al. 2008, Dawson and Schrama 2016). There is evidence that exotic plants are often released from their natural soil-borne enemies (Mitchell and Power 2003, Kulmatiski et al. 2008, but see van Kleunen and Fischer 2009). Alternatively, they may encounter avirulent strains of pathotrophs that are virulent in the species' native range (Reinhart et al. 2010). The release from pathotrophs may lead to weaker feedback effects (Klironomos 2002) and/or evolution of increased competitive ability of exotic plant species (Maron et al. 2004), e.g. via developing novel allelopathic and defensive compounds (Zheng et al. 2015). However, there is only little support from advanced molecular methods, such as high-throughput sequencing, to single out the role of different fungal trophic modes in the invasion process (Dawson and Schrama 2016, but see Gundale et al. 2016).

While invasive plants are able to cause rapid shifts in soil community structure and diversity (Hawkes et al. 2005, Gibbons et al. 2017), effects of invididual abundant plant species on the overall composition of the soil microbial community are inconclusive and hard to quantify (Leff et al. 2018). While studies found that functional characteristics of abundant plant species can be important determinants of soil microbial communities in grasslands (Bardgett et al. 1999, Lentendu et al. 2011), there seems to be no general pattern predicting relationships between individual plant species and their specific soil microbial communities (Berg and Smalla 2009, Lucero et al. 2020). Moreover, although there is evidence for changes in soil microbial composition through shifts in plant community composition on landscape (de Vries et al. 2012) and global scales (Prober et al. 2015), it remains unclear if these effects are caused by locally abundant species (Leff et al. 2018).

We set up a field experiment in a grassland in Central Germany and used fungal ITS high-troughput sequencing to (1) better understand the effects of fungicide application in an ecological context, and to (2) quantify the influence of plant species origin, i. e. native or exotic, and abundant plant species on soil fungal communities. We addressed the following hypotheses:

i) Fungicide application will decrease soil fungal diversity and abundance and alter the composition of non-target soil fungal communities.
ii) Exotic plants accumulate fewer pathotrophs in their soil community compared to native plants.
iii) Soil fungal community composition is affected by the identity of the most abundant plant species.

4.3 Methods

Study site

The field experiment was located approximately 20 km west of the city of Halle in central Germany (State of Saxony-Anhalt, 51°29'N 11°49'E, 119 m above sea level). The area is characterized by a sub-continental climate with an average annual precipitation of 488 mm and an average annual temperature of 9.0 °C (German Meteorological Office, DWD). The former arable field site was used as an occasionally mulched grassland since 1992. The soil is a shallow rendzina with marl and characterized by high amounts of clay (16.48%), carbonate (14.60%), magnesium (1.20%), calcium (6.73%) and relatively low amounts of organic carbon (1.74%) and nitrogen (0.17%, C/N ratio = 10.49, Schubert 2010). Soil reaction is neutral (pH = 7.26). The vegetation before setting up the experiment consisted of a mix of semi-dry grassland and ruderal species, interspersed by shrubs and small trees. This vegetation was removed by plowing in fall 2013, six weeks before sowing, in order to create similar starting conditions for the addition of native and exotic (henceforth termed 'plant species origin') seed mixtures. The field site was fenced as protection against naturally occurring wild boars (*Sus scrofa*), and had an area of ca. 3000 m².

Experimental design

Although this experiment was part of a larger experiment, studying the effect of herbivorous insects in addition to fungal pathotrophs, we focus here only on the treatments relevant for the questions raised above and ignore insecticide-treated plots. For a full description of the experiment, including information on the insecticide treatment, see methods and appendices in Schmidt et al. (2020b).

On the experimental site, we established 120 plots of 1 m² size, arranged in five blocks of 24 plots, in November 2013. Plots were spaced at a distance of 1.5 m from each other. Distance between blocks varied between 1.5 m and 5 m. We selected six native plant species (*Arrhenatherum elatius*, *Lotus corniculatus*, *Medicago falcata*, *Tragopogon pratensis*, *Hypericum perforatum*, *Falcaria vulgaris*) as typical species of semi-dry to moist central German grasslands and six exotic plant species (*Lolium multiflorum*, *Onobrychis viciifolia*, *Medicago* x *varia*, *Pimpinella peregrina*, *Dianthus giganteus*, *Bunias orientalis*), for being naturalized exotic species in grasslands of Germany, with the exception of *P. peregrina*, that is considered to be a casual close to naturalization (floraweb.de, Bundesamt für Naturschutz 2019). Most of these species are perennials, except for *T. pratensis*, which is either biennal or perennial, and *L. multiflorum*, which is either annual, biennial or perennial (floraweb.de, Bundesamt für Naturschutz, 2019). Focal species were selected in such a way that each species origin contained

one grass, two legumes and three non-legume forbs, thereby reflecting the proportions of functional groups in natural grasslands but also being limited by the rarity of exotic grasses in Central Europe (Floraweb.de, Bundesamt für Naturschutz 2019). In addition to these focal species, a random sample of four out of ten "matrix species" was added to each plot at low abundance in order to assemble species-rich grassland communities. The identity of these four matrix species was the same within each block, but varied among the five blocks. Likewise, we allowed spontaneous establishment of additional species from the local species pool, to let the emerging communities form a vegetation as natural as possible.

Within each of the five blocks, 12 randomly selected plots were sown with a seed mixture containing the six native plant species, and 12 plots were sown with a seed mixture containing the six exotic plant species. Each of the plant species was sown at high frequency on two of the 12 plots, while the other five plant species were sown at low frequency on the same plots. Hence, a high frequency plot for a particular plant species (henceforth termed 'abundant species') at the same time represented a low frequency plot for the other five plant species (henceforth termed 'rare species'), and vice versa. Plant species were either sown at 2.4 g seeds m^{-2} for abundant species and 0.04 g seeds m^{-2} for each rare species. To ensure an equal dispensation of the seeds across the plot, 50 g of quartz sand was added to each seed mixture prior to sowing. Seed mixtures were filled in 50 ml Falcon® tubes and dispensed manually across the whole plot, as evenly as possible. Sowing took place in December 2013. Plots were not weeded. Seeds of focal and matrix species were either self-collected or purchased from local seed traders from regional (within a distance of <100 km around the experimental site), wild-growing populations (one to four sampled populations). Seeds of all legume species where scarified with fine sandpaper prior to sowing.

Within each block, the two high frequency plots of each plant species were randomly assigned to two fungicide application treatments: no application of fungicide ('control') and fungicide application. The resulting 24 treatment combinations (2 species origins × 6 abundant species (nested within species origin) × 2 fungicide application treatments) were replicated five times according to the number of blocks. See also Appendix 7.2.2 in Schmidt et al. (2020b) for a schematic overview of the treatment structure.

Fungicide application

In order to exclude fungi we used the commercially available fungicides Acrobat® WG (Dimethomorph and Mancozeb) and a combination of Champion® (Boscalid and Epoxiconazol) and Diamant® (Pyraclostrobin, Epoxiconazol and Fenpropimorph). We chose this combination of fungicides to control a broad range of potential pathotrophs in our experiment, which was

designed to study the effect of pathotrophic fungi on the coexistence of plant species in natural communities. The fungicides were applied alternately for the same number of times per vegetation period: every three weeks, starting in April and ending in September, in concentrations according to the instruction manuals. For reasons of practicability, each plot received the respective amount of the fungicide stock solution (according to the instruction manual), diluted with tap water at a final volume of one liter. Control plots received the same volume of tap water. For application, we spread the liquid evenly across the plot using a watering can. This procedure assured that all aboveground plant parts were sprinkled with the fungicide solution. Due to the systemic nature of all used fungicides and as the fungicide solutions penetrated the soil, we ensured to reduce both above- and belowground fungi. For detailed information on the applied fungicides see Table 4.1.

Soil sample collection

Soil samples were collected from each fungicide-treated and each control plot in late November and early December 2014, i.e. after one season of fungicide application. On each of these 120 plots, four soil cores with a diameter of 1 cm were taken from the upper 10 cm of soil. Living plant parts and plant litter were removed before taking the soil cores. The four samples of each plot were pooled immediately after collection and homogenized. Of the pooled soil samples, aliquots of 50 g were stored at -80°C for further molecular analysis.

DNA Extraction, Amplicon Library Preparation and Pyrosequencing

Microbial genomic DNA was extracted from each pooled soil sample using a MO BIO Power Soil DNA isolation kit (MO BIO Laboratories, Carlsbad, CA, USA) following the manufacturer's protocol. DNA concentrations were quantified using a NanoDrop UV-Vis spectrophotometer (Peqlab Biotechnologie GmbH, Erlangen, Germany). The fungal ITS rDNA barcode region was amplified using custom ITS1F primers (Gardes and Bruns 1993) containing Roche 454 pyrosequencing adaptor B and the universal primer ITS4 (White et al. 1990) containing Roche 454 pyrosequencing adaptor A and a sample-specific multiplex identifier (MID). The PCR reactions were performed as described previously (Wubet et al. 2012), in a total volume of 50 µl reaction mix containing 1 µl DNA template (7–15 ng), 25 µl Go Taq Green Master mix (Promega, Mannheim, Germany) and 1 µl of a solution containing 25 pmol of each of the ITS region-specific primers.

All samples were amplified in triplicate and purified using a Qiagen gel extraction kit (Qiagen, Hilden, Germany). DNA concentrations were then measured using a fluorescence spectrophotometer (Cary Eclipse, Agilent Technologies, Waldbronn, Germany) and the samples

were pooled to yield equimolar representation of each. Unidirectional pyrosequencing from the ITS4 end of the amplicons was performed using a 454 Titanium amplicon sequencing kit and the Roche GS-FLX + 454 pyrosequencer (Roche, Mannheim, Germany) at the Department of Soil Ecology, Helmholtz Centre of Environmental Research (UFZ, Halle, Germany). The raw ITS rDNA sequences were deposited at the NCBI BioSample database (SRA number PRJNA694534).

Bioinformatic analyses

Sequence processing and quality filtering were conducted applying an in-house developed metabarcode analysis pipeline for grid engines mainly based on MOTHUR (version 1.39.5, Schloss et al. 2009) and OBITools (version 1.2.11, Boyer et al. 2016) software suites. Sequences with more than one barcode mismatch or more than six primer mismatches were removed. All primer and barcode sequences were discarded. Sequences with a read length of less than 335 nucleotides, an average quality score lower than 30, any ambiguous bases or homopolymers exceeding 12 nucleotides were removed. Flows were denoised and reads were trimmed using FlowClus (Gaspar and Thomas 2015) to a uniform ITS2 rDNA read fragments of 335 nucleotides long. Chimeric reads were detected and removed from each sample using the UCHIME algorithm as implemented in MOTHUR (Edgar et al. 2011). Dereplicated quality-filtered sequences were clustered into operational taxonomic units (OTUs) based on the vsearch algorithm (version 2.4.4, Rognes et al. 2016) with a sequence similarity threshold of 97%. Representative sequences for each OTU (the most abundant sequence in each OTU) were taxonomically assigned based on the reference sequences from the UNITE database (version v7.2, dynamic clustered, Kõljalg et al. 2013) using the naïve Bayesian classifier (Wang et al. 2007), as implemented in MOTHUR, at a consensus threshold of 60%. The sequences identified as of fungal origin were further classified against the UNITE database including singletons in order to improve taxonomic annotation and to detect non-target OTUs. Putative functions were annotated using the FUNGuild fungal database (version 1.1, Nguyen et al. 2016).

Statistical analyses

The statistical analyses were conducted in R version 3.6.2 (R Development Core Team 2019). Data pre-processing and analysis was mainly performed using the phyloseq package (McMurdie and Holmes 2013). The per sample read count was normalized to the sample with the lowest read counts using the 'rarefy_even_depth' function. As fungal α-diversity measures OTU richness, Shannon diversity index, and Pielou's evenness were estimated. Shannon diversity index was calculated as $H = \sum(P_i \times \ln P_i)$ and Pielou's evenness was calculated as $J = \sum(P_i \times \ln P_i)/\ln S$, where

P_i is the proportional cover of species *i*, and *S* is the species richness of the particular plot. All α-diversity measures were tested for normality and homogeneity of variance using the Shapiro-Wilk test in R. The impact of fungicide application and origin of the abundant plant species on the fungal α-diversity measures was tested using the function 'compare_means' of the ggpubr package (Kassambara 2020) and the pairwise.adonis function of the pairwiseAdonis package (Arbizu 2017). The effect of fungicide application on the fungal β-diversity was assessed by permutational multivariate analysis of variance (PERMANOVA) with 999 permutations using the function 'adonis' of the vegan package (Oksanen et al. 2019) using Bray-Curtis distances. Relationships of the fungal communities with fungicide treatment and abundant species were visualized using Principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity index using the function 'ordinate' in the phyloseq package. Pairwise comparisons of the effects of fungicide treatment and abundant species on the fungal community were tested using the 'pairwise.adonis' function of the package pairwiseAdonis and p-values were adjusted for the false discovery rate (FDR) using the method of Benjamini and Hochberg (Benjamini and Hochberg 1995). Indicator species analysis was used to identify OTUs specific to fungicide treatment of exotic and native plant species using the *indicspecies* package ver. 1.7.9 (De Cáceres and Legendre 2020), where OTUs with $P < 0.05$ were considered as indicator taxa.

Table 4.1 Overview of the applied fungicides. All fungicides were manufactured by BASF SE, Ludwigshafen, Germany.

Trade name	**Fungicidal agent**	**Compound class**	**Amount applied**	**Mode of action**
Acrobat® WG	Dimethomorph	Cinnamic acid amides	90 g/kg	Cellulose synthase inhibitor

	Mancozeb	Dithiocarbamates	600 g/kg	Multi site activity
Champion®	Boscalid	Carboxamides	233 g/l	Succinate dehydrogenase inhibitor
	Epoxiconazol	Azoles	67 g/l	Sterol biosynthesis inhibitor
Diamant®	Pyraclostrobin (F500®)	Strobilurines	114 g/l	Quinone outside inhibitor
	Epoxiconazol	Azoles	43 g/l	Sterol biosynthesis inhibitor
	Fenpropimorph	Morpholines	214 g/l	Sterol biosynthesis inhibitor

4.4 Results

Sequence quality control and characterization of soil fungal community

Of the 120 soil samples, a total of 388,425 DNA sequence reads were obtained. After sequence quality filtering and normalization, 340,793 reads remained, representing 2,840 reads per sample on average and ranging from 1,006 to 4,771 reads per sample (see Appendix 7.3.1 for rarefaction curves). Further removal of 3,370 potentially chimeric and 5,197 non-fungal sequences resulted in 332,226 fungal reads, clustered into 2,388 fungal OTUs (Figures 4.1 and 4.2), of which 1,039 were abundant (represented by at least four reads) and used for analyses (with a total of 330,544 reads). Taxonomic assignment revealed that Ascomycota represented the most diverse fungal phylum at our study site, followed by Basidiomycota, Glomeromycota, Mortierellamycota, Chytridiomycota and Rozellomycota (Figures 4.1a, c). Ascomycota were also the most abundant fungi, followed by Basidiomycota, Mortierellamycota, Chytridiomycota, Glomeromycota and Rozellomycota (Figures 4.2a, c). Saprotrophic fungi were the most diverse and abundant trophic group, followed by mixed trophy, symbiotrophs and pathotrophs (Figures 4.1b, d and Figures 4.2b, d). The majority of the detected fungal OTUs were assigned to the major taxonomic levels of class (903 OTUs, 86.9%), order (806 OTUs, 77.6%), family (664 OTUs, 63.9%) and genus (556 OTUs, 54.5%). Mycorrhizal fungi had to be excluded from further statistical analysis, because of a low sample size. This may be due to poor amplification of arbuscular mycorrhizal fungi by the primers we used in our study (Tedersoo et al. 2015, 2018).

Effects of fungicide application, plant species origin and abundant plant species on α-diversity of soil fungi communities

The fungal α-diversity measures (i.e. OTU richness, Shannon diversity index and Pielou's evenness) did neither differ significantly between fungicide-treated plots and control plots, nor between native plots and plots dominated by exotic plant species (Fig. 4.2). Similarly, the interaction of fungicide application and plant species origin did not show any effect on α–diversity measures (Appendix 7.3.2).

OTU richness, Shannon diversity index and Pielou's evenness of soil fungal communities did differ among abundant plant species (Fig. 4.3). However, we found no differences for these measures of fungal α-diversity between fungicide-treated and control plots across abundant plant species, suggesting that fungicide application had, surprisingly, no effect on the mean species diversity (Fig. 4.4 and Appendix 7.3.3).

Effects of fungicide application, plant species origin and abundant plant species on β-diversity of soil fungal communities

PERMANOVA showed that fungicide application had a significantly decreasing effect on the β-diversity of soil fungal communities, while plant species origin showed no effect (Table 4.2). However, PCoA revealed that only 18.8 % of variance observed in β-diversity can be explained by the first two axis (Fig. 4.5A). With respect to the fungal trophic modes analyzed, the proportion of variance explained by the first two axes varied between 29.6% for pathotrophic fungi and 25.5% for saprotrophic fungi (Fig. 4.5B and C).

PERMANOVA revealed a significant effect of the most abundant plant species on OTU community composition, which was largely driven by a change in OTU abundance of pathotrophs (Table 4.2). Consequently, PCoA showed that fungal community composition differed between abundant plant species, and, furthermore, also between fungicide-treated and control plots of certain plant species, especially legumes and the exotic forb *D. giganteus* (Appendices 7.3.4A-7.3.4C). Pairwise species comparisons after PERMANOVA revealed that differences in OTU abundances existed between soil fungi communities associated with different abundant plant species, but were rare (8 out of 66 comparisons or 12%, see Table 4.3). Notably, all of these eight significant comparisons included either *Arrhenatherum elatius* or *Bunias orientalis* as the abundant plant species (but not both). These data indicate that soil fungal communities of these two species differed markedly from other species, but not between these two species. In the case of *B. orientalis* it is possible that this result was confounded by poor seed germination, and hence low realized abundance of the species in the field (see Schmidt et al. 2020b). Indicator analysis of all analyzed

2388 OTUs yielded only 34 OTUs specific for native or exotic plant communities subjected to fungicide or control treatments (Table 4.4). Furthermore, we found 22 OTUs that were specific for a particular exotic plant species (Appendix 7.3.5A) and 13 OTUs specific for a particular native plant species (Appendix 7.3.5B).

Table 4.2 PERMANOVA results for the effects of fungicide application, plant species origin, and identity of the most abundant plant species (nested within origin) as well as their interactions on OTU abundances of the whole fungal soil community and for two fungal trophic modes (saprotrophic and pathotrophic fungi). Terms were added sequentially (first to last) to the model and the number of permutations was restricted to 999. The five replications ('block') were treated as strata. We used Bray-Curtis as dissimilarity matrix. Abbreviations for factors are given in brackets. Significant effects are marked in bold. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

		Fungicide application [F]	Origin [O]	F x O	Plant species identity [I]	F x I	Residuals
Whole community	R^2	0.016	0.008	0.007	0.097	0.080	0.806
	F	1.948	0.965	0.859	1.049	0.869	-
	p	**0.002**	0.489	0.741	**0.031**	0.834	-
Saprotrophic fungi	R^2	0.015	0.007	0.008	0.093	0.077	0.815
	F	1.810	0.888	0.960	0.994	0.823	-
	p	**0.034**	0.579	0.468	0.115	0.822	-
Pathotrophic fungi	R^2	0.019	0.009	0.008	0.103	0.074	0.804
	F	2.226	1.100	1.013	1.114	0.807	-
	p	**0.009**	0.361	0.426	**0.033**	0.818	-

Table 4.3 Dissimilarity matrix of the soil fungi communities of 12 grassland plant species, when sown as an abundant species. These pairwise comparisons were conducted after a PERMANOVA analysis. Values shown are F-values. Significant differences between soil fungi communities of two species are marked in bold ($0.01 < \boldsymbol{P} < 0.05$). Species abbreviations: *Arrhenatherum elatius*, *Lotus corniculatus*, *Medicago falcata*, *Tragopogon pratensis*, *Hypericum perforatum*, *Falcaria vulgaris*, *Lolium multiflorum*, *Onobrychis viciifolia*, *Medicago* x *varia*, *Pimpinella peregrina*, *Dianthus giganteus*, *Bunias orientalis*

	Arr_ela	*Lot_cor*	*Med_fal*	*Tra_pra*	*Hyp_per*	*Fal_vul*	*Lol_mul*	*Ono_vic*	*Med_var*	*Pim_per*	*Dia_gig*	*Bun_ori*
Arr_ela	-	-	-	-	-	-	-	-	-	-	-	-
Lot_cor	1.125	-	-	-	-	-	-	-	-	-	-	-
Med_fal	**1.490**	1.072	-	-	-	-	-	-	-	-	-	-
Tra_pra	1.374	1.039	1.081	-	-	-	-	-	-	-	-	-
Hyp_per	**1.573**	0.776	0.746	0.944	-	-	-	-	-	-	-	-
Fal_vul	**1.533**	0.755	0.843	1.119	0.932	-	-	-	-	-	-	-
Lol_mul	**1.509**	1.262	0.922	0.994	0.912	1.401	-	-	-	-	-	-
Ono_vic	0.932	0.886	0.914	0.905	0.954	0.991	0.836	-	-	-	-	-
Med_var	1.347	0.885	0.798	0.825	0.681	1.133	0.841	0.674	-	-	-	-
Pim_per	1.069	1.032	1.120	1.164	1.188	0.829	1.331	0.937	1.002	-	-	-
Dia_gig	1.223	0.653	0.831	0.944	0.708	0.737	1.055	0.846	0.795	1.109	-	-
Bun_ori	1.148	**1.565**	1.078	**1.665**	1.383	**1.537**	1.239	1.102	1.050	**1.533**	1.204	-

Table 4.4 Results of indicator species analysis to identify OTUs specific to soil fungal communities of native vs. exotic plant species subjected to fungicide treatment using the *indicspecies* package (ver. 1.7.9), where OTUs with $P < 0.05$ were considered as indicator taxa. 0 and 1 indicate absence and presence, respectively, of the OTUs in exotic vs. native plant communities with or without fungicide treatment. E: Exotic, N: Native, F-: no fungicide application, F+: fungicide application, P: Pathotroph, SA: Saprotrop, SY: Symbiotroph, na: information not available. See also Appendices 7.3.5A and 7.3.5B.

OTU Nr.	Taxonomy	Trophic mode	E F-	E F+	N F-	N F+	Stat	p-value
0084	*Monodictys* sp.	P-SA	0	0	0	1	0.2927	0.013
0169	Hypocreales	SA	0	0	0	1	0.2666	0.013
0231	*Gymnoascus reessii*	SA	0	0	0	1	0.2466	0.027
0014	*Pyrenochaetopsis* sp.	SA	0	0	1	0	0.2405	0.036
0027	Sordariales	na	0	0	1	0	0.2657	0.020
0040	*Pyrenochaetopsis leptospora*	SA	0	0	1	0	0.2550	0.020
0105	Pleosporales	na	0	0	1	0	0.3347	0.003
0269	Ascomycota	na	0	0	1	0	0.2426	0.045
0388	Ascomycota	na	0	0	1	0	0.2190	0.034
0471	*Phaeosphaeria podocarpi*	na	0	0	1	0	0.2520	0.022
0570	Unclassified fungi	na	0	0	1	0	0.3086	0.016
0622	Unclassified fungi	na	0	0	1	0	0.2611	0.048
0651	Herpotrichiellaceae	na	0	0	1	0	0.3612	0.004
0663	Unclassified fungi	na	0	0	1	0	0.2730	0.009
0321	*Tubaria conspersa*	SA	0	0	1	1	0.2593	0.016
0086	*Fusarium solani*	P-SA-SY	0	1	0	0	0.2703	0.019
0119	Arthoniomycetes	na	0	1	0	0	0.3347	0.001
0239	Glomeromycota	SY	0	1	0	0	0.2290	0.048
0271	Pyronemataceae	na	0	1	0	0	0.2586	0.016
0453	Lasiosphaeriaceae	na	0	1	0	0	0.2680	0.036
0521	*Golubevia pallescens*	na	0	1	0	0	0.2928	0.009
0538	*Mycoarthris corallina*	SA	0	1	0	0	0.3471	0.005
0102	Pyronemataceae	na	0	1	0	1	0.2447	0.044
0129	*Acremonium rutilum*	P-SA-SY	0	1	0	1	0.2243	0.035
0195	*Mortierella exigua*	SA	0	1	1	0	0.2528	0.025
0525	Unclassified fungi	na	0	1	1	0	0.2673	0.030
0056	*Tetracladium marchalianum*	na	0	1	1	1	0.2469	0.040
0137	Ceratobasidiaceae	na	1	0	0	0	0.2462	0.020
0050	*Paraphoma chrysanthemicola*	na	1	0	1	0	0.3219	0.005
0092	*Stachybotrys chartarum*	SA	1	0	1	0	0.2177	0.046
0417	*Funneliformis geosporum*	SY	1	0	1	0	0.2681	0.025
0019	*Gibberella intricans*	P	1	0	1	1	0.2449	0.030
0248	Microascales	na	1	1	0	0	0.2972	0.004
0031	Microascales	na	1	1	0	1	0.2382	0.048

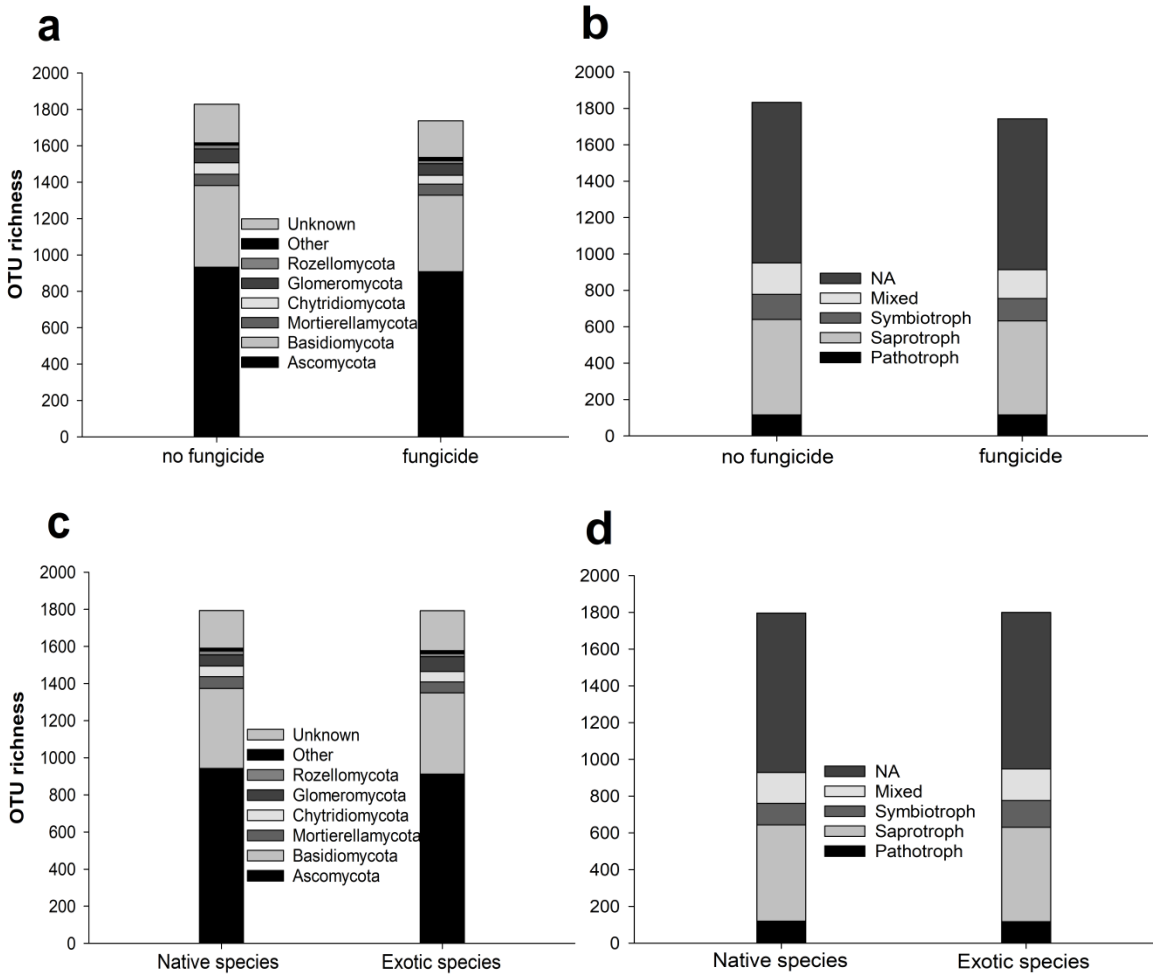

Figure 4.1 Richnesses of soil fungal community OTUs assigned to taxonomic groups (a and c) and trophic modes (b and d), dependent on fungicide application (a and b) and the origin of plant species (c and d). Of all 2,388 fungal OTUs, 1,207 (50.5%) represented Ascomycota, 592 (24.8%) Basidiomycota, 85 (3.6%) Mortierellamycota, 76 (3.2%) Chytridiomycota, 91 (4.1%) Glomeromycota and 24 (1.0%) Rozellomycota. 22 OTUs (0.9%) were assigned to other taxa and 285 (11.9%) OTUs remained unclassified within the kingdom of Fungi. Pathotrophs constituted 149 (6.8%), saprotrophs 691 (31.6%) and symbiotrophs 185 (8.5%) OTUs, while 214 (9.8%) OTUs displayed a mixed trophic mode and for 1,149 (52.5%) reads no trophic mode information was available.

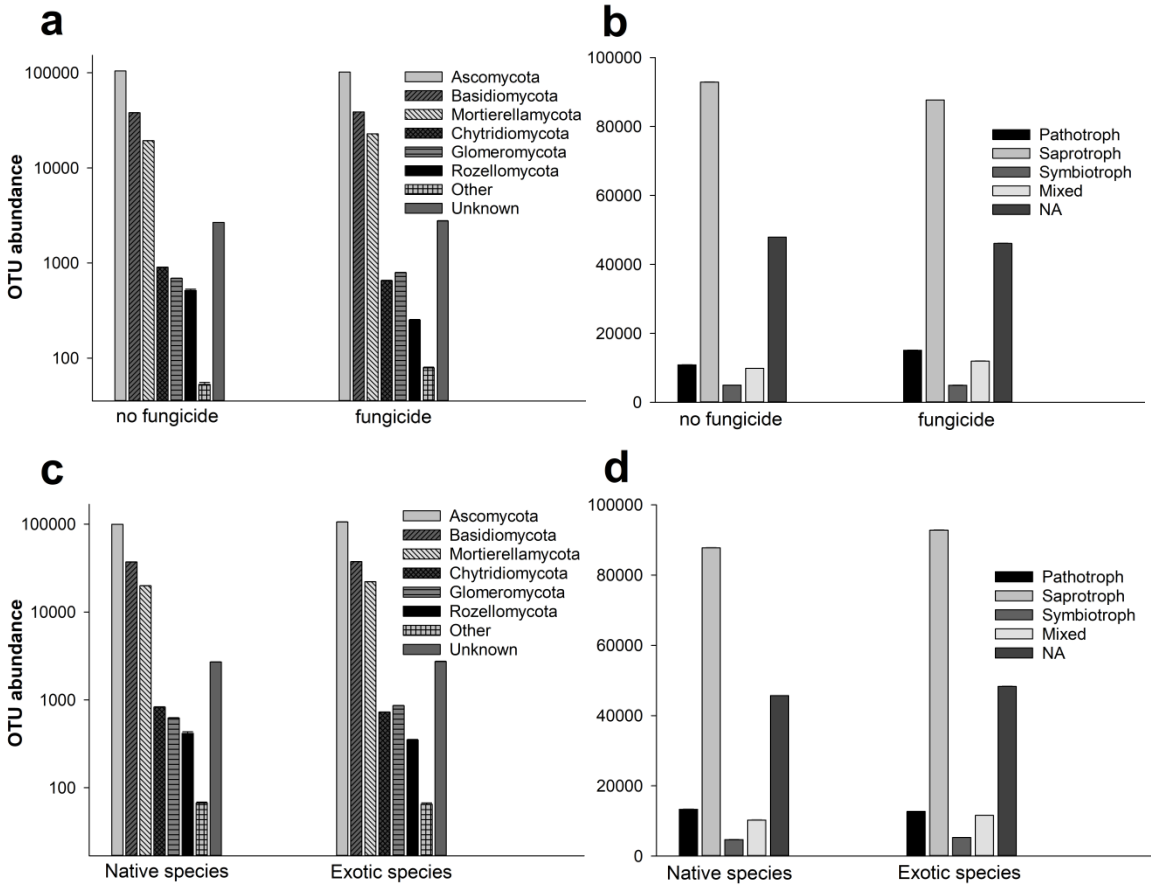

Figure 4.2 Abundances of soil fungal community OTUs assigned to taxonomic groups (a and c) and trophic modes (b and d), dependent on fungicide application (a and b) and the origin of plant species (c and d). Of all 332,226 reads of fungal OTUs, 205,996 (61.6%) represented Ascomycota, 74,711 (23.0%) Basidiomycota, 42,136 (12.6%) Mortierellamycota, 1,554 (0.5%) Chytridiomycota, 1,482 (0.4%) Glomeromycota and 766 (0.2%) Rozellomycota. 132 reads (<0.1%) were assigned to other taxa and 5,332 (1.6%) reads remained unclassified within the kingdom of Fungi. Pathotrophs constituted 25,989 (7.8%), saprotrophs 180,581 (54.0%) and symbiotrophs 9,938 (3.0%) reads, while 21,811 (6.5%) reads displayed a mixed trophic mode and for 93,907 (28.1%) reads no trophic mode information was available. Note the logarithmic scale in a and c.

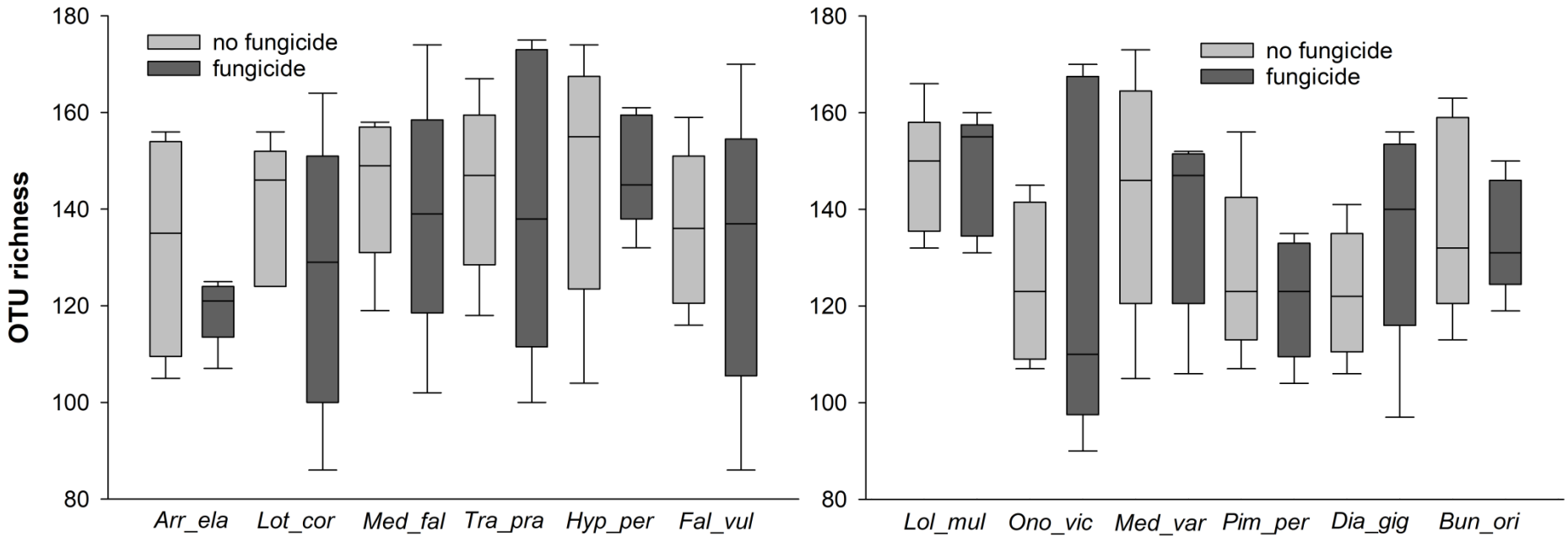

Figure 4.3 Effects of fungicide application on the OTU richness of soil fungal communities depending on the abundant plant species. Boxes represent 25th and 75th percentiles, error bars show 10th and 90th percentiles. The median is indicated by the horizontal line in each box. Every outlier is shown. Results of within-species comparisons using t-tests are not shown because all of these comparisons yielded non-significant results (with $\alpha = 0.05$). N for each species is 10. Because of the low sample size we also conducted a non-parametric Mann-Whitney U test, which yielded a non-significant result as well. Species abbreviations: *Arrhenatherum elatius*, *Lotus corniculatus*, *Medicago falcata*, *Tragopogon pratensis*, *Hypericum perforatum*, *Falcaria vulgaris*, *Lolium multiflorum*, *Onobrychis viciifolia*, *Medicago* x *varia*, *Pimpinella peregrina*, *Dianthus giganteus*, *Bunias orientalis*

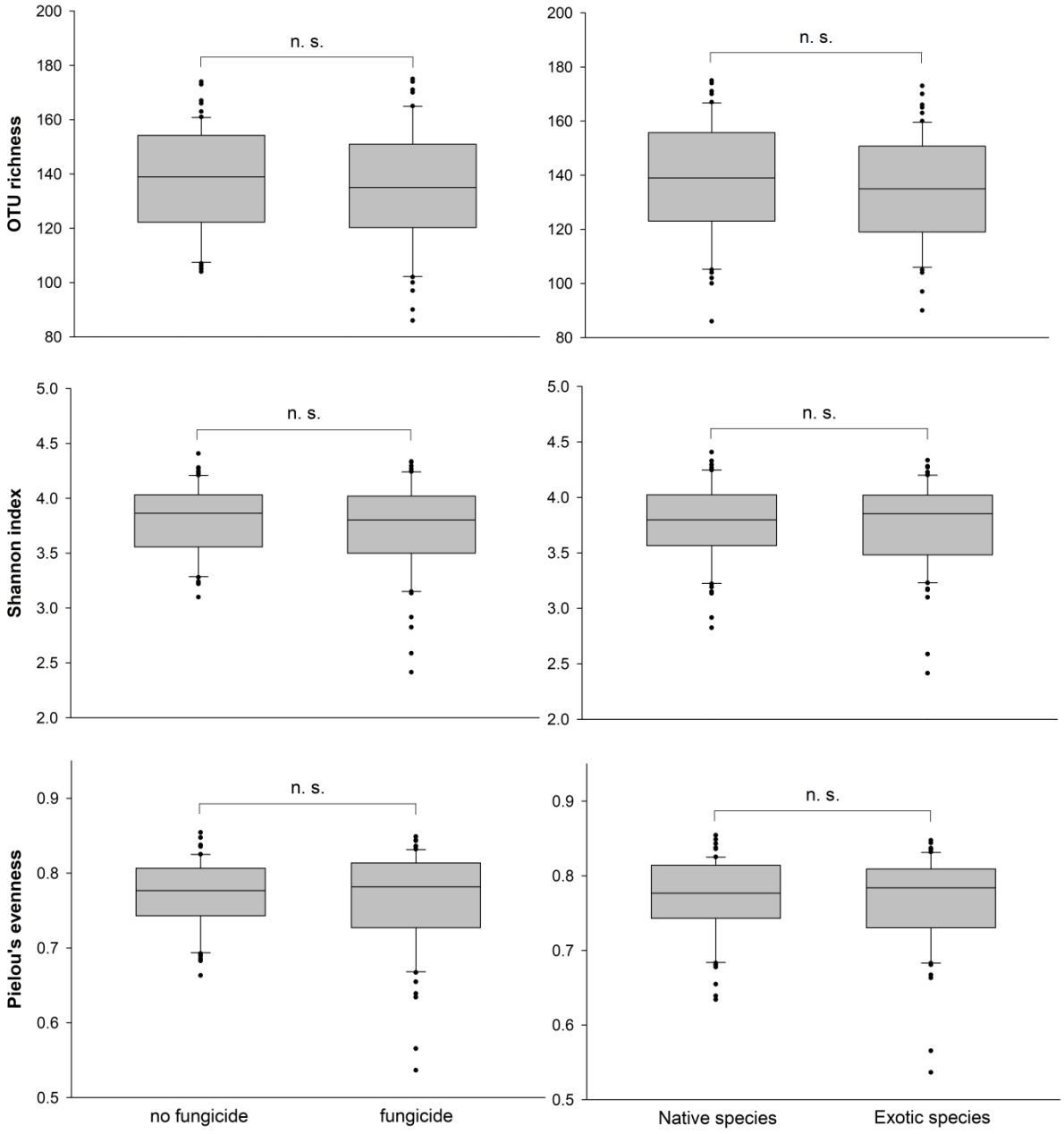

Figure 4.4 Effects of fungicide application (left half of the panel) and plant species origin (right half of the panel) on the α-diversity measures OTU richness, Shannon diversity index and Pielou's evenness of soil fungal communities. Boxes represent 25th and 75th percentiles, error bars show 10th and 90th percentiles. The median is indicated by the horizontal line in each box. Each outlier is shown. $N_{no\ fungicide} = N_{fungicide} = N_{Native\ species} = N_{Exotic\ species} = 60$.

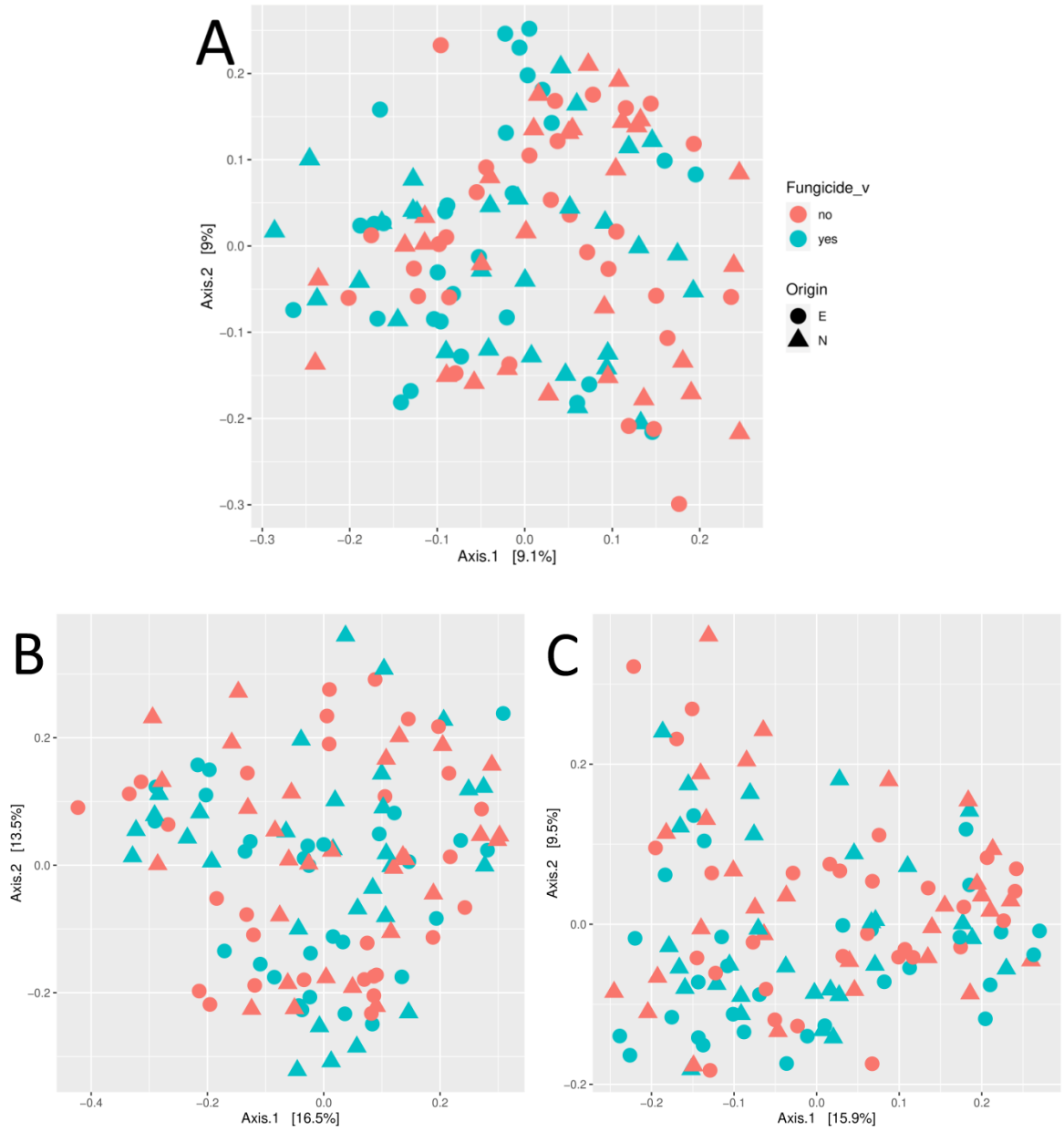

Figure 4.5 Results of Principal Coordinate Analysis (PCoA) for the effects of fungicide application and plant species origin on fungal OTU composition of A) the whole fungal community, B) pathotrophic and C) saprotrophic fungi. E = exotic plant species, N = native plant species.

4.5 Discussion

Using a fungal ITS rDNA pyrotag sequencing approach, our study revealed a decrease in fungal β-diversity of soil fungal communities in experimental grassland communities treated with fungicide, but no such effect on fungal α-diversity (hypothesis i). In contrast, we found no effect of plant species origin (hypothesis ii) and only few effects of single abundant plant species on the soil fungal community (hypothesis iii). These results suggest that soil fungi communities associated with native versus exotic plant species do neither differ in abundance nor composition and respond similarly to fungicide application. Our findings are consistent with results of the same field experiment that showed a positive effect of fungicide application on plant community productivity but no difference in the responses of exotic versus native plant communities (Schmidt et al. 2020b). We found that 86.0% of the OTUs and 85.7% of the reads belonged to either Ascomycota or Basidiomycota that constitute the largest fungal phyla. The primer pairs used in this study are frequently used to amplify the whole fungal ITS rDNA region (Ihrmark et al. 2012, Wubet et al. 2012) because they offer advantages over other molecular markers in terms of high information content and ease of amplification. However, they have appear to favor Asco- and Basidiomycota over other fungal taxa (Goldmann et al. 2015, Schadt and Rosling 2015). Ascomycota contain the majority of all fungal species worldwide (James et al. 2006) and it has been shown to be the most common taxon in grassland soil fungal communities, also when other primer pairs were used (Prober et al. 2015, Egidi et al. 2019).

Our results are supported by studies that found decreases in fungal β-diversity of fungal communities in the rhizosphere of natural ecosystems (Shi et al. 2019) and in the phyllosphere of plants in agricultural ecosystems (Karlsson et al. 2014) resulting from fungicide application. As in these experiments, in our study a low but significant decrease in fungal β-diversity could be attributed to fungicide application. Intriguingly, differences in α-diversity in response to fungicide application were not detected, which may be explained by the frequent application of six different fungicidal agents that addressed targets present in all species and thus simultaneously reduced a broad range of fungi. However, a change in β-diversity occurring without effects on α-diversity implies that fungicide application decreased fungal species turnover between plots, which in turn results in an increased spatial homogeneity of fungal communities. A limited number of fungal OTUs indicative for exotic or native plant species origin and/or fungicide treatment shows that the majority of fungal taxa are shared by all plant species. Interactions between different trophic modes, modulated by single fungicidal agents, may therefore be levelled. This result likely also explains the similar responses of pathotrophic and saprotrophic fungi to fungicide application. In contrast, other studies have shown, that when single fungicides were applied, antagonistic

interactions between different pathotrophic fungi (Birzele et al. 2002, Henriksen and Elen 2005), pathotrophs and saprotrophs (Henriksen and Elen 2005) or pathotrophs and phyllosphere fungi (Newton et al. 2010) can occur. Thus, treatment with single fungicides can lead to increases in pathotroph attack after fungicide application in some cases. For example, when differences in fungicide sensitivity among taxa exist, less sensitive taxa may increase relative to more sensitive taxa as a result of fungicide application (Karlsson et al. 2014). However, populations of pathotrophic fungi may have experienced previous fungicide applications that led to fungicide sensitivity shifts and an advantage in colonizing fungicide-treated plants (Reimann and Deising 2005, Becher et al. 2010, Lucas et al. 2015). Thus, depending on the particular hypotheses to be tested in ecological experiments, ecologists may want to use either a mixture of different fungicides targeting different groups of fungi if the role of the whole fungal community will be studied, or a more specific fungicide if a certain group of fungi should be excluded.

As found in other studies and reviews (Köhler and Triebskorn 2013, Zubrod et al. 2019, Halbach et al. 2021), our results suggest that soil fungal communities in natural ecosystems are susceptible to fungicide exposure, with effects on the community structure of soil fungi. However, exposure to agricultural fungicide application varies greatly among ecosystems, with aquatic ecosystems reported as the most severely affected (Zubrod et al. 2019), while exposure to terrestrial ecosystems like grasslands depends heavily on air concentration of fungicides, wind speed and direction, air temperature and spatial heterogeneity (Klöppel and Kördel 1997).

In our study, soil fungal communities associated with communities of native and exotic plants did not differ significantly in their composition, suggesting that exotic plants host similar pathotrophs, saprotrophs and mycorrhizal fungi as native plants and show, with respect to fungi, no effects of enemy release (Keane and Crawley 2002). This contrasts with results from studies that showed a release from fungal pathotrophs in exotic plants in their introduced range (Mitchell and Power 2003, Agrawal et al. 2005, Uddin et al. 2021) or strong effects of invasive plants on soil microbial communities (van der Putten et al. 2007). However, our findings are in line with studies showing that co-introduction of ubiquitous generalist pathotrophs and loss of rare, mostly specialist pathotrophs, is rather common (Parker and Gilbert 2007, van Kleunen and Fischer 2009, Blaisdell and Roy 2014, Schmidt et al. 2020a), and frequently result in only weak effects on plant population fitness. Other possible mechanisms to explain the observed lack of differences between soil fungal communities of native and exotic plant species include the transience of enemy release via accumulation of natural enemies with increasing time since introduction (Brändle et al. 2008, Mordecai 2011) and effective infection of exotic plants by native pathotrophs (Elton 1958). Especially the former mechanism seems likely to support our findings because the exotic species

we used, with the exception of *D. giganteus*, were introduced to Central Europe more than 200 years ago. Furthermore, we are also aware that only two of our exotic species are considered invasive (Bundesamt für Naturschutz 2019b).

Importantly, our results are also consistent with the findings from the same field experiment, that showed no different responses of native and exotic plant communities to fungicide application even after two and three years of fungicide application. This indicates that, at the community level, plant-soil interactions do not contribute to the observed negative frequency-dependent population growth, an indicator for stable coexistence among species (Schmidt et al. 2020b).

We found that soil fungal β-diversity is influenced by the most abundant plant species and that this effect was present in pathotrophic fungi, but not in saprotrophs. These results are supported by a recent study in temperate grasslands in Europe, which found that soil fungal pathotrophs are often limited to single or few plant species while saprotrophs are rather ubiquitous and less influenced by the identity of abundant plant species (Leff et al. 2018). The significant dissimilarity of soil fungal communities associated with the exotic *B. orientalis* compared to other plant species may be caused by its poor germination. However, the observed differences between soil fungi of native *A. elatius*, which was the most frequent and productive species in the experiment (see Schmidt et al. 2020b), compared to those of four other plant species resembles the reported dissimilarity between soil microbial communities of grasses and those of other plant functional groups, like legumes (Chen et al. 2008). Reasons for different soil microbe interactions among plant functional groups are, for example, differences in plant C/N ratios (Chen et al. 2008) or root exudates (Mathesius 2001) that attract or repel different soil microbes. Interestingly, three of these dissimilarities between soil fungal communities of *A. elatius* and other species occurred in native plant species, suggesting that co-evolution of native plants and pathotrophs, symbionts and saprotrophs may lead to distinct plant-soil interactions and thus, soil microbial communities (Gilbert and Parker 2016, Hoeksema et al. 2018). We observed differences between soil fungal communities in a rather small percentage of the tested species pairs, which supports previous results showing that abundant plant species affect the composition of soil fungal communities less than those of bacteria (Costa et al. 2006, Berg and Smalla 2009). Plant functional traits and phylogeny are often invoked to explain effects of individual abundant plant species. For example, plant species distributions and community diversity are often predictable by plant functional traits (Wardle et al. 2004) and soil microbes are thought to associate with plant species based on these traits (Ben-Hur et al. 2012, Leff et al. 2018). In addition, more closely related plants are known to be associated with more similar soil microbe communities (Bouffaud et al. 2014, Barberán et al. 2015). Our study was not designed to distinguish between these mechanisms, however, as all of

our exotic species, except for *B. orientalis*, had at least one relative from the same family in the experimental plant communities, our results support the latter notion.

Conclusion

Using a high-throughput sequencing approach, our study covers the entire soil fungal community and not only particular fungal trophic modes. We found a significant negative impact of fungicide application on β-diversity but not α-diversity of soil fungi. This underscores previous findings of direct fungicidal effects on soil fungal communities of natural plant communities, and thus, needs to be considered when environmental risks of fungicides are assessed. The inclusion of different plant communities allowed for a general assessment of their effects on soil fungal communities. Notably, the origin of plant species did not influence the soil fungal community, indicating that the effects of enemy release are transitory or non-existent. This assumption is confirmed by the result that dissimilarities among fungal communities were not related to changes in fungal species number, i.e. there were only few fungi exhibiting a specific association to the origin of a plant or to fungicide treatment. The identity of the most abundant plant species had a prominent and significant effect on fungal β-diversity, largely in pathotrophic but not saprotrophic fungi. This identity effect was primarily caused by differences in soil fungi communities of the native grass *A. elatius* to those of other native plants, reflecting dissimilarities of soil fungi communities among plant species groups. Co-evolution of the native plant community with soil fungi, especially pathotrophs may also such identity effects. Future studies should include phylogenetically and trait-based approaches to better understand the effects of fungicide application on fungal communities of natural plant communities. Furthermore, disentangling the mechanisms responsible for the success, or failure, of biological invasions and the effects of individual abundant plant species is an important asset in the context of other drivers of global changes.

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5

General discussion

In my thesis I aimed to investigate the role of interactions between plants and their natural enemies and how these interactions influence species coexistence in plant communities of different origins. Combining an observational study with manipulative experiments, this thesis provides important insights into the mechanisms underlying maintenance of species diversity, multitrophic dynamics of native and exotic plant communities and ecosystem functioning in temperate grasslands. The findings of my thesis illustrate the **differentiated importance of two antagonistic groups for plant coexistence in novel communities of native and exotic species**. The key results of the preceding chapters were as follows (see Figure 5.1 for a graphical summary of the results):

Chapter 2: Plant abundance did not influence pathogen diversity and infection. I found no evidence for the release of exotic plants from pathogens. Ubiquitous generalists dominated the pathogen community of exotic plants. Pathogen diversity and infection was not related to phylogenetic affiliation of plant species.

Chapter 3: Native and exotic-dominated communities did not differ in productivity and diversity. Pesticide application increased plant community productivity independent of plant species origin. Fungicide application reduced diversity and infection of fungal leaf pathogens. Insecticide application reduced diversity and abundances of soil arthropod communities. Negative frequency-dependence across life cycle stages was present in the majority of focal species but not linked to interactions with natural enemies.

Chapter 4: α-diversity and composition of soil fungal communities were affected by the identity of the most abundant plant species. Fungicide application reduced β-diversity and overall abundance of soil fungal communities, but had no effect on α-diversity. Changes in β-diversity and community composition were mainly explained by changes in abundances of pathotrophic fungi. Plant species origin did not influence α- or β-diversity, nor community composition of soil fungi.

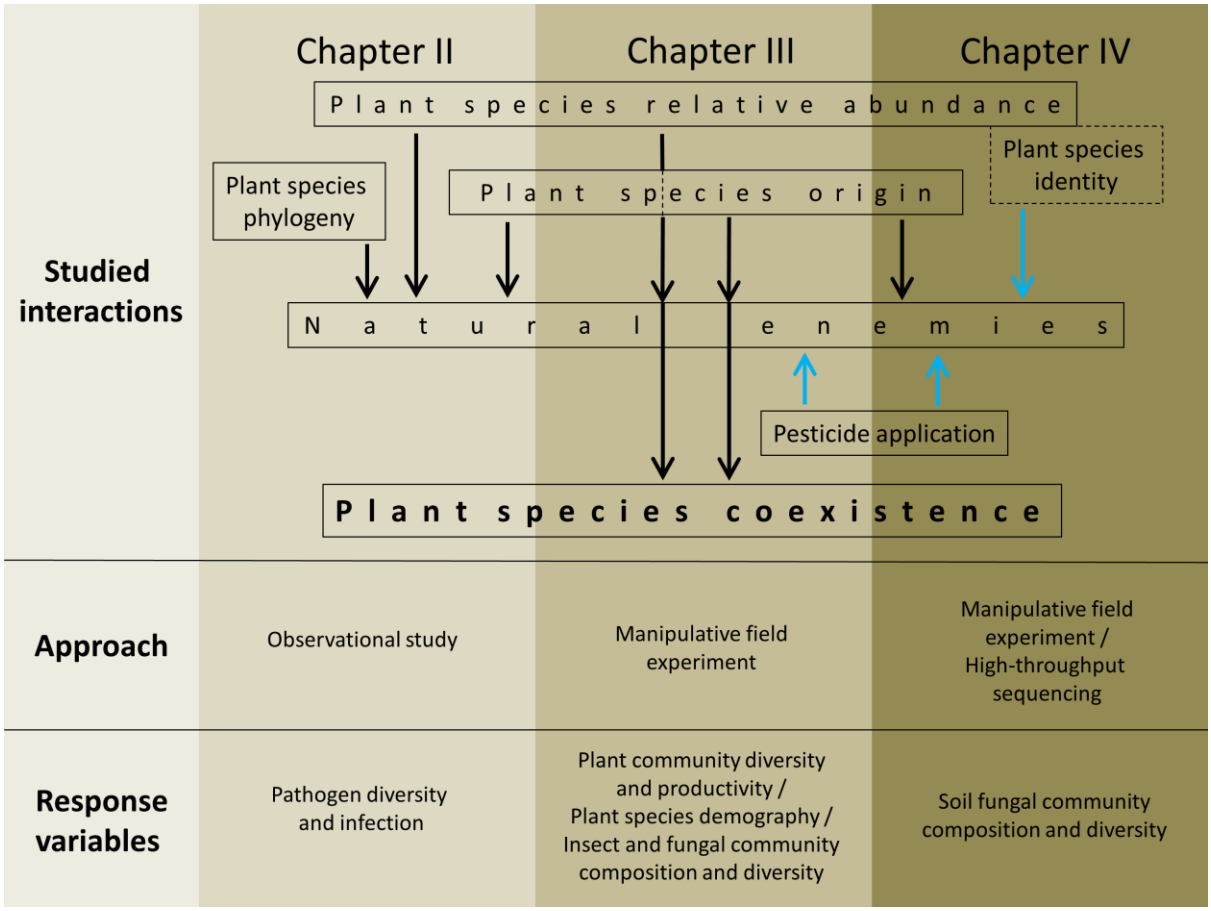


Figure 5.1 Graphical summary of the results of my thesis, based on Fig. 1.3. Colored arrows indicate effects for which I found evidence in my thesis.

5.1 Synthesis

How do the relative abundance, origin and phylogenetic affiliation of plant species influence interactions between plants and their natural enemies?

Pathogen diversity and infection were not related to the abundance of host plants in species-rich grasslands of the central United states (**chapter 2**). Furthermore, in the manipulative field experiment in central Germany, effects of natural enemies on per capita rate of increase were only present at low relative abundances of most plant species and disappeared at high relative abundance. This indicates that interactions among plants on the one hand, and insect herbivores and fungal pathogens on the other hand, were not linked to a plant species' relative abundance in the community (**chapter 3**). These findings are in contrast to studies that showed that accumulation of fungal pathogens and insect herbivores are correlated with and/or caused by high plant abundances (Janzen 1970, Connell 1971, Packer and Clay 2000, Mangan et al. 2010, Bagchi et al. 2014, Parker et al. 2015, Parker and Gilbert 2018). This accumulation of natural enemies with

increasing host abundance creates negative frequency-dependent patterns of population growth, thereby having a stabilizing effect and allowing rare species to persist in the community. In diverse plant communities, such as temperate grasslands, it is known that, due to a lower realized host density, levels of pathogen infection (Mitchell et al. 2002, Keesing et al. 2006, Schnitzer et al. 2011, Rottstock et al. 2014) and insect herbivory (Lau et al. 2008) are frequently reduced (Thrall et al. 2007). Furthermore, species-rich communities often favor generalist pathogens (Power and Mitchell 2004, Spear et al. 2015, Halliday et al. 2017) and insect herbivores (Joshi et al. 2004, Unsicker et al. 2006, Loranger et al. 2014) over specialists. This may unlink the frequency of antagonistic interactions from the relative abundance of individual plant species.

One possible mechanism to explain this pattern is that spillover of generalists across multiple plant species may frequently occur (Power and Mitchell 2004, Halliday et al. 2017) and that herbivore abundance often increases with productivity (Ritchie 2000, Haddad et al. 2001), which in turn increases with plant diversity (Mittelbach et al. 2001, Fraser et al. 2015). Yet, effects of plant species richness on antagonistic interactions are often not direct but, for example, the result of multitrophic interactions between herbivores and their enemies (Root 1973, Unsicker et al. 2006), driving complex herbivory patterns. Nutrient availability can also have large effects on interactions between plants and their natural enemies (Lau et al. 2008, Ebeling et al. 2022), independent of a plant species' relative abundance (Mitchell et al. 2003). Poor soil conditions, such as those at the experimental site in central Germany (**chapters 3** and **4**), are known to limit levels of herbivory (Coupe et al. 2009, Körner et al. 2014) and plant disease (Dordas 2008, Blumenthal et al. 2009, Liu et al. 2017), and thus, enhance the role of competition for resources among plant species. Furthermore, disease transmission rates of particular pathogen species may be intrinsically low or high (Deacon 2006), affected by the identity of neighboring plants (Ampt et al. 2018), modified by landscape characteristics (Moore and Borer 2012) or depend on a vector (Hogenhout et al. 2008, Cronin et al. 2010). To a certain degree, all of these factors would make transmission of these pathogens irrelative of the abundance of their host species.

Native and exotic plant species did not differ consistently in the magnitude of pathogen infection (**chapter 2**), in their response to a reduction of fungi and insects (**chapter 3**) nor in the composition of their soil fungal communities (**chapter 4**). These results together suggest that there is no release of exotic species from their natural enemies, yet may have multiple reasons:

- First, the advantages of enemy release for exotic plants could be transitory and natural enemies accumulate with time since their introduction (Hawkes 2007, Flory and Clay 2013). This has been shown for several groups of plant antagonists, including pathogens

(van Kleunen and Fischer 2009, Mitchell et al. 2010, but see McGinn et al. 2018) and insect herbivores (Brändle et al. 2008).

- Second, enemy release could be non-existent in my investigated grasslands, because of the confounding presence of close relatives of the exotic species (**chapter 3**, Darwin 1859, Mack 1996, Harvey et al. 2012, Cadotte et al. 2018) that facilitate spillover events (Parker et al. 2015), encounters with ubiquitous enemies with broad host ranges (**chapter 2**, Parker and Gilbert 2007, Sikes et al. 2018) or co-introduction of enemies (Blaisdell and Roy 2014, Allen et al. 2015). All of these factors would lead to an assumedly rapid equalization of disease and herbivory pressure for exotic plants (Parker and Gilbert 2007).
- Third, plant species identity can have effects on plant disease and on fungal communities in grasslands (Burns et al. 2015, Sweeney et al. 2021), that are more important than plant species origin (Majewska et al. 2018). For example, although I found no effects of plant species origin on diversity and composition of soil fungal communities (**chapter 4**), there were few, but distinct differences in soil fungal community diversity and composition among plant species, mainly driven by changes in fungal pathogens. Studies in grasslands (Leff et al. 2018) and tropical forests (Peay et al. 2013) support these findings, as they show that mutualists and pathogens are stronger associated with distinct plant species than ubiquitous saprotrophic fungi. However, although the identity of individual plant species can be a strong predictor of soil fungal community diversity and composition (Cardinale et al. 2006), it is arguable that the magnitude and directionality of these effects are context-dependent, e.g. soil pH and nutrients, especially on local scales (Tedersoo et al. 2016).
- Fourth, mutualists can have profound impacts on plant invasions (Richardson et al. 2000a, Dickie et al. 2017) for multiple reasons. For example, mutualists that limit invasion may be absent in the introduced range (Catford et al. 2009), mutualists from the native range may be co-introduced with their hosts (Dickie et al. 2010), or native fungi can establish novel associations with exotic hosts (Callaway et al. 2004). Although I found mycorrhizal OTUs in soil fungal communities (**chapter 4**), analysis of these data was not possible because of poor amplification of mycorrhizal fungi with the general fungal ITS primers I used (Tedersoo et al. 2015, 2018).
- Fifth, if generalists dominate pathogen (Lindblad 2000, Gilbert 2002, Gilbert et al. 2002) and herbivore (Wolfe 2002, Biswas et al. 2015, Korell et al. 2016b) communities, it is more likely that native and exotic species share the same enemies and experience equal levels of herbivory and disease.

- Sixth, similar levels of enemy damage and diversity on native and exotic plants may be linked to habitat productivity and caused by pre-adaptation of exotic species to the fertility of the invaded soil (Dostál et al. 2013). The study systems I investigated were characterized by a rather moderate productivity and could therefore explain the observed similar effects of enemy reduction on productivity of native and exotic-dominated communities. This would require pre-adaptation of exotic species to these levels of soil fertility in their native range.
- Finally, multiple introductions are rather common in biological invasions (Novak 2007, Suarez and Tsutsui 2008, Dutech et al. 2010, 2012) and could therefore increase the probability, with each separate introductory event, that enemies of exotic species are co-introduced into the invaded range (Lester et al. 2014).

I found no signal in pathogen diversity and infection related to the phylogenies of the studied plant communities (**chapter 2**). This finding contradicts with several research detecting effects of the phylogenetic structure of plant communities on interactions between plants and their enemies (Gilbert et al. 2015, Parker et al. 2015, Těšitel et al. 2021, Karunarathna et al. 2021), but could be explained by the following reasons. First, for several plant clades, it has been shown that environmental variation is more important in determining defenses against natural enemies than phylogenetically constrained defense traits (Koricheva et al. 2004, Haak et al. 2014). For example, Hahn et al. (2021) were able to show that resource availability is the strongest predictor for the shape (i. e. positive or negative) of trade-offs between growth and defense against pathogens and herbivores among populations of *Monarda fistulosa*, a perennial herb that also occurs in the plant communities of Missouri that were part of my study. Second, the dynamics of pathogen metapopulations are highly complex, variable and have been identified to be an important driver of pathogen virulence by local adaptation (Papaïx et al. 2021). There is indeed evidence for various fungal pathogen groups, such as rusts (Burdon et al. 1995, Thrall et al. 2002, Smith et al. 2011), mildews (Laine and Hanski 2006, Lebeda et al. 2008) and smuts (Carlsson-Granér 1997, Antonovics 2004) that spatial patchiness in plant distribution may generate differentiation in pathogen virulence on local scales. Third, such effects of local adaptation are, in principle, also applicable to host plants (Forester et al. 2016). In fact, empirical evidence for local host adaptation to selection pressure by pathogens (Springer 2007, Salvaudon et al. 2008, Laine et al. 2011, Parratt and Laine 2018), and local host adaptation mediated by spatial variation in soil biota (Mursinoff and Tack 2017) has been found. The matrix of forest and grasslands suggests that local adaptation of both host and pathogen populations may play an important role in determining susceptibility and virulence (Thrall et al. 2002, Forester et al. 2016) in my study system in the central United

States (**chapter 2**). Spatial heterogeneity may thus override potential effects of plant phylogeny on pathogen infection (Strauss et al. 2002, Haak et al. 2014). However, recent plant-microbiome studies in crops revealed that host susceptibility is not only determined by host defense traits linked to the genotype, but is also directly conferred by microbiota (Carrión et al. 2019, Matsumoto et al. 2021). It is argued that analogous findings are very likely in other plant species (Cernava and Berg 2022). Further, hyperparasitism of plant pathogens can be an additional, confounding aspect of the complexity of plant-pathogen interactions, shaping patterns of host susceptibility and pathogen virulence (Davelos and Jarosz 2004, Tollenaere et al. 2014, Parratt and Laine 2018).

Although I did not study the effects of phylogenetic plant community context in the manipulative field experiment in central Europe, one possible explanation for the similarity between soil fungal communities of different plant species is the phylogenetic relatedness of individual plant species (**chapter 4**).

How do pathogens and insect herbivores mediate the coexistence of plant species in grassland ecosystems?

A key result of my thesis is evidence that plant species coexistence is mediated by stabilizing and equalizing mechanisms in a manipulated semi-natural central European grassland (**chapter 3** and **4**). Fungal pathogens and insect herbivores did exert an effect on community productivity, an important indicator of ecosystem function (Melillo et al. 1993). Antagonistic interactions are regarded to affect community productivity, particularly in diverse communities (HilleRisLambers et al. 2004), where natural enemies substantially contribute to coexistence (Chase et al. 2002, Bartomeus and Godoy 2018). However, my thesis could consistently show that the presence of stabilizing forces, indicated by negative frequency-dependent per capita rates of increase, is not, to a large part, the result of antagonistic interactions.

Furthermore, varying and offsetting responses to natural enemies of species in exotic-dominated communities suggest the presence of equalizing forces that minimize competitive and frequency-independent fitness differences (Gross et al. 2014, Kandlikar et al. 2019). Indeed, evidence is growing that especially (soil) fungi frequently promote coexistence among plant species by trade-offs between plants' competitive ability and susceptibility to pathogens or mutualists (Bever et al. 2015, Laliberté et al. 2015, Lekberg et al. 2018, Ke and Wan 2019, Cappelli et al. 2020). Assuming that equalizing mechanisms are relevant in our studied communities, neutral processes may contribute to the abundances of native and exotic plants (Volkov et al. 2003, Daleo et al. 2009). According to niche models of community assembly, a prerequisite for the operationality of neutral

processes is functional equivalence[2] among native and exotic plants (Shea and Chesson 2002, Fargione et al. 2003, Gurevitch et al. 2011). Empirical evidence for functional equivalence among natives and exotics is equivocal (Leffler et al. 2014, Ordonez 2014). This evidence emphasizes, that in addition to plant traits, species identity (**chapter 4**, Mokany et al. 2008, Mason et al. 2016), environmental context such as nitrogen availability (Maron and Connors 1996, Holdredge et al. 2010, Zhang et al. 2022), and community composition (Gerhold et al. 2011) contribute to responses of native and exotic plants to natural enemies and mutualists. Similar evidence for insect herbivores promoting coexistence among plant species in novel communities by means of trade-offs, e.g. competition-defense or growth-preference, has been found (Heard and Sax 2013, Gross et al. 2014, Kempel et al. 2015). However, these trade-offs are less frequently investigated in herbivores compared to soil biota.

The negative frequency-dependence and a coincidental lack of effects from antagonistic interactions observed in my field experiment suggest that, particularly, native communities were predominantly governed by competition for resources (**chapter 3**, McKane et al. 2002, Chu and Adler 2015, Kuster et al. 2016, Ceulemans et al. 2017). Resource competition is a strong indicator for stabilizing niche differences that maintain species diversity in these plant communities (Chesson 2000, Adler et al. 2007, Levine and HilleRisLambers 2009, Broekman et al. 2019). Strong competition for resources is common in soils with low fertility (Tilman 1985, 2007, Herms and Mattson 1992, Miller et al. 2005), similar to my experimental site in central Germany (**chapters 3** and **4**), and could favor resource partitioning over plants' responses to natural enemies in shaping niche differences. However, in species-rich communities such as temperate grasslands, there is theoretical (Adler et al. 2007, Chisholm and Pacala 2010, Leibold et al. 2019, Pinsky 2019) and empirical (Chisholm and Pacala 2010, Spear et al. 2015) evidence that, despite the presence of strong niche structures and negative frequency-dependence, coexistence is independent of a species' relative abundance. Interestingly, neutral processes can generate identical community-level patterns. According to recent modelling approaches this may be due to stronger influences of

[2] This functional equivalence (i.e. in fitness) among species should result from equalizing mechanisms that promote ecological similarities in species' responses to environmental heterogeneities (Cadotte 2007). In contrast to classic neutral theory (Hubbell 2001), which poses that species do not differ in ways that distinguish their population dynamics, it has been shown that the existence of niche differences does not negate the importance of ecological similarity (i.e. through equalizing mechanisms) in structuring overall community dynamics (Chesson 2000, Leibold and McPeek 2006). In other words, analysing species abundance patterns does not suffice to distinguish among the relative contributions of niche-based and neutral processes (Adler et al. 2007). Rather, direct examination of the underlying stabilizing and equalizing mechanisms is necessary to resolve the often simultaneous contributions of each, niche and neutral processes, to species coexistence and the maintenance of diversity (Adler et al. 2007, Zhang et al. 2009, Godoy et al. 2020).

demographic stochasticity, compared to stabilizing niche differences in resource use, in generating patterns at the community scale (D'Andrea et al. 2020, Gupta et al. 2021). At the same time, effects of natural enemies on plant community assembly appear to be stronger at (enemy) family level compared to (enemy) species level (D'Andrea et al. 2020).

How are natural communities of fungal pathogens and insect herbivores altered by the application of pesticides?

Application of pesticides modified composition and abundances of fungal and insect herbivore communities in a central European semi-natural grassland ecosystem (**chapter 3** and **4**). This is not surprising, given that these compounds are developed to control fungal and insect pests in agricultural crops. In addition to this desired effect, the unintended side effects of pesticide application on non-target organisms in natural ecosystems are largely not understood (Köhler and Triebskorn 2013). In the context of ecological experiments, the efficacy of pesticides to exclude fungi and insects needs to be assessed when applying them to study the importance of these antagonists for the dynamics of natural plant communities (Allan et al. 2010). Remarkably, fungicide application had no effect on species richness of fungal aboveground or belowground communities, but significantly altered abundance, composition and overall infection levels of these communities in natural ecosystems (**chapter 3** and **4**). Saprotrophs and pathotrophs of soil fungal communities both experienced significant decreases in their abundances due to fungicide application, however, this effect was more pronounced in pathotrophs. Thus, resistance to fungicides, a problem affecting agro-ecosystems globally (Hahn 2014, Lucas et al. 2015, Fisher et al. 2018), was likely not present in soil pathotrophic fungi of my studied communities, potentially due to high plant diversity (Yang et al. 2021)[3] and application of multiple fungicides. The combined use of different fungicidal agents could also explain why I did not find evidence for interactions between different groups or between species within a particular group of pathotrophs. Several studies using single fungicidal agents report such interactions and show that they could lead to an increase in plant disease, despite fungicide application (Birzele et al. 2002, Henriksen and Elen 2005, Newton et al. 2010). However, the most striking effect of fungicide application was the reduced fungal species turnover between plots, i.e. a decrease in β-diversity, resulting in a biotic homogenization of soil fungal communities (Lockwood and McKinney 2001, Olden et al. 2004, Gossner et al. 2016, Blowes et al. 2019). Agricultural intensification, to which pesticide application significantly contributes, is a major driver of biotic homogenization (Elek et al. 2020), that is known

[3] High plant diversity can mitigate the effects of pathogens on their hosts by promoting selection for pathogen strains that differ in the ability of fungicide influxes, effluxes or detoxification rates, rather than mutations in target genes of fungicides.

to decrease multiple ecosystem functions at local, regional and global scales (Dornelas et al. 2014, Wang and Loreau 2016, Wang et al. 2021).

Insecticide application led to a significant decrease in arthropod diversity and abundance (**chapter 3**). This was primarily driven by a strong decrease in abundances of springtails, that made up the majority of hexapods in my studied soil arthropod communities. Springtails are considered indicators for the functioning of soil ecosystem (Thimm et al. 1998, van Gestel et al. 2017), because they contribute to the fragmentation of detritus, stimulating its degradation by microorganisms and therefore enhancing nutrient cycling (Hanlon 1981, Elkins and Whitford 1982, Seastedt 1984). My findings support that springtails have a high sensitivity to insecticides in general (Frampton et al. 2006, Fountain et al. 2007), and to imidacloprid, the insecticide I used, in particular (de Lima e Silva et al. 2017, Mabubu et al. 2017). A possible explanation for these toxicological effects is the phylogenetic proximity of springtails to insects (Bandeira et al. 2021). Imidacloprid belongs to the class of neonicotinoids, that are currently the most widely used insecticides in the world, because of their remarkable effectiveness against a broad spectrum of sucking and chewing insect pests, persistence, systemic mode of action and low mammalian toxicity (Jeschke et al. 2011, Casida 2018). Nonetheless, these characteristics also increase the probability of exposure to nontarget organisms and environmental contamination (Bonmatin et al. 2015). For a long time, pollinators have been the major focus of research on the effects of neonicotinoid exposure to nontarget organism (Godfray et al. 2014, Woodcock et al. 2017, Hladik et al. 2018). However, evidence is growing that survival and reproduction of soil biota, especially springtails, are also severely affected by exposure to neonicotinoids, in particular imidacloprid, with effects lasting for multiple generations after the application (Pisa et al. 2021).

My investigations on the effects of pesticide application on natural communities of fungal pathogens and insect herbivores strongly support the long-held view that toxicity-conferring mechanisms of pesticides are not restricted to target pests. These effects should be critically considered when environmental impacts of pesticides are assessed, to further mitigate damage to biodiversity and ecosystem health (Köhler and Triebskorn 2013, Carvalho 2017, Zubrod et al. 2019). Besides, the results of **chapter 3** and **4** show that application of fungicides and insecticides are an effective tool to exclude two important groups of plant antagonists. This allowed studying the importance of these groups for plant community dynamics in a factorially designed, ecological experiment (Paul et al. 1989, Siemann et al. 2004).

5.2 Conclusions and future challenges

Although modern coexistence theory provides a detailed framework of how interactions among plants and their natural enemies contribute to species coexistence, there have been surprisingly few empirical applications on this research topic. In particular, theories of mechanisms driving coexistence within novel communities, consisting of native species and introduced exotics, need tangible data to understand how global change impacts ecosystem functioning. In my thesis, I combined an observational study with a field experiment and molecular techniques in temperate grasslands of the central United States and central Germany, consisting of native and exotic species, to contribute to filling this knowledge gap. The implications of my results are:

(1) Interactions between plants and their natural enemies are **independent of plant species' relative abundances** within the community, suggesting the importance of **abiotic soil conditions** and **spatial heterogeneity** in decoupling antagonistic effects from host abundances.

Native and exotic species do not differ in their interactions with fungal pathogens and insect herbivores, indicating the **absence or transience of enemy release.** Instead, the accumulation of enemies with time since introduction, the confounding presence of closely related native plants, co-introductions of (ubiquitous) enemies or the dominance of generalists in the pathogen and herbivore communities may have contributed to the observed patterns.

Phylogeny of plant communities **does not drive infection patterns** of plant disease. Instead, environmental resource availability may be a stronger predictor of plant defense. Furthermore, spatial patchiness in plant distributions can lead to rapid local adaptation of pathogens and host plants, creating unique patterns of virulence and susceptibility that result in the observed levels of pathogen diversity and infection.

(2) **Negative frequency-dependent rates of increase** govern predominantly **native communities** and indicate the presence of **stabilizing niche differences**. These, however, are largely **not the result of interactions between plants and their natural enemies**, but rather point to the importance of resource competition in soils with low fertility. A contribution of neutral processes to the observed community-level patterns of species' abundances is possible, due to the influence of demographic stochasticity in species-rich communities.

Varying and off-setting responses to natural enemies in exotic-dominated communities are indicators for the presence of **equalizing and frequency-**

independent fitness differences. Thus, coexistence among native and exotic species in novel communities may be promoted by trade-offs, e.g. between plants' competitive ability and susceptibility to pathogens and herbivores.

(3) **Fungicide application** leads to **decreases in fungal OTU abundances**, to a larger part in pathotrophs compared to saprotrophs, and to **biotic homogenization** of natural soil fungal communities, driven by a **decrease in fungal β-diversity**. **Fungal α-diversity is not affected** by fungicide application.

Insecticide application has a **negative effect on arthropod diversity and abundance**, driven primarily by **strong decreases in springtail abundances**, suggesting a high toxicity of neonicotinoids to this important group of soil biota.

Empirical insights into biotic controls of species coexistence are still limited (Bartomeus and Godoy 2018). My thesis clearly emphasizes the differential importance of two groups of natural enemies for the coexistence between native and exotic species in novel grassland communities. I found evidence for the joint effects of both concepts of Chesson's (2000, 2018) framework for species coexistence: stabilizing niche differences and equalizing fitness differences. This highlights the need for reconciling neutral and niche theory, that has already been put forward by modern coexistence theory (Adler et al. 2007, Song et al. 2019). More experimental tests of modern coexistence theory in multitrophic systems, combined with advances in data-driven modeling and network analyses, are needed to disentangle context-dependencies of interactions between plants and their natural enemies, from general underlying mechanisms (Levine et al. 2017). The results of my thesis help to elucidate how these context-dependencies can be addressed: experimental manipulations of the abiotic and biotic environment, evaluating relationships between plant phylogeny, natural enemies and the environment, and the quantification of frequency-dependent per capita rates of increase (Valladares et al. 2015). Biological invasions are an important driver of global change and are better understood within a framework of niche and fitness differences, while other drivers of global change, such as climate change and land-use intensification, are able to modulate the outcome of biological invasions (Valladares et al. 2015). Thus, future experimental approaches to understand the mechanisms by which invasions occur need to combine modern coexistence theory with insights from invasion ecology if we aim to control invasive species. In this regard, my thesis provides an exemplary experimental approach of how to gain a more complete understanding about the mechanisms underlying the maintenance of species diversity and ecosystem functioning in these novel communities.

6

References

Adler, P. B., S. P. Ellner, and J. M. Levine. 2010. Coexistence of perennial plants: An embarrassment of niches. Ecology Letters 13:1019–1029.

Adler, P. B., J. HilleRisLambers, P. C. Kyriakidis, Q. Guan, and J. M. Levine. 2006. Climate variability has a stabilizing effect on the coexistence of prairie grasses. Proceedings of the National Academy of Sciences of the United States of America 103:12793–12798.

Adler, P. B., J. HilleRislambers, and J. M. Levine. 2007. A niche for neutrality. Ecology Letters 10:95–104.

Agrawal, A. A. 2007. Macroevolution of plant defense strategies. Trends in Ecology and Evolution 22:103–109.

Agrawal, A. A., and M. Fishbein. 2006. Plant defense syndromes. Ecology 87:132–49.

Agrawal, A. A., and P. M. Kotanen. 2003. Herbivores and the success of exotic plants: A phylogenetically controlled experiment. Ecology Letters 6:712–715.

Agrawal, A. A., P. M. Kotanen, C. E. Mitchell, A. G. Power, W. Godsoe, and J. Klironomos. 2005. Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. Ecology 86:2979–2989.

Ahrens, W. H., D. J. Cox, and G. Budhwar. 1990. Use of the Arcsine and Square Root Transformations for Subjectively Determined Percentage Data. Weed Science 38:452–458.

Alexander, H. M. 2010. Disease in natural plant populations, communities, and ecosystems: Insights into ecological and evolutionary processes. Plant Disease 94:492–503.

Alexander, H. M., and R. D. Holt. 1998. The interaction between plant competition and disease. Perspectives in Plant Ecology, Evolution and Systematics 1:206–220.

Allan, E., J. Van Ruijven, and M. J. Crawley. 2010. Foliar fungal pathogens and grassland biodiversity. Ecology 91:2572–2582.

Allen, W. J., R. E. Young, G. P. Bhattarai, J. R. Croy, A. M. Lambert, L. A. Meyerson, and J. T. Cronin. 2015. Multitrophic enemy escape of invasive Phragmites australis and its introduced herbivores in North America. Biological Invasions 17:3419–3432.

Ampt, E. A., J. van Ruijven, J. M. Raaijmakers, A. J. Termorshuizen, and L. Mommer. 2018. Linking ecology and plant pathology to unravel the importance of soil-borne fungal pathogens in species-rich grasslands. European Journal of Plant Pathology 154:141–156.

Anacker, B. L., N. E. Rank, D. Hüberli, M. Garbelotto, S. Gordon, T. Harnik, R. Whitkus, and R. Meentemeyer. 2008. Susceptibility to Phytophthora ramorum in a key infectious host: Landscape variation in host genotype, host phenotype, and environmental factors. New Phytologist 177:756–766.

Andrade, G. S., W. F. Barbosa, A. M. Milanez, F. N. Celestino, R. A. Polanczyk, and D. Pratissoli. 2010. Side Effects of Fungicides Used in Cucurbitaceous Crop on Trichogrammaatopovirilia Oatman & Platner (Hymenoptera: Trichogramatidae). Chilean journal of agricultural research 70:323–327.

Antonovics, J. 2004. Long-Term Study of a Plant-Pathogen Metapopulation. Pages 471–488 *in* I. Hanski and O. E. Gaggiotti, editors. Ecology, Genetics and Evolution of Metapopulations. Academic Press, San Diego, CA.

Arbizu, M. 2017. Pairwiseadonis: Pairwise multilevel comparison using adonis. R Package Version 0.0.1.

Augspurger, C. K. 1983. Seed Dispersal of the Tropical Tree, Platypodium Elegans, and the Escape of its Seedlings from Fungal Pathogens. The Journal of Ecology 71:759–771.

Bagchi, R., R. E. Gallery, S. Gripenberg, S. J. Gurr, L. Narayan, C. E. Addis, R. P. Freckleton, and O. T. Lewis. 2014. Pathogens and insect herbivores drive rainforest plant diversity and composition. Nature 506:85–88.

Bagchi, R., T. Swinfield, R. E. Gallery, O. T. Lewis, S. Gripenberg, L. Narayan, and R. P. Freckleton. 2010. Testing the Janzen-Connell mechanism: Pathogens cause overcompensating density dependence in a tropical tree. Ecology Letters 13:1262–1269.

Bandeira, F. O., P. R. L. Alves, T. B. Hennig, J. Brancalione, D. J. Nogueira, and W. G. Matias. 2021. Chronic effects of clothianidin to non-target soil invertebrates: Ecological risk assessment using the species sensitivity distribution (SSD) approach. Journal of Hazardous Materials 419:126491.

Barberán, A., K. L. Mcguire, J. A. Wolf, F. A. Jones, S. J. Wright, B. L. Turner, A. Essene, S. P. Hubbell, B. C. Faircloth, and N. Fierer. 2015. Relating belowground microbial composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest. Ecology Letters 18:1397–1405.

Bardgett, R. D., J. L. Mawdsley, S. Edwards, P. J. Hobbs, J. S. Rodwell, and W. J. Davies. 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Functional Ecology

13:650–660.
Bartomeus, I., and O. Godoy. 2018. Biotic controls of plant coexistence. Journal of Ecology 106:1767–1772.
Becher, R., U. Hettwer, P. Karlovsky, H. B. Deising, and S. G. R. Wirsel. 2010. Adaptation of Fusarium graminearum to tebuconazole yielded descendants diverging for levels of fitness, fungicide resistance, virulence, and mycotoxin production. Phytopathology 100:444–453.
Ben-Hur, E., O. Fragman-Sapir, R. Hadas, A. Singer, and R. Kadmon. 2012. Functional trade-offs increase species diversity in experimental plant communities. Ecology Letters 15:1276–1282.
Benham, K. E. 1982. Soil Survey of St. Louis County and St. Louis City, Missouri. U.S. Dept. of Agriculture, Soil Conservation Science, Washington, D.C., USA.
Benítez, M. S., M. H. Hersh, R. Vilgalys, and J. S. Clark. 2013. Pathogen regulation of plant diversity via effective specialization. Trends in Ecology and Evolution 28:705–711.
Benjamini, Y., and Y. Hochberg. 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society: Series B (Methodological) 57:289–300.
Bennett, J. A., and J. F. Cahill. 2016. Fungal effects on plant-plant interactions contribute to grassland plant abundances: Evidence from the field. Journal of Ecology 104:755–764.
Bennett, J. A., H. Maherali, K. O. Reinhart, Y. Lekberg, M. M. Hart, and J. Klironomos. 2017. Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. Science 355:181–184.
Berg, G., and K. Smalla. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology 68:1–13.
Bever, J. D. 2003. Soil community feedback and the coexistence of competitors: Conceptual frameworks and empirical tests. New Phytologist 157:465–473.
Bever, J. D., S. A. Mangan, and H. M. Alexander. 2015. Maintenance of Plant Species Diversity by Pathogens. Annual Review of Ecology, Evolution, and Systematics 46:305–325.
Birzele, B., A. Meier, H. Hindorf, J. Krämer, and H. W. Dehne. 2002. Epidemiology of Fusarium infection and deoxynivalenol content in winter wheat in the Rhineland, Germany. European Journal of Plant Pathology 108:667–673.
Biswas, S. R., P. M. Kotanen, D. Kambo, and H. H. Wagner. 2015. Context-dependent patterns, determinants and demographic consequences of herbivory in an invasive species. Biological Invasions 17:165–178.
Blaisdell, G. K., and B. A. Roy. 2014. Two tests of enemy release of commonly co-occurring bunchgrasses native in Europe and introduced in the United States. Biological Invasions 16:833–842.
Blanton, C. M., and J. J. Ewel. 1985. Leaf-cutting and herbivory in successional and agricultural tropical ecosystems. Ecology 66:861–869.
Blomberg, S. P., T. Garland, and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. Evolution 57:717–745.
Blowes, S. A., S. R. Supp, L. H. Antão, A. Bates, H. Bruelheide, J. M. Chase, F. Moyes, A. Magurran, B. McGill, I. H. Myers-Smith, M. Winter, A. D. Bjorkman, D. E. Bowler, J. E. K. Byrnes, A. Gonzalez, J. Hines, F. Isbell, H. P. Jones, L. M. Navarro, P. L. Thompson, M. Vellend, C. Waldock, and M. Dornelas. 2019. The geography of biodiversity change in marine and terrestrial assemblages. Science 366:339–345.
Blumenthal, D., C. E. Mitchell, P. Pysek, and V. Jarosik. 2009. Synergy between pathogen release and resource availability in plant invasion. PNAS 106:7899–7904.
Bonmatin, J. M., C. Giorio, V. Girolami, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. Marzaro, E. A. Mitchell, D. A. Noome, N. Simon-Delso, and A. Tapparo. 2015. Environmental fate and exposure; neonicotinoids and fipronil. Environmental Science and Pollution Research 22:35–67.
Borer, E. T., W. S. Harpole, P. B. Adler, C. A. Arnillas, M. N. Bugalho, M. W. Cadotte, M. C. Caldeira, S. Campana, C. R. Dickman, T. L. Dickson, I. Donohue, A. Eskelinen, J. L. Firn, P. Graff, D. S. Gruner, R. W. Heckman, A. M. Koltz, K. J. Komatsu, L. S. Lannes, A. S. MacDougall, J. P. Martina, J. L. Moore, B. Mortensen, R. Ochoa-Hueso, H. Olde Venterink, S. A. Power, J. N. Price, A. C. Risch, M. Sankaran, M. Schütz, J. Sitters, C. J. Stevens, R. Virtanen, P. A. Wilfahrt, and E. W. Seabloom. 2020. Nutrients cause grassland biomass to outpace herbivory. Nature Communications 11:6036.
Borer, E. T., P. R. Hosseini, E. W. Seabloom, and A. P. Dobson. 2007. Pathogen-induced reversal of native

dominance in a grassland community. Proceedings of the National Academy of Sciences 104:5473–8.

Borgström, P., J. Strengbom, M. Viketoft, and R. Bommarco. 2016. Aboveground insect herbivory increases plant competitive asymmetry, while belowground herbivory mitigates the effect. PeerJ 4:e1867.

Bouffaud, M. L., M. A. Poirier, D. Muller, and Y. Moënne-Loccoz. 2014. Root microbiome relates to plant host evolution in maize and other Poaceae. Environmental Microbiology 16:2804–2814.

Boyer, F., C. Mercier, A. Bonin, Y. Le Bras, P. Taberlet, and E. Coissac. 2016. obitools : a unix -inspired software package for DNA metabarcoding. Molecular Ecology Resources 16:176–182.

Brandenburger, W. 1985. Parasitische Pilze an Gefaeßpflanzen in Europa. Gustav Fischer Verlag, Stuttgart, Germany.

Brändle, M., I. Kühn, S. Klotz, C. Belle, and R. Brandl. 2008. Species richness of herbivores on exotic host plants increases with time since introduction of the host. Diversity and Distributions 14:905–912.

Broekman, M. J. E., H. C. Muller-Landau, M. D. Visser, E. Jongejans, S. J. Wright, and H. de Kroon. 2019. Signs of stabilisation and stable coexistence. Ecology Letters 22:1957–1975.

Brohmer, P. 2018. Fauna von Deutschland. Quelle and Meyer, Heidelberg.

Brown, J. K. M., and M. S. Hovmøll. 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science 297:537–541.

Bundesamt für Naturschutz. 2019a. FloraWeb, www.floraweb.de, accessed on 11.03.2019.

Bundesamt für Naturschutz. 2019b. Neobiota, bfn.neobiota.de, accessed on 11.03.2019.

Burdon, J. J., and G. A. Chilvers. 1982. Host Density as a Factor in Plant Disease Ecology. Annual Review of Phytopathology 20:143–166.

Burdon, J. J., L. Ericson, and W. J. Muller. 1995. Temporal and Spatial Changes in a Metapopulation of the Rust Pathogen Triphragmium Ulmariae and its Host, Filipendula Ulmaria. The Journal of Ecology 83:979–989.

Burkey, T. V. 1994. Tropical tree species diversity: a test of the Janzen-Connell model. Oecologia 97:533–540.

Burns, J. H., B. L. Anacker, S. Y. Strauss, and D. J. Burke. 2015. Soil microbial community variation correlates most strongly with plant species identity, followed by soil chemistry, spatial location and plant genus. AoB PLANTS 7:1–10.

De Cáceres, M., and P. Legendre. 2020. Package "indicspecies." CRAN Repository.

Cadotte, M. W. 2007. Concurrent niche and neutral processes in the competition-colonization model of species coexistence. Proceedings of the Royal Society B: Biological Sciences 274:2739–2744.

Cadotte, M. W., S. E. Campbell, S. Li, D. S. Sodhi, and N. E. Mandrak. 2018. Preadaptation and Naturalization of Nonnative Species: Darwin's Two Fundamental Insights into Species Invasion. Annual Review of Plant Biology 69:661–684.

Cahill, J. F., and M. D. Coupe. 2003. Effects of insects on primary production in temperate herbaceous communities: a meta-analysis. Ecological Entomology 28:511–521.

Callaway, R. M., G. C. Thelen, A. Rodriguez, and W. E. Holben. 2004. Soil biota and exotic plant invasion. Nature 427:731–733.

Cappelli, S. L., N. A. Pichon, A. Kempel, and E. Allan. 2020. Sick plants in grassland communities: a growth-defense trade-off is the main driver of fungal pathogen abundance. Ecology Letters 23:1349–1359.

Cardinale, B. J., D. S. Srivastava, J. E. Duffy, J. P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature 443:989–992.

Carlsson-Granér, U. 1997. Anther-smut disease in silene dioica: Variation in susceptibility among genotypes and populations, and patterns of disease within populations. Evolution 51:1416– 1426.

Carniel, L. S. C., J. C. Niemeyer, L. C. Iuñes de Oliveira Filho, D. Alexandre, L. Gebler, and O. Klauberg-Filho. 2019. The fungicide mancozeb affects soil invertebrates in two subtropical Brazilian soils. Chemosphere 232:180–185.

Carrión, V. J., J. Perez-Jaramillo, V. Cordovez, V. Tracanna, M. de Hollander, D. Ruiz-Buck, L. W. Mendes, W. F. van Ijcken, R. Gomez-Exposito, S. S. Elsayed, P. Mohanraju, A. Arifah, J. van der Oost, J. N. Paulson, R. Mendes, G. P. van Wezel, M. H. Medema, and J. M. Raaijmakers. 2019. Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. Science 366:606–612.

Carson, W. P., and R. B. Root. 1999. Top-down effects of insect herbivores during early succession:

Influence on biomass and plant dominance. Oecologia 121:260–272.
Carson, W. P., and R. B. Root. 2000. Herbivory and plant species coexistence: Community regulation by an outbreaking phytophagous insect. Ecological Monographs 70:73–99.
Carvalho, F. P. 2017. Pesticides, environment, and food safety. Food and Energy Security 6:48–60.
Casida, J. E. 2018. Neonicotinoids and Other Insect Nicotinic Receptor Competitive Modulators: Progress and Prospects. Annual Review of Entomology 63:125–144.
Caswell, H. 1978. Predator-Mediated Coexistence: A Nonequilibrium Model. The American Naturalist 112:127–154.
Catford, J. A., R. Jansson, and C. Nilsson. 2009. Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Diversity and Distributions 15:22–40.
Cernava, T., and G. Berg. 2022. The emergence of disease-preventing bacteria within the plant microbiota. Environmental Microbiology.
Ceulemans, T., S. Bodé, J. Bollyn, S. Harpole, K. Coorevits, G. Peeters, K. Van Acker, E. Smolders, P. Boeckx, and O. Honnay. 2017. Phosphorus resource partitioning shapes phosphorus acquisition and plant species abundance in grasslands. Nature Plants 3:16224.
Chase, J. M., P. A. Abrams, J. P. Grover, S. Diehl, P. Chesson, R. D. Holt, S. A. Richards, R. M. Nisbet, and T. J. Case. 2002. The interaction between predation and competition: A review and synthesis. Ecology Letters 5:302–315.
Chase, J. M., and M. A. Leibold. 2003. Ecological Niches. University of Chicago Press, Chicago.
Chazdon, R. L., S. Careaga, C. Webb, and O. Vargas. 2003. Community and phylogenetic structure of reproductive traits of woody species in wet tropical forests. Ecological Monographs 73:331–348.
Chen, M., B. Chen, Marschner, and Petra. 2008. Plant growth and soil microbial community structure of legumes and grasses grown in monoculture or mixture. Journal of Environmental Sciences 20:1231–1237.
Chen, X., B. Zheng, and H. Liu. 2011. Optical and digital microscopic imaging techniques and applications in pathology. Analytical Cellular Pathology 34:5–18.
Chen, Y. L., T. Le Xu, S. D. Veresoglou, H. W. Hu, Z. P. Hao, Y. J. Hu, L. Liu, Y. Deng, M. C. Rillig, and B. D. Chen. 2017. Plant diversity represents the prevalent determinant of soil fungal community structure across temperate grasslands in northern China. Soil Biology and Biochemistry 110:12–21.
Chesson, P. 1994. Multispecies Competition in Variable Environments. Theoretical Population Biology 45:227–276.
Chesson, P. 2000. Mechanisms of Maintenance of Species Diversity. Annu. Rev. Ecol. Syst 31:343–66.
Chesson, P. 2018. Updates on mechanisms of maintenance of species diversity. Journal of Ecology 106:1773–1794.
Chesson, P., and J. J. Kuang. 2008. The interaction between predation and competition. Nature 456:235–238.
Chesson, P. L., and R. R. Warner. 1981. Environmental Variability Promotes Coexistence in Lottery Competitive Systems. The American Naturalist 117:923–943.
Chisholm, R. A., and S. W. Pacala. 2010. Niche and neutral models predict asymptotically equivalent species abundance distributions in high-diversity ecological communities. Proceedings of the National Academy of Sciences of the United States of America 107:15821–15825.
Chu, C., and P. B. Adler. 2015. Large niche differences emerge at the recruitment stage to stabilize grassland coexistence. Ecological Monographs 85:373–392.
Chun, Y. J., M. van Kleunen, and W. Dawson. 2010. The role of enemy release, tolerance and resistance in plant invasions: Linking damage to performance. Ecology Letters 13:937–946.
Chung, Y. A., and J. A. Rudgers. 2016. Plant–soil feedbacks promote negative frequency dependence in the coexistence of two aridland grasses. Proceedings of the Royal Society B: Biological Sciences 283.
Civitello, D. J., J. Cohen, H. Fatima, N. T. Halstead, J. Liriano, T. A. McMahon, C. N. Ortega, E. L. Sauer, T. Sehgal, S. Young, and J. R. Rohr. 2015. Biodiversity inhibits parasites: Broad evidence for the dilution effect. Proceedings of the National Academy of Sciences of the United States of America 112:8667–8671.
St. Clair, S. B., R. O'Connor, R. Gill, and B. McMillan. 2016. Biotic resistance and disturbance: Rodent consumers regulate post-fire plant invasions and increase plant community diversity. Ecology 97:1700–1711.
Clark, J. S., and J. S. McLachlan. 2003. Stability of forest biodiversity. Nature 423:635–638.
Clark, N. J., and S. M. Clegg. 2017. Integrating phylogenetic and ecological distances reveals new insights

into parasite host specificity. Molecular Ecology 26:3074–3086.
Cobb, R. C., R. K. Meentemeyer, and D. M. Rizzo. 2010. Apparent competition in canopy trees determined by pathogen transmission rather than susceptibility. Ecology 91:327–333.
Cody, M. L. 1991. Niche theory and plant growth form. Vegetatio 97:39–55.
Colautti, R. I., A. Ricciardi, I. A. Grigorovich, and H. J. MacIsaac. 2004. Is invasion success explained by the enemy release hypothesis? Ecology Letters 7:721–733.
Comita, L. S., H. C. Muller-Landau, S. Aguilar, and S. P. Hubbell. 2010. Asymmetrie density dependence shapes species abundances in a tropical tree community. Science 329:330–332.
Connell, J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. Pages 298–312 *in* P. J. den Boer and G. R. Gradwell, editors. Dynamics of populations. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.
Cordonnier, T., B. Courbaud, and A. Franc. 2006. The effect of colonization and competition processes on the relation between disturbance and diversity in plant communities. Journal of Theoretical Biology 243:1–12.
Costa, R., M. Götz, N. Mrotzek, J. Lottmann, G. Berg, and K. Smalla. 2006. Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. FEMS Microbiology Ecology 56:236–249.
Coupe, M. D., J. N. Stacey, and J. F. Cahill. 2009. Limited effects of above- And belowground insects on community structure and function in a species-rich grassland. Journal of Vegetation Science 20:121–129.
Craine, J. M., and R. Dybzinski. 2013. Mechanisms of plant competition for nutrients, water and light. Functional Ecology 27:833–840.
Cramer, N. F., and R. M. May. 1972. Interspecific competition, predation and species diversity: A comment. Journal of Theoretical Biology 34:289–293.
Crandall, R., and T. M. Knight. 2015. Positive frequency dependence undermines the success of restoration using historical disturbance regimes. Ecology Letters 18:883–891.
Crawley, M. 1989. Insect Herbivores And Plant Population Dynamics. Annual Review of Entomology 34:531–564.
Cronin, J. P., M. E. Welsh, M. G. Dekkers, S. T. Abercrombie, and C. E. Mitchell. 2010. Host physiological phenotype explains pathogen reservoir potential. Ecology Letters 13:1221–1232.
Crous, P. W., W. Gams, J. A. Stalpers, V. Robert, and G. Stegehuis. 2004. MycoBank: An online initiative to launch mycology into the 21st century. Studies in Mycology 50:19–22.
D'Andrea, R., T. Gibbs, and J. P. O'Dwyer. 2020. Emergent neutrality in consumer-resource dynamics. PLoS Computational Biology 16:1–17.
Daleo, P., J. Alberti, and O. Iribarne. 2009. Biological invasions and the neutral theory. Diversity and Distributions 15:547–553.
Darwin, C. 1859. On the origin of species by means of natural selection. John Murray, London.
Davelos, A. L., and A. M. Jarosz. 2004. Demography of American chestnut populations: Effects of a pathogen and a hyperparasite. Journal of Ecology 92:675 – 685.
Davis, M. A. 2006. Invasion biology 1958-2005: The pursuit of science and conservation. Pages 35–64 *in* M. W. Cadotte, S. M. McMahon, and T. Fukami, editors. Conceptual Ecology and Invasion Biology: Reciprocal Approaches to Nature. Springer, Dordrecht, The Netherlands.
Dawson, W., D. Moser, M. Van Kleunen, H. Kreft, J. Pergl, P. Pyšek, P. Weigelt, M. Winter, B. Lenzner, T. M. Blackburn, E. E. Dyer, P. Cassey, S. L. Scrivens, E. P. Economo, B. Guénard, C. Capinha, H. Seebens, P. García-Díaz, W. Nentwig, E. García-Berthou, C. Casal, N. E. Mandrak, P. Fuller, C. Meyer, and F. Essl. 2017. Global hotspots and correlates of alien species richness across taxonomic groups. Nature Ecology and Evolution 1:0186.
Dawson, W., and M. Schrama. 2016. Identifying the role of soil microbes in plant invasions. Journal of Ecology 104:1211–1218.
Deacon, J. 2006. Fungal Biology: 4th edition. Blackwell Publishing Ltd, New York, USA.
Deising, H. B., I. Gase, and Y. Kubo. 2017. The unpredictable risk imposed by microbial secondary metabolites: how safe is biological control of plant diseases? Journal of Plant Diseases and Protection 124:413–419.
Diamond, J. M. 1975. The island dilemma: Lessons of modern biogeographic studies for the design of natural reserves. Biological Conservation 7:129–146.

Dickie, I. A., N. Bolstridge, J. A. Cooper, and D. A. Peltzer. 2010. Co-invasion by Pinus and its mycorrhizal fungi. New Phytologist 187:475–484.

Dickie, I. A., J. L. Bufford, R. C. Cobb, M. L. Desprez-Loustau, G. Grelet, P. E. Hulme, J. Klironomos, A. Makiola, M. A. Nuñez, A. Pringle, P. H. Thrall, S. G. Tourtellot, L. Waller, and N. M. Williams. 2017. The emerging science of linked plant–fungal invasions. New Phytologist 215:1314–1332.

Dietz, H., T. Steinlein, and I. Ullmann. 1999. Establishment of the invasive perennial herb Bunias orientalis L.: An experimental approach. Acta Oecologica 20:621–632.

Dinnage, R. 2013. Phylogenetic diversity of plants alters the effect of species richness on invertebrate herbivory. PeerJ 1:e93.

Dinoor, A., and N. Eshed. 1984. The Role and Importance of Pathogens in Natural Plant Communities. Annual Review of Phytopathology 22:443–466.

Dobson, A. 2004. Population Dynamics of Pathogens with Multiple Host Species. The American Naturalist 164:S64–S78.

Dordas, C. 2008. Role of nutrients in controlling plant diseases in sustainable agriculture. A review. Agronomy for Sustainable Development 28:33–46.

Dormann, C. F., and S. H. Roxburgh. 2005. Experimental evidence rejects pairwise modelling approach to coexistence in plant communities. Proceedings of the Royal Society B: Biological Sciences 272:1279–1285.

Dornelas, M., N. J. Gotelli, B. McGill, H. Shimadzu, F. Moyes, C. Sievers, and A. E. Magurran. 2014. Assemblage time series reveal biodiversity change but not systematic loss. Science 344:296–299.

Dostál, P., E. Allan, W. Dawson, M. van Kleunen, I. Bartish, and M. Fischer. 2013. Enemy damage of exotic plant species is similar to that of natives and increases with productivity. Journal of Ecology 101:388–399.

Duncan, R. P., and P. A. Williams. 2002. Ecology: Darwin's naturalization hypothesis challenged. Nature 417:608–609.

Dunger, J. W., and H. J. Fiedler. 1999. Methoden der Bodenbiologie. VEB Gustav Fisher Verlag Jena.

Dutech, C., B. BarrÈs, J. Bridier, C. Robin, M. G. Milgroom, and V. RavignÉ. 2012. The chestnut blight fungus world tour: Successive introduction events from diverse origins in an invasive plant fungal pathogen. Molecular Ecology 21:3931–3946.

Dutech, C., O. Fabreguettes, X. Capdevielle, and C. Robin. 2010. Multiple introductions of divergent genetic lineages in an invasive fungal pathogen, Cryphonectria parasitica, in France. Heredity 105:220–228.

Ebeling, A., A. T. Strauss, P. B. Adler, C. A. Arnillas, I. C. Barrio, L. A. Biederman, E. T. Borer, M. N. Bugalho, M. C. Caldeira, M. W. Cadotte, P. Daleo, N. Eisenhauer, A. Eskelinen, P. A. Fay, J. Firn, P. Graff, N. Hagenah, S. Haider, K. J. Komatsu, R. L. McCulley, C. E. Mitchell, J. L. Moore, J. Pascual, P. L. Peri, S. A. Power, S. M. Prober, A. C. Risch, C. Roscher, M. Sankaran, E. W. Seabloom, H. Schielzeth, M. Schütz, K. L. Speziale, M. Tedder, R. Virtanen, and D. M. Blumenthal. 2022. Nutrient enrichment increases invertebrate herbivory and pathogen damage in grasslands. Journal of Ecology 110:327–339.

Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200.

Egidi, E., M. Delgado-Baquerizo, J. M. Plett, J. Wang, D. J. Eldridge, R. D. Bardgett, F. T. Maestre, and B. K. Singh. 2019. A few Ascomycota taxa dominate soil fungal communities worldwide. Nature Communications 10:2369.

Elek, Z., J. Růžičková, R. Ádám, K. Bereczki, G. Boros, F. Kádár, A. Kovács-Hostyánszki, L. Somay, O. Szalkovszki, and A. Báldi. 2020. Mixed effects of ecological intensification on natural pest control providers: A short-term study for biotic homogenization in winter wheat fields. PeerJ 2020:e8746.

Elkins, N. Z., and W. G. Whitford. 1982. The role of microarthropods and nematodes in decomposition in a semi-arid ecosystem. Oecologia 55:303–310.

Ellenberg, H., and C. Leuschner. 2010. Wirtschaftswiesen und -weiden, Tritt- und Flutrasen. Pages 953–1043 *in* C. Leuschner, editor. Vegetation Mitteleuropas mit den Alpen in ökologischer, dynamischer und historischer Sicht. Sixth edition. Verlag Eugen Ulmer, Stuttgart.

Elton, C. S. 1958. The Ecology of Invasions By Animals and Plants. Methuen, London.

Engelkes, T., A. Meisner, E. Morriën, O. Kostenko, W. H. Van der Putten, and M. Macel. 2016. Herbivory and dominance shifts among exotic and congeneric native plant species during plant community establishment. Oecologia 180.

Fagan, W. F., M. Lewis, M. G. Neubert, C. Aumann, J. L. Apple, and J. G. Bishop. 2005. When Can Herbivores Slow or Reverse the Spread of an Invading Plant? A Test Case from Mount St. Helens. The American Naturalist 166:669–685.

Fargione, J., C. S. Brown, and D. Tilman. 2003. Community assembly and invasion: An experimental test of neutral versus niche processes. Proceedings of the National Academy of Sciences of the United States of America 100:8916– 8920.

Farr, D. F.; Bills, G. F.; Chamuris, G. P.; Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. APS, St Paul, Minnesota.

Farr, D. F., and A. Y. Rossman. 2019. Fungal Databases, accessed on 11.03.2019. http://nt.ars-grin.gov/fungaldatabases/.

Farrell, B. D. 2001. Evolutionary Assembly of the Milkweed Fauna: Cytochrome Oxidase I and the Age of TetraopesBeetles. Molecular Phylogenetics and Evolution 18:467–478.

Fisher, M. C., N. J. Hawkins, D. Sanglard, and S. J. Gurr. 2018. Worldwide emergence of resistance to antifungal drugs challenges human health and food security. Science 742:739–742.

Flory, S. L., C. Alba, K. Clay, R. D. Holt, and E. M. Goss. 2018. Emerging pathogens can suppress invaders and promote native species recovery. Biological Invasions 19:469–77.

Flory, S. L., and K. Clay. 2013. Pathogen accumulation and long-term dynamics of plant invasions.

Forester, B. R., M. R. Jones, S. Joost, E. L. Landguth, and J. R. Lasky. 2016. Detecting spatial genetic signatures of local adaptation in heterogeneous landscapes. Molecular Ecology 25:104–120.

Fountain, M. T., V. K. Brown, A. C. Gange, W. O. C. Symondson, and P. J. Murray. 2007. The effects of the insecticide chlorpyrifos on spider and Collembola communities. Pedobiologia 51:147–158.

Frampton, G. K., S. Jänsch, J. J. Scott-Fordsmand, J. Römbke, and P. J. Van Den Brink. 2006. Effects of pesticides on soil invertebrates in laboratory studies: A review and analysis using species sensitivity distributions. Environmental Toxicology and Chemistry 25:2480–2489.

Frank, D., and H. John. 2007. Bunte Blumenwiesen – Erhöhung der Biodiversität oder Verstoß gegen Naturschutzrecht? Mitt. florist. Kart. Sachsen-Anhalt 12:31–45.

Fraser, L. H., and J. P. Grime. 1998. Top-down control and its effect on the biomass and composition of three grasses at high and low soil fertility in outdoor microcosms. Oecologia 113:239–246.

Fraser, L. H., J. Pither, A. Jentsch, M. Sternberg, M. Zobel, D. Askarizadeh, S. Bartha, C. Beierkuhnlein, J. A. Bennett, A. Bittel, B. Boldgiv, I. I. Boldrini, E. Bork, L. Brown, M. Cabido, J. Cahill, C. N. Carlyle, G. Campetella, S. Chelli, O. Cohen, A. M. Csergo, S. Díaz, L. Enrico, D. Ensing, A. Fidelis, J. D. Fridley, B. Foster, H. Garris, J. R. Goheen, H. A. L. Henry, M. Hohn, M. H. Jouri, J. Klironomos, K. Koorem, R. Lawrence-Lodge, R. Long, P. Manning, R. Mitchell, M. Moora, S. C. Müller, C. Nabinger, K. Naseri, G. E. Overbeck, T. M. Palmer, S. Parsons, M. Pesek, V. D. Pillar, R. M. Pringle, K. Roccaforte, A. Schmidt, Z. Shang, R. Stahlmann, G. C. Stotz, S. I. Sugiyama, S. Szentes, D. Thompson, R. Tungalag, S. Undrakhbold, M. Van Rooyen, C. Wellstein, J. B. Wilson, and T. Zupo. 2015. Worldwide evidence of a unimodal relationship between productivity and plant species richness. Science 349:302–305.

García-Guzmán, G., and M. Heil. 2014. Life histories of hosts and pathogens predict patterns in tropical fungal plant diseases. New Phytologist 201:1106–1120.

Gardes, M., and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113–118.

Gaspar, J. M., and W. K. Thomas. 2015. FlowClus: Efficiently filtering and denoising pyrosequenced amplicons. BMC Bioinformatics 16:1–9.

Gause, G. F. 1934. Experimental analysis of Vito Volterra's mathematical theory of the struggle for existence. Science 79:16–17.

Gerhold, P., M. Pärtel, O. Tackenberg, S. M. Hennekens, I. Bartish, J. H. J. Schaminée, A. J. F. Fergus, W. A. Ozinga, and A. Prinzing. 2011. Phylogenetically poor plant communities receive more alien species, which more easily coexist with natives. American Naturalist 177:668–680.

van Gestel, C. A. M., C. de Lima e Silva, T. Lam, J. C. Koekkoek, M. H. Lamoree, and R. A. Verweij. 2017. Multigeneration toxicity of imidacloprid and thiacloprid to Folsomia candida. Ecotoxicology 26:320–328.

Gibbons, S. M., Y. Lekberg, D. L. Mummey, N. Sangwan, P. W. Ramsey, and J. A. Gilbert. 2017. Invasive Plants Rapidly Reshape Soil Properties in a Grassland Ecosystem. mSystems 2:e00178-16.

Gilbert, B., and J. M. Levine. 2017. Ecological drift and the distribution of species diversity. Proceedings of the Royal Society B: Biological Sciences 284:20170507.

Gilbert, G. S. 2002. Evolutionary ecology of plant diseases in natural ecosystems. Annual Review of Phytopathology 40:13–43.

Gilbert, G. S. 2005. Dimensions of plant disease in tropical forests. Pages 141–164 *in* D. Burslem, M. Pinard, and S. Hartley, editors. Biotic interactions in the tropics: their role in the maintenance of species diversity. Cambridge University Press, New York, New York, USA.

Gilbert, G. S., H. M. Briggs, and R. Magarey. 2015. The impact of plant enemies shows a phylogenetic signal. PLoS ONE 10:1–11.

Gilbert, G. S., A. Ferrer, and J. Carranza. 2002. Polypore fungal diversity and host density in a moist tropical forest. Biodiversity and Conservation 11:947–957.

Gilbert, G. S., R. B. Foster, and S. P. Hubbell. 1994. Density and distance-to-adult effects of a canker disease of trees in a moist tropical forest. Oecologia 98:100–108.

Gilbert, G. S., R. Magarey, K. Suiter, and C. O. Webb. 2012. Evolutionary tools for phytosanitary risk analysis: Phylogenetic signal as a predictor of host range of plant pests and pathogens. Evolutionary Applications 5:869–878.

Gilbert, G. S., and I. M. Parker. 2006. Invasions and the regulation of plant populations by pathogens. Pages 289–305 *in* M. W. Cadotte, S. M. McMahon, and T. Fukami, editors. Conceptual Ecology and Invasion Biology: Reciprocal Approaches to Nature. Springer, Dordrecht, The Netherlands.

Gilbert, G. S., and I. M. Parker. 2016. The Evolutionary Ecology of Plant Disease: A Phylogenetic Perspective. Annual Review of Phytopathology 54:549–578.

Gilbert, G. S., and C. O. Webb. 2007. Phylogenetic signal in plant pathogen-host range. Proceedings of the National Academy of Sciences USA 104:4979–4983.

Godfray, H. C. J., T. Blacquière, L. M. Field, R. S. Hails, G. Petrokofsky, S. G. Potts, N. E. Raine, A. J. Vanbergen, and A. R. McLean. 2014. A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. Proceedings of the Royal Society B: Biological Sciences 281:20140558.

Godoy, O. 2019. Coexistence theory as a tool to understand biological invasions in species interaction networks: Implications for the study of novel ecosystems. Functional Ecology 33:1190–1201.

Godoy, O., L. Gómez-Aparicio, L. Matías, I. M. Pérez-Ramos, and E. Allan. 2020. An excess of niche differences maximizes ecosystem functioning. Nature Communications 11:1–10.

Goldmann, K., I. Schöning, F. Buscot, and T. Wubet. 2015. Forest management type influences diversity and community composition of soil fungi across temperate forest ecosystems. Frontiers in Microbiology 6:1300.

Goodland, R. J. 1975. The Tropical Origin of Ecology: Eugen Warming's Jubilee. Oikos 26:240–245.

Gossner, M. M., T. M. Lewinsohn, T. Kahl, F. Grassein, S. Boch, D. Prati, K. Birkhofer, S. C. Renner, J. Sikorski, T. Wubet, H. Arndt, V. Baumgartner, S. Blaser, N. Blüthgen, C. Börschig, F. Buscot, T. DIekötter, L. R. Jorge, K. Jung, A. C. Keyel, A. M. Klein, S. Klemmer, J. Krauss, M. Lange, J. Müller, J. Overmann, E. Pašali, C. Penone, D. J. Perovic, O. Purschke, P. Schall, S. A. Socher, I. Sonnemann, M. Tschapka, T. Tscharntke, M. Türke, P. C. Venter, C. N. Weiner, M. Werner, V. Wolters, S. Wurst, C. Westphal, M. Fischer, W. W. Weisser, and E. Allan. 2016. Land-use intensification causes multitrophic homogenization of grassland communities. Nature 540:266–269.

Griffin, E. A., M. B. Traw, P. J. Morin, J. N. Pruitt, S. J. Wright, and W. P. Carson. 2016. Foliar bacteria and soil fertility mediate seedling performance: A new and cryptic dimension of niche differentiation. Ecology 97:2998–3008.

Grinnell, J. 1904. The Origin and Distribution of the Chest-Nut-Backed Chickadee. The Auk 21:364–382.

Grinnell, J. 1924. Geography and Evolution. Ecology 5:225–229.

Gross, K. 2008. Positive interactions among competitors can produce species-rich communities. Ecology Letters 11:929–936.

Gross, N., P. Liancourt, R. Butters, R. P. Duncan, and P. E. Hulme. 2014. Functional equivalence, competitive hierarchy and facilitation determine species coexistence in highly invaded grasslands. New Phytologist 206:175–186.

Gundale, M. J., J. P. Almeida, H. Wallander, D. A. Wardle, P. Kardol, M. C. Nilsson, A. Fajardo, A. Pauchard, D. A. Peltzer, S. Ruotsalainen, B. Mason, and N. Rosenstock. 2016. Differences in endophyte communities of introduced trees depend on the phylogenetic relatedness of the receiving forest. Journal of Ecology 104:1219–1232.

Gupta, D., S. Garlaschi, S. Suweis, S. Azaele, and A. Maritan. 2021. Effective Resource Competition Model for Species Coexistence. Physical Review Letters 127:208101.

Gurevitch, J., G. A. Fox, G. M. Wardle, Inderjit, and D. Taub. 2011. Emergent insights from the synthesis of conceptual frameworks for biological invasions. Ecology Letters 14:407–418.

Haak, D. C., B. A. Ballenger, and L. C. Moyle. 2014. No evidence for phylogenetic constraint on natural defense evolution among wild tomatoes. Ecology 95:1633–1641.

Haddad, N. M., D. Tilman, J. Haarstad, M. Ritchie, and J. M. H. Knops. 2001. Contrasting effects of plant richness and composition on insect communities: A field experiment. American Naturalist 158:17–35.

Hage-Ahmed, K., K. Rosner, and S. Steinkellner. 2019. Arbuscular mycorrhizal fungi and their response to pesticides. Pest Management Science 75:583–590.

Hahn, M. 2014. The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as a case study. Journal of Chemical Biology 7:133–141.

Hahn, P. G., K. Keefover-Ring, L. M. N. Nguyen, and J. L. Maron. 2021. Intraspecific correlations between growth and defence vary with resource availability and differ within and among populations. Functional Ecology 35:2387–2396.

Halbach, K., M. Möder, S. Schrader, L. Liebmann, R. B. Schäfer, A. Schneeweiss, V. C. Schreiner, P. Vormeier, O. Weisner, M. Liess, and T. Reemtsma. 2021. Small streams–large concentrations? Pesticide monitoring in small agricultural streams in Germany during dry weather and rainfall. Water Research 203:117535.

Halliday, F. W., R. W. Heckman, P. A. Wilfahrt, and C. E. Mitchell. 2017. A multivariate test of disease risk reveals conditions leading to disease amplification. Proceedings of the Royal Society B: Biological Sciences 284:20171340.

Hambäck, P. A., and G. Englund. 2005. Patch area, population density and the scaling of migration rates: The resource concentration hypothesis revisited. Ecology Letters 8:1057–1065.

Hanlon, R. D. G. 1981. Some factors influencing microbial growth on soil animal faeces. II. Bacterial and fungal growth on soil animal faeces. Pedobiologia 21.

Harvey, K. J., D. A. Nipperess, D. R. Britton, and L. Hughes. 2012. Australian family ties: Does a lack of relatives help invasive plants escape natural enemies? Biological Invasions 14:2423–2434.

Hawkes, C. V. 2007. Are invaders moving targets? The generality and persistence of advantages in size, reproduction, and enemy release in invasive plant species with time since introduction. American Naturalist 170:832–843.

Hawkes, C. V., I. F. Wren, D. J. Herman, and M. K. Firestone. 2005. Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. Ecology Letters 8:976–985.

Heard, M. J., and D. F. Sax. 2013. Coexistence between native and exotic species is facilitated by asymmetries in competitive ability and susceptibility to herbivores. Ecology Letters 16:206–213.

Hector, A., J. Joshi, M. Scherer-Lorenzen, B. Schmid, E. M. Spehn, L. Wacker, M. Weilenmann, E. Bazeley-White, C. Beierkuhnlein, M. C. Caldeira, P. G. Dimitrakopoulos, J. A. Finn, K. Huss-Danell, A. Jumpponen, P. W. Leadley, M. Loreau, C. P. H. Mulder, C. Neßhöver, C. Palmborg, D. J. Read, A. S. D. Siamantziouras, A. C. Terry, and A. Y. Troumbis. 2007. Biodiversity and ecosystem functioning: Reconciling the results of experimental and observational studies. Functional Ecology 21:998–1002.

Van Der Heijden, M. G. A., R. D. Bardgett, and N. M. Van Straalen. 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11:296–310.

Henderson, I. F., and R. O. Clements. 1979. Differential Susceptibility To Pest Damage In Agricultural Grasses. The Journal of Agricultural Science 93:465–472.

Hendriks, M., J. M. Ravenek, A. E. Smit-Tiekstra, J. W. van der Paauw, H. de Caluwe, W. H. van der Putten, H. de Kroon, and L. Mommer. 2015. Spatial heterogeneity of plant-soil feedback affects root interactions and interspecific competition. New Phytologist 207:830–840.

Henriksen, B., and O. Elen. 2005. Natural Fusarium grain infection level in wheat, barley and oat after early application of fungicides and herbicides. Journal of Phytopathology 153:214–220.

Herms, D. A., and W. J. Mattson. 1992. The dilemma of plants: To grow or defend. Quarterly Review of Biology 67:283–335.

Heusinkveld, H. J., J. Molendijk, M. Van den Berg, and R. H. S. Westerink. 2013. Azole fungicides disturb intracellular Ca2+ in an additive manner in dopaminergic PC12 cells. Toxicological Sciences 134:374–381.

Hierro, J. L., J. L. Maron, and R. M. Callaway. 2005. A biogeographical approach to plant invasions: The importance of studying exotics in their introduced and native range. Journal of Ecology 93:5–15.

Hierro, J. L., D. Villarreal, O. Eren, J. M. Graham, and R. M. Callaway. 2006. Disturbance facilitates invasion: the effects are stronger abroad than at home. The American Naturalist 168:144–156.

HilleRisLambers, J., P. B. Adler, W. S. Harpole, J. M. Levine, and M. M. Mayfield. 2012. Rethinking Community Assembly through the Lens of Coexistence Theory. Annual Review of Ecology, Evolution, and Systematics 43:227–248.

HilleRisLambers, J., W. S. Harpole, D. Tilman, J. Knops, and P. B. Reich. 2004. Mechanisms responsible for the positive diversity-productivity relationship in Minnesota grasslands. Ecology Letters 7:661–668.

Hladik, M. L., A. R. Main, and D. Goulson. 2018. Environmental Risks and Challenges Associated with Neonicotinoid Insecticides. Environmental Science and Technology 52:3329–3335.

Hoeksema, J. D., J. D. Bever, S. Chakraborty, V. B. Chaudhary, M. Gardes, C. A. Gehring, M. M. Hart, E. A. Housworth, W. Kaonongbua, J. N. Klironomos, M. J. Lajeunesse, J. Meadow, B. G. Milligan, B. J. Piculell, A. Pringle, M. A. Rúa, J. Umbanhowar, W. Viechtbauer, Y. W. Wang, G. W. T. Wilson, and P. C. Zee. 2018. Evolutionary history of plant hosts and fungal symbionts predicts the strength of mycorrhizal mutualism. Communications biology 1:116.

Hogenhout, S. A., E. D. Ammar, A. E. Whitfield, and M. G. Redinbaugh. 2008. Insect vector interactions with persistently transmitted viruses. Annual Review of Phytopathology 46:327–359.

Holdredge, C., M. D. Bertness, E. Von Wettberg, and B. R. Silliman. 2010. Nutrient enrichment enhances hidden differences in phenotype to drive a cryptic plant invasion. Oikos 119:1776–1784.

von Holle, B., and D. Simberloff. 1999. Positive interactions of nonindigenous species: invasional meltdown? Biological invasions 1:21–32.

Holt, R. D. 1984. Spatial Heterogeneity, Indirect Interactions, and the Coexistence of Prey Species. The American Naturalist 124:377–406.

Holt, R. D., and A. P. Dobson. 2006. Extending the principles of community ecology to address the epidemiology of hostpathogen systems. Pages 6–27 *in* S. K. Collinge and C. Ray, editors. Disease Ecology: Community Structure and Pathogen Dynamics. Oxford University Press, Oxford, UK.

Holt, R. D., and J. Pickering. 1985. Infectious disease and species coexistence: a model of Lotka- Volterra form. American Naturalist 126:196–211.

Hubbell, S. P. 2001. The Unified Neutral Theory of Biodiversity and Biogeography. Princeton University Press, Princeton, New Jersey, USA.

Hutchinson, G. E. 1957. A treatise on limnology. Vol. 1. Geography, physics, chemistry. Page John Wiley & Sons. New York City, NY, USA.

Hutchinson, G. E. 1959. Homage to Santa Rosalia or Why Are There So Many Kinds of Animals? The American Naturalist 93:145–159.

Ihrmark, K., I. T. M. Bödeker, K. Cruz-Martinez, H. Friberg, A. Kubartova, J. Schenck, Y. Strid, J. Stenlid, M. Brandström-Durling, K. E. Clemmensen, and B. D. Lindahl. 2012. New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiology Ecology 82:666–677.

James, T. Y., F. Kauff, C. L. Schoch, P. B. Matheny, V. Hofstetter, C. J. Cox, G. Celio, C. Gueidan, E. Fraker, J. Miadlikowska, H. T. Lumbsch, A. Rauhut, V. Reeb, A. E. Arnold, A. Amtoft, J. E. Stajich, K. Hosaka, G. H. Sung, D. Johnson, B. O'Rourke, M. Crockett, M. Binder, J. M. Curtis, J. C. Slot, Z. Wang, A. W. Wilson, A. Schüßler, J. E. Longcore, K. O'Donnell, S. Mozley-Standridge, D. Porter, P. M. Letcher, M. J. Powell, J. W. Taylor, M. M. White, G. W. Griffith, D. R. Davies, R. A. Humber, J. B. Morton, J. Sugiyama, A. Y. Rossman, J. D. Rogers, D. H. Pfister, D. Hewitt, K. Hansen, S. Hambleton, R. A. Shoemaker, J. Kohlmeyer, B. Volkmann-Kohlmeyer, R. A. Spotts, M. Serdani, P. W. Crous, K. W. Hughes, K. Matsuura, E. Langer, G. Langer, W. A. Untereiner, R. Lücking, B. Büdel, D. M. Geiser, A. Aptroot, P. Diederich, I. Schmitt, M. Schultz, R. Yahr, D. S. Hibbett, F. Lutzoni, D. J. McLaughlin, J. W. Spatafora, and R. Vilgalys. 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443:818–822.

Janzen, D. H. 1970. Herbivores and the number of tree species in tropical forests. American Naturalist 104:501–528.

Jarosz, A. M., and A. L. Davelos. 1995. Effects of disease in wild plant populations and the evolution of pathogen aggressiveness. New Phytologist 129:371–387.

Jeschke, P., R. Nauen, M. Schindler, and A. Elbert. 2011. Overview of the status and global strategy for neonicotinoids. Journal of Agricultural and Food Chemistry 59:2897–2908.

Jongejans, E., M. B. Soons, and H. De Kroon. 2006. Bottlenecks and spatiotemporal variation in the sexual

reproduction pathway of perennial meadow plants. Basic and Applied Ecology 7:71–81.
Jorritsma-Wienk, L. D., E. Ameloot, J. P. M. Lenssen, and H. De Kroon. 2007. Differential responses of germination and seedling establishment in populations of Tragopogon pratensis (Asteraceae). Plant Biology 9:109–115.
Joshi, J., S. J. Otway, J. Koricheva, A. B. Pfisterer, J. Alphei, B. A. Roy, M. Scherer-Lorenzen, B. Schmid, E. Spehn, and A. Hector. 2004. Bottom-Up Effects and Feedbacks in Simple and Diverse Experimental Grassland Communities. Pages 115–134 *in* S. E. Weisser WW, editor. Insects and Ecosystem Function. Springer, Berlin.
Kandlikar, G. S., C. A. Johnson, X. Yan, N. J. B. Kraft, and J. M. Levine. 2019. Winning and losing with microbes: how microbially mediated fitness differences influence plant diversity. Ecology Letters 22:1178–1191.
Karlsson, I., H. Friberg, C. Steinberg, and P. Persson. 2014. Fungicide effects on fungal community composition in the wheat phyllosphere. PLoS ONE 9:e111786.
Karunarathna, A., S. Tibpromma, R. S. Jayawardena, C. Nanayakkara, S. Asad, J. Xu, K. D. Hyde, S. C. Karunarathna, S. L. Stephenson, S. Lumyong, and J. Kumla. 2021. Fungal Pathogens in Grasslands. Frontiers in Cellular and Infection Microbiology 11:695087.
Kassambara, A. 2020. Package 'ggpubr': "ggplot2" Based Publication Ready Plots. R package version 0.4.0.
Ke, P., and J. Wan. 2019. Effects of soil microbes on plant competition: a perspective from modern coexistence theory. Ecological Monographs 90:e01391.
Keane, R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. Trends Ecol. Evol. 17:164–170.
Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters 9:485–98.
Kempel, A., M. Razanajatovo, C. Stein, S. B. Unsicker, H. Auge, W. W. Weisser, M. Fischer, and D. Prati. 2015. Herbivore preference drives plant community composition. Ecology 96:2923–2934.
Kempel, A., H. Vincent, D. Prati, and M. Fischer. 2020. Context dependency of biotic interactions and its relation to plant rarity. Diversity and Distributions 26:758–768.
Kempson, D., M. Lloyd, and R. Ghelardi. 1963. A new extractor for woodland litter. Pedobiologia 3:1–21.
Kieltyk, P., and Z. Mirek. 2015. Importance of molehill disturbances for invasion by Bunias orientalis in meadows and pastures. Acta Oecologica 64:29–34.
Kirichenko, N., C. Péré, Y. Baranchikov, U. Schaffner, and M. Kenis. 2013. Do alien plants escape from natural enemies of congeneric residents? Yes but not from all. Biological Invasions 15:2105–2113.
Klenke, F., and M. Scholler. 2015. Pflanzenparasitische Kleinpilze Bestimmungsbuch für Brand-, Rost-, Mehltau-, Flagellatenpilze und Wucherlingsverwandte in Deutschland, Österreich, der Schweiz und Südtirol. Springer Spektrum, Berlin Heidelberg, Germany.
van Kleunen, M., W. Dawson, F. Essl, J. Pergl, M. Winter, E. Weber, H. Kreft, P. Weigelt, J. Kartesz, M. Nishino, L. A. Antonova, J. F. Barcelona, F. J. Cabezas, D. Cárdenas, J. Cárdenas-Toro, N. Castaño, E. Chacón, C. Chatelain, A. L. Ebel, E. Figueiredo, N. Fuentes, Q. J. Groom, L. Henderson, Inderjit, A. Kupriyanov, S. Masciadri, J. Meerman, O. Morozova, D. Moser, D. L. Nickrent, A. Patzelt, P. B. Pelser, M. P. Baptiste, M. Poopath, M. Schulze, H. Seebens, W. S. Shu, J. Thomas, M. Velayos, J. J. Wieringa, and P. Pyšek. 2015. Global exchange and accumulation of non-native plants. Nature 525:100–103.
van Kleunen, M., and M. Fischer. 2009. Release from foliar and floral fungal pathogen species does not explain the geographic spread of naturalized North American plants in Europe. Journal of Ecology 97:385–392.
Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67–70.
Klironomos, J. N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301.
Klöppel, H., and W. Kördel. 1997. Pesticide volatilization and exposure of terrestrial ecosystems. Chemosphere 35:1271–1289.
Klotz, S., I. Kühn, and W. Durka. 2002. BIOLFLOR - Eine Datenbank mit biologisch-ökologischen Merkmalen zur Flora von Deutschland. Schriftenreihe für Vegetationskunde.
Koffel, T., T. Daufresne, and C. A. Klausmeier. 2021. From competition to facilitation and mutualism: a general theory of the niche. Ecological Monographs 91:e01458.
Köhler, H. R., and R. Triebskorn. 2013. Wildlife ecotoxicology of pesticides: Can we track effects to the

population level and beyond? Science 341:759–765.
Kõljalg, U., R. H. Nilsson, K. Abarenkov, L. Tedersoo, A. F. S. Taylor, M. Bahram, S. T. Bates, T. D. Bruns, J. Bengtsson-Palme, T. M. Callaghan, B. Douglas, T. Drenkhan, U. Eberhardt, M. Dueñas, T. Grebenc, G. W. Griffith, M. Hartmann, P. M. Kirk, P. Kohout, E. Larsson, B. D. Lindahl, R. Lücking, M. P. Martín, P. B. Matheny, N. H. Nguyen, T. Niskanen, J. Oja, K. G. Peay, U. Peintner, M. Peterson, K. Põldmaa, L. Saag, I. Saar, A. Schüßler, J. A. Scott, C. Senés, M. E. Smith, A. Suija, D. L. Taylor, M. T. Telleria, M. Weiss, and K. H. Larsson. 2013. Towards a unified paradigm for sequence-based identification of fungi. Molecular Ecology 22:5271–5277.
Korell, L., H. Auge, M. Schädler, R. Brandl, and S. Schreiter. 2019. Release from above-and belowground insect herbivory mediates invasion dynamics and impact of an exotic plant. Plants 8:544.
Korell, L., R. Schmidt, H. Bruelheide, I. Hensen, and H. Auge. 2016a. Mechanisms driving diversity–productivity relationships differ between exotic and native communities and are affected by gastropod herbivory. Oecologia 180:1025–1036.
Korell, L., C. Stein, I. Hensen, H. Bruelheide, K. N. Suding, and H. Auge. 2016b. Stronger effect of gastropods than rodents on seedling establishment, irrespective of exotic or native plant species origin. Oikos 125:1467–1477.
Koricheva, J., H. Nykänen, and E. Gianoli. 2004. Meta-analysis of trade-offs among plant antiherbivore defenses: are plants jacks-of-all-trades, masters of all? The American naturalist 163:64–75.
Körner, K., H. Pfestorf, F. May, and F. Jeltsch. 2014. Modelling the effect of belowground herbivory on grassland diversity. Ecological Modelling 273:79–85.
Krupinsky, J. M., K. L. Bailey, M. P. McMullen, B. D. Gossen, and T. Kelly Turkington. 2002. Managing plant disease risk in diversified cropping systems. Agronomy Journal 94:198–209.
Kulmatiski, A., K. H. Beard, J. R. Stevens, and S. M. Cobbold. 2008. Plant-soil feedbacks: A meta-analytical review. Ecology Letters 11:980–992.
Kuster, T. M., A. Wilkinson, P. W. Hill, D. L. Jones, and R. D. Bardgett. 2016. Warming alters competition for organic and inorganic nitrogen between co-existing grassland plant species. Plant and Soil 406:117–129.
Laine, A. L., J. J. Burdon, P. N. Dodds, and P. H. Thrall. 2011. Spatial variation in disease resistance: From molecules to metapopulations. Journal of Ecology 99:96–112.
Laine, A. L., and I. Hanski. 2006. Large-scale spatial dynamics of a specialist plant pathogen in a fragmented landscape. Journal of Ecology 94:217–226.
Laliberté, E., H. Lambers, T. I. Burgess, and S. J. Wright. 2015. Phosphorus limitation, soil-borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. New Phytologist 206:507–521.
Lanuza, J. B., I. Bartomeus, and O. Godoy. 2018. Opposing effects of floral visitors and soil conditions on the determinants of competitive outcomes maintain species diversity in heterogeneous landscapes. Ecology Letters 21:865–874.
Lau, J. A., J. Strengbom, L. R. Stone, P. B. Reich, and P. Tiffin. 2008. Direct and indirect effects of CO2, nitrogen, and community diversity on plant-enemy interactions. Ecology 89:226–236.
Lebeda, A., I. Petrželová, and Z. Maryška. 2008. Structure and variation in the wild-plant pathosystem: Lactuca serriola-Bremia lactucae. European Journal of Plant Pathology 122:127–146.
Leff, J. W., R. D. Bardgett, A. Wilkinson, B. G. Jackson, W. J. Pritchard, J. R. De Long, S. Oakley, K. E. Mason, N. J. Ostle, D. Johnson, E. M. Baggs, and N. Fierer. 2018. Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. ISME Journal 12:1794–1805.
Leffler, A. J., J. J. James, T. A. Monaco, and R. L. Sheley. 2014. A new perspective on trait differences between native and invasive exotic plants. Ecology 95:298–305.
Leibold, M. A. 1995. The niche concept revisited: Mechanistic models and community context. Ecology 76:1371–1382.
Leibold, M. A., and M. A. McPeek. 2006. Coexistence of the niche and neutral perspectives in community ecology. Ecology 87:1399–1410.
Leibold, M. A., M. C. Urban, L. De Meester, C. A. Klausmeier, and J. Vanoverbeke. 2019. Regional neutrality evolves through local adaptive niche evolution. Proceedings of the National Academy of Sciences of the United States of America 116:2612–2617.
Lekberg, Y., J. D. Bever, R. A. Bunn, R. M. Callaway, M. M. Hart, S. N. Kivlin, J. Klironomos, B. G. Larkin, J. L. Maron, K. O. Reinhart, M. Remke, and W. H. van der Putten. 2018. Relative importance of competition and plant–soil feedback, their synergy, context dependency and implications for

coexistence. Ecology Letters 21:1268–1281.
Lentendu, G., L. Zinger, S. Manel, E. Coissac, P. Choler, R. A. Geremia, and C. Melodelima. 2011. Assessment of soil fungal diversity in different alpine tundra habitats by means of pyrosequencing. Fungal Diversity 49:113–123.
Lester, P. J., M. A. M. Gruber, E. C. Brenton-Rule, M. Archer, J. C. Corley, L. Dvořák, M. Masciocchi, and A. Van Oystaeyen. 2014. Determining the origin of invasions and demonstrating a lack of enemy release from microsporidian pathogens in common wasps (Vespula vulgaris). Diversity and Distributions 20:964–974.
Leung, M. R., and M. Kot. 2015. Models for the spread of white pine blister rust. Journal of Theoretical Biology 382:328–36.
Levine, J. M., P. B. Adler, and S. G. Yelenik. 2004. A meta-analysis of biotic resistance to exotic plant invasions. Ecology Letters 7:975–989.
Levine, J. M., J. Bascompte, P. B. Adler, and S. Allesina. 2017. Beyond pairwise mechanisms of species coexistence in complex communities. Nature 546:57–64.
Levine, J. M., and J. HilleRisLambers. 2009. The importance of niches for the maintenance of species diversity. Nature 461:254–257.
Lewis, S. L., and M. A. Maslin. 2015. Defining the Anthropocene. Nature 519:171–180.
de Lima e Silva, C., N. Brennan, J. M. Brouwer, D. Commandeur, R. A. Verweij, and C. A. M. van Gestel. 2017. Comparative toxicity of imidacloprid and thiacloprid to different species of soil invertebrates. Ecotoxicology 26:555–564.
Lindblad, I. 2000. Host specificity of some wood-inhabiting fungi in a tropical forest. Mycologia 92:399–405.
Liu, H., and P. Stiling. 2006. Testing the enemy release hypothesis: A review and meta-analysis. Biological Invasions 8:1535–1545.
Liu, X., S. Lyu, D. Sun, C. J. A. Bradshaw, and S. Zhou. 2017. Species decline under nitrogen fertilization increases community-level competence of fungal diseases. Proceedings of the Royal Society B: Biological Sciences 284:20162621.
Lockwood, J., and M. McKinney. 2001. Biotic homogenization. J. Lockwood and M. McKinney, Eds.). Springer, Boston, Massachusetts, USA.
Lombardero, M. J., M. Alonso-Rodríguez, and E. P. Roca-Posada. 2012. Tree insects and pathogens display opposite tendencies to attack native vs. non-native pines. Forest Ecology and Management 281:121–129.
Loranger, H., W. W. Weisser, A. Ebeling, T. Eggers, E. De Luca, J. Loranger, C. Roscher, and S. T. Meyer. 2014. Invertebrate herbivory increases along an experimental gradient of grassland plant diversity. Oecologia 174:183–193.
Lotka, A. J. 1932. The growth of mixed populations: Two species competing for a common food supply. J. Wash. Acad. Sci. 22:461–469.
Lozon, J. D., and H. J. MacIsaac. 1997. Biological invasions: are they dependent on disturbance? Environmental Reviews 5:131–144.
Lucas, J. A., N. J. Hawkins, and B. A. Fraaije. 2015. The Evolution of Fungicide Resistance. Advances in Applied Microbiology 90:29–92.
Lucero, J. E., N. M. Arab, S. T. Meyer, R. W. Pal, R. A. Fletcher, D. U. Nagy, R. M. Callaway, and W. W. Weisser. 2020. Escape from natural enemies depends on the enemies, the invader, and competition. Ecology and Evolution 10:10818–10828.
Ma, J., C. Cheng, Z. Du, B. Li, J. Wang, J. Wang, Z. Wang, and L. Zhu. 2019. Toxicological effects of pyraclostrobin on the antioxidant defense system and DNA damage in earthworms (Eisenia fetida). Ecological Indicators 101:111–116.
Mabubu, J. I., M. Nawaz, W. Cai, J. Zhao, Y. He, and H. Hua. 2017. Ecotoxicity of the Neonicotinoid Insecticides Imidacloprid and Thiacloprid to the Soil-Dwelling Arthropod Folsomia candida (Collembola). Journal of the Kansas Entomological Society 90:323–333.
MacArthur, R. H. 1958. Population Ecology of Some Warblers of Northeastern Coniferous Forests. Ecology 39:599–619.
MacDougall, A. S., B. Gilbert, and J. M. Levine. 2009. Plant invasions and the niche. Journal of Ecology 97:609–615.
Mack, R. N. 1996. Biotic barriers to plant naturalization. Pages 39–46 Proceedings of the 9th International Symposium on Biological Control of Weeds.

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. Ecological Applications 10:689–710.

MacKay, J., and P. M. Kotanen. 2008. Local escape of an invasive plant, common ragweed (Ambrosia artemisiifolia L.), from above-ground and below-ground enemies in its native area. Journal of Ecology 96:1152–1161.

Majewska, M. L., K. Rola, A. M. Stefanowicz, M. Nobis, J. Błaszkowski, and S. Zubek. 2018. Do the impacts of alien invasive plants differ from expansive native ones? An experimental study on arbuscular mycorrhizal fungi communities. Biology and Fertility of Soils 54:631–643.

Mangan, S. A., S. A. Schnitzer, E. A. Herre, K. M. L. Mack, M. C. Valencia, E. I. Sanchez, and J. D. Bever. 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466:752–5.

Maron, J. L., K. C. Baer, and A. L. Angert. 2014. Disentangling the drivers of context-dependent plant-animal interactions. Journal of Ecology 102:1485–1496.

Maron, J. L., and P. G. Connors. 1996. A native nitrogen-fixing shrub facilitates weed invasion. Oecologia 105:302–312.

Maron, J. L., and E. Crone. 2006. Herbivory: Effects on plant abundance, distribution and population growth. Proceedings of the Royal Society B: Biological Sciences 273:2575–2584.

Maron, J. L., M. Marler, J. N. Klironomos, and C. C. Cleveland. 2011. Soil fungal pathogens and the relationship between plant diversity and productivity. Ecology Letters 14:36–41.

Maron, J. L., and M. Vila. 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. Oikos 95:361–373.

Maron, J. L., M. Vilà, R. Bommarco, S. Elmendorf, and P. Beardsley. 2004. Rapid evolution of an invasive plant. Ecological Monographs 74:261–280.

Mason, N. W. H., K. Orwin, S. Lambie, S. L. Woodward, T. Mccready, and P. Mudge. 2016. Leaf economics spectrum-productivity relationships in intensively grazed pastures depend on dominant species identity. Ecology and Evolution 6:3079–3091.

Massoni, J., M. Bortfeld-Miller, L. Jardillier, G. Salazar, S. Sunagawa, and J. A. Vorholt. 2019. Consistent host and organ occupancy of phyllosphere bacteria in a community of wild herbaceous plant species. ISME Journal 14:245–258.

Mathesius, U. 2001. Flavonoids induced in cells undergoing nodule organogenesis in white clover are regulators of auxin breakdown by peroxidase. Journal of Experimental Botany 52:419–426.

Matson, P. A., W. J. Parton, A. G. Power, and M. J. Swift. 1997. Agricultural intensification and ecosystem properties. Science 277:504–509.

Matsumoto, H., X. Fan, Y. Wang, P. Kusstatscher, J. Duan, S. Wu, S. Chen, K. Qiao, Y. Wang, B. Ma, G. Zhu, Y. Hashidoko, G. Berg, T. Cernava, and M. Wang. 2021. Bacterial seed endophyte shapes disease resistance in rice. Nature Plants 7:60–72.

May, R. M. 1972. Will a large complex system be stable? Nature 238:412–413.

May, R. M. 1991. A fondness for fungi. Nature 352:475–476.

McGinn, K. J., W. H. van der Putten, P. E. Hulme, N. Shelby, C. Weser, and R. P. Duncan. 2018. The influence of residence time and geographic extent on the strength of plant–soil feedbacks for naturalised Trifolium. Journal of Ecology 106:207–217.

McKane, R. B., L. C. Johnson, G. R. Shaver, K. J. Nadelhoffer, E. B. Rastetter, B. Fry, A. E. Giblin, K. Kielland, B. L. Kwlatkowski, J. A. Laundre, and G. Murray. 2002. Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. Nature 425:68–71.

McMurdie, P. J., and S. Holmes. 2013. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE 8:e61217.

Meemken, E. M., and M. Qaim. 2018. Organic Agriculture, Food Security, and the Environment. Annual Review of Resource Economics 10:39–63.

Meentemeyer, R., D. Rizzo, W. Mark, and E. Lotz. 2004. Mapping the risk of establishment and spread of sudden oak death in California. Forest Ecology and Management 200:195–214.

Meijer, K., M. Schilthuizen, L. Beukeboom, and C. Smit. 2016. A review and meta-analysis of the enemy release hypothesis in plant–herbivorous insect systems. PeerJ 4:e2778.

Melillo, J. M., A. D. McGuire, D. W. Kicklighter, B. Moore, C. J. Vorosmarty, and A. L. Schloss. 1993. Global climate change and terrestrial net primary production. Nature 363:234–240.

Metz, M. R., W. P. Sousa, and R. Valencia. 2010. Widespread density-dependent seedling mortality promotes species coexistence in a highly diverse Amazonian rain forest. Ecology 91:3675–3685.

Miller, T. E., J. H. Burns, P. Munguia, E. L. Walters, J. M. Kneitel, P. M. Richards, N. Mouquet, and H. L. Buckley. 2005. A critical review of twenty years' use of the resource-ratio theory. American Naturalist 165:439–448.

Mills, K. E., and J. D. Bever. 1998. Maintenance of diversity within plant communities: Soil pathogens as agents of negative feedback. Ecology 79:1595–1601.

Mishra, S., S. Hättenschwiler, and X. Yang. 2020. The plant microbiome: A missing link for the understanding of community dynamics and multifunctionality in forest ecosystems. Applied Soil Ecology 145:103345.

Mitchell, C. E., A. A. Agrawal, J. D. Bever, G. S. Gilbert, R. A. Hufbauer, J. N. Klironomos, J. L. Maron, W. F. Morris, I. M. Parker, A. G. Power, E. W. Seabloom, M. E. Torchin, and D. P. Vázquez. 2006. Biotic interactions and plant invasions. Ecology Letters 9:726–740.

Mitchell, C. E., D. Blumenthal, V. Jarošík, E. E. Puckett, and P. Pyšek. 2010. Controls on pathogen species richness in plants' introduced and native ranges: Roles of residence time, range size and host traits. Ecology Letters 13:1525–1535.

Mitchell, C. E., and A. G. Power. 2003. Release of invasive plants from fungal and viral pathogens. Nature 421:625–627.

Mitchell, C. E., P. B. Reich, D. Tilman, and J. V. Groth. 2003. Effects of elevated CO2, nitrogen deposition, and decreased species diversity on foliar fungal plant disease. Global Change Biology 9:438–451.

Mitchell, C. E., D. Tilman, and J. V. Groth. 2002. Effects of grassland plant species diversity, abundance, and composition on foliar fungal disease. Ecology 83:1713–1726.

Mittelbach, G. G., C. F. Steiner, S. M. Scheiner, K. L. Gross, H. L. Reynolds, R. B. Waide, M. R. Willig, S. I. Dodson, and L. Gough. 2001. What is the observed relationship between species richness and productivity? Ecology 82:2381–2396.

Mokany, K., J. Ash, and S. Roxburgh. 2008. Functional identity is more important than diversity in influencing ecosystem processes in a temperate native grassland. Journal of Ecology 96:884 –893.

Mommer, L., T. E. A. Cotton, J. M. Raaijmakers, A. J. Termorshuizen, J. van Ruijven, M. Hendriks, S. Q. van Rijssel, J. E. van de Mortel, J. W. van der Paauw, E. G. W. M. Schijlen, A. E. Smit-Tiekstra, F. Berendse, H. de Kroon, and A. J. Dumbrell. 2018. Lost in diversity: the interactions between soil-borne fungi, biodiversity and plant productivity. New Phytologist 218:542–553.

Mooney, H. A., and R. J. Hobbs. 2000. Invasive Species in a changing world. Island Press, Washington, D.C., USA.

Moore, S. M., and E. T. Borer. 2012. The influence of host diversity and composition on epidemiological patterns at multiple spatial scales. Ecology 93:1095–1105.

Mordecai, E. A. 2011. Pathogen impacts on plant communities: unifying theory, concepts, and emirical work. Ecological Monographs 81:429–441.

Mordecai, E. A. 2013. Despite spillover, a shared pathogen promotes native plant persistence in a cheatgrass-invaded grassland. Ecology 94:2744–2753.

Moreby, S. J., N. W. Sotherton, and P. C. Jepson. 1997. The effects of pesticides on species of non-target heteroptera inhabiting cereal fields in southern England. Pesticide Science 51:39–47.

Moretti, M., F. De Bello, S. Ibanez, S. Fontana, G. B. Pezzatti, F. Dziock, C. Rixen, and S. Lavorel. 2013. Linking traits between plants and invertebrate herbivores to track functional effects of land-use changes. Journal of Vegetation Science 24:949–962.

Mulé, R., G. Sabella, L. Robba, and B. Manachini. 2017. Systematic review of the effects of chemical insecticides on four common butterfly families. Frontiers in Environmental Science 5:32.

Mundt, C. C., and J. A. Browning. 1985. 17 - Genetic Diversity and Cereal Rust Management. Pages 527–560 *in* A. P. Roelfs and W. R. Bushnell, editors. The Cereal Rusts - Volume II - Diseases, Distribution, Epidemiology, and Control. Academic Press, Inc., Orlando, USA.

Murdoch, W. W. 1969. Switching in General Predators: Experiments on Predator Specificity and Stability of Prey Populations. Ecological Monographs 39:335–354.

Mursinoff, S., and A. J. M. Tack. 2017. Spatial variation in soil biota mediates plant adaptation to a foliar pathogen. New Phytologist 214:644–654.

Myers, J. H., and R. M. Sarfraz. 2017. Impacts of Insect Herbivores on Plant Populations. Annual Review of Entomology 62:207–230.

Newton, A. C., C. Gravouil, and J. M. Fountaine. 2010. Managing the ecology of foliar pathogens: Ecological tolerance in crops. Annals of Applied Biology 157:343–359.

Nguyen, N. H., Z. Song, S. T. Bates, S. Branco, L. Tedersoo, J. Menke, J. S. Schilling, and P. G. Kennedy.

2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20:241–248.
Nicholls, C. I., and M. A. Altieri. 2013. Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A review. Agronomy for Sustainable Development 33:257–274.
Norghauer, J. M., A. R. Martin, E. E. Mycroft, A. James, and S. C. Thomas. 2011. Island invasion by a threatened tree species: Evidence for natural enemy release of mahogany (Swietenia macrophylla) on Dominica, Lesser Antilles. PLoS ONE 6:e18790.
Novak, M., J. D. Yeakel, A. E. Noble, D. F. Doak, M. Emmerson, J. A. Estes, U. Jacob, M. T. Tinker, and J. T. Wootton. 2016. Characterizing Species Interactions to Understand Press Perturbations: What Is the Community Matrix? Annual Review of Ecology, Evolution, and Systematics 47:409–432.
Novak, S. J. 2007. The role of evolution in the invasion process. Proceedings of the National Academy of Sciences of the United States of America 104:3671–3672.
Oberforster, M. 2001. Bonitieren: den Krankheitsbefall von Getreide richtig einschaetzen. Oesterreichische Agentur fuer Gesundheit und Ernaehrungssicherheit GmbH, Vienna, Austria.
Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. Mcglinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2019. vegan: Community Ecology Package. R package version 2.4-2.
Olden, J. D., N. L. R. Poff, M. R. Douglas, M. E. Douglas, and K. D. Fausch. 2004. Ecological and evolutionary consequences of biotic homogenization. Trends in Ecology and Evolution 19:18–24.
Ordonez, A. 2014. Functional and phylogenetic similarity of alien plants to co-occurring natives. Ecology 95:191–1202.
Ostfeld, R. S., G. E. Glass, and F. Keesing. 2005. Spatial epidemiology: An emerging (or re-emerging) discipline. Trends in Ecology and Evolution 20:328–336.
Ostfeld, R. S., and F. Keesing. 2000. The function of biodiversity in the ecology of vector-borne zoonotic diseases. Canadian Journal of Zoology 78:2061–2078.
Packer, A., and K. Clay. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Nature 404:278–281.
Paine, R. T. 1969. A Note on Trophic Complexity and Community Stability. The American Naturalist 103:91–93.
Papaïx, J., J. J. Burdon, E. Walker, L. G. Barrett, and P. H. Thrall. 2021. Metapopulation structure predicts population dynamics in the cakile maritima–alternaria brassicicola host-pathogen interaction. American Naturalist 197:E55–E71.
Parker, I. M., and G. S. Gilbert. 2004. The Evolutionary Ecology of Novel Plant-Pathogen Interactions. Annual Review of Ecology, Evolution, and Systematics 35:675–700.
Parker, I. M., and G. S. Gilbert. 2007. When there is no escape: The effects of natural enemies on native, invasive, and noninvasive plants. Ecology 88:1210–1224.
Parker, I. M., and G. S. Gilbert. 2018. Density-dependent disease, life-history trade-offs, and the effect of leaf pathogens on a suite of co-occurring close relatives. Journal of Ecology 106:1829–1838.
Parker, I. M., M. Saunders, M. Bontrager, A. P. Weitz, R. Hendricks, R. Magarey, K. Suiter, and G. S. Gilbert. 2015. Phylogenetic structure and host abundance drive disease pressure in communities. Nature 520:542–544.
Parratt, S. R., and A. L. Laine. 2018. Pathogen dynamics under both bottom-up host resistance and top-down hyperparasite attack. Journal of Applied Ecology 55:2976–2985.
Paul, N. D., P. G. Ayres, and L. E. Wyness. 1989. On the Use of Fungicides for Experimentation in Natural Vegetation. Functional Ecology 3:759–769.
Pearse, I. S., and A. L. Hipp. 2009. Phylogenetic and trait similarity to a native species predict herbivory on non-native oaks. Proceedings of the National Academy of Sciences of the United States of America 106:18097–18102.
Pearse, W. D., M. W. Cadotte, J. Cavender-Bares, A. R. Ives, C. M. Tucker, S. C. Walker, and M. R. Helmus. 2015. Pez: Phylogenetics for the environmental sciences. Bioinformatics 31:2888–2890.
Peay, K. G., C. Baraloto, and P. V. A. Fine. 2013. Strong coupling of plant and fungal community structure across western Amazonian rainforests. ISME Journal 7:1852–1861.
Petermann, J. S., A. J. F. Fergus, L. A. Turnbull, and B. Schmid. 2008. Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. Ecology 89:2399–2406.
Pimentel, D., and D. A. Andow. 1984. Pest management and pesticide impacts. International Journal of Tropical Insect Science 5:141–149.

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. BioScience 50:53–65.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52:273–288.

Pinsky, M. L. 2019. Species coexistence through competition and rapid evolution. Proceedings of the National Academy of Sciences of the United States of America 116:2407–2409.

Pinto, C., D. Pinho, S. Sousa, M. Pinheiro, C. Egas, and A. C. Gomes. 2014. Unravelling the diversity of grapevine microbiome. PLoS ONE 9:e85622.

Pirozzi, A. V. A., A. Stellavato, A. La Gatta, M. Lamberti, and C. Schiraldi. 2016. Mancozeb, a fungicide routinely used in agriculture, worsens nonalcoholic fatty liver disease in the human HepG2 cell model. Toxicology Letters 249:1–4.

Pisa, L., D. Goulson, E. C. Yang, D. Gibbons, F. Sánchez-Bayo, E. Mitchell, A. Aebi, J. van der Sluijs, C. J. K. MacQuarrie, C. Giorio, E. Y. Long, M. McField, M. Bijleveld van Lexmond, and J. M. Bonmatin. 2021. An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 2: impacts on organisms and ecosystems. Environmental Science and Pollution Research 28:11749–11797.

Power, A. G., and C. E. Mitchell. 2004. Pathogen Spillover in Disease Epidemics. The American Naturalist 164:S79–S89.

Price, J. N., A. Gazol, R. Tamme, I. Hiiesalu, and M. Pärtel. 2014. The functional assembly of experimental grasslands in relation to fertility and resource heterogeneity. Functional Ecology 28:509–519.

Prober, S. M., J. W. Leff, S. T. Bates, E. T. Borer, J. Firn, W. S. Harpole, E. M. Lind, E. W. Seabloom, P. B. Adler, J. D. Bakker, E. E. Cleland, N. M. Decrappeo, E. Delorenze, N. Hagenah, Y. Hautier, K. S. Hofmockel, K. P. Kirkman, J. M. H. Knops, K. J. La Pierre, A. S. Macdougall, R. L. Mcculley, C. E. Mitchell, A. C. Risch, M. Schuetz, C. J. Stevens, R. J. Williams, and N. Fierer. 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. Ecology Letters 18:85–95.

Prospero, S., and M. Cleary. 2017. Effects of host variability on the spread of invasive forest diseases. Forests 8:80.

van der Putten, W. H., R. D. Bardgett, J. D. Bever, T. M. Bezemer, B. B. Casper, T. Fukami, P. Kardol, J. N. Klironomos, A. Kulmatiski, J. A. Schweitzer, K. N. Suding, T. F. J. Van de Voorde, and D. A. Wardle. 2013. Plant-soil feedbacks: The past, the present and future challenges. Journal of Ecology 101:265–276.

van der Putten, W. H., C. van Dijk, and B. A. M. Peters. 1993. Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature 362:53–56.

van der Putten, W. H., J. N. Klironomos, and D. A. Wardle. 2007. Microbial ecology of biological invasions. ISME Journal 1:28–37.

Pyšek, P., J. Pergl, F. Essl, B. Lenzner, W. Dawson, H. Kreft, P. Weigelt, M. Winter, J. Kartesz, M. Nishino, L. A. Antonova, J. F. Barcelona, F. J. Cabezas, D. Cárdenas, J. Cárdenas-Toro, N. Castaño, E. Chacón, C. Chatelain, S. Dullinger, A. L. Ebel, E. Figueiredo, N. Fuentes, P. Genovesi, Q. J. Groom, L. Henderson, Inderjit, A. Kupriyanov, S. Masciadri, N. Maurel, J. Meerman, O. Morozova, D. Moser, D. Nickrent, P. M. Nowak, S. Pagad, A. Patzelt, P. B. Pelser, H. Seebens, W. S. Shu, J. Thomas, M. Velayos, E. Weber, J. J. Wieringa, M. P. Baptiste, and M. Van Kleunen. 2017. Naturalized alien flora of the world: Species diversity, taxonomic and phylogenetic patterns, geographic distribution and global hotspots of plant invasion. Preslia 89:203–274.

R Development Core Team. 2019. R Version 3.6.2. Page R: A Language and Environmental for Estatistical Computing.

R Development Core Team 3.0.1. 2013. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing 2:https://www.R-project.org.

Raynaud, X., and P. W. Leadley. 2004. Soil characteristics play a key role in modeling nutrient competition in plant communities. Ecology 85:2200–2214.

Rees, M., and V. K. Brown. 1992. Interactions between invertebrate herbivores and plant competition. Journal of Ecology 80:353–360.

Reimann, S., and H. B. Deising. 2005. Inhibition of efflux transporter-mediated fungicide resistance in Pyrenophora tritici-repentis by a derivative of 4'-hydroxyflavone and enhancement of fungicide activity. Applied and Environmental Microbiology 71:3269–3275.

Reinhart, K. O., A. Packer, W. H. Van Der Putten, and K. Clay. 2003. Plant-soil biota interactions and

spatial distribution of black cherry in its native and invasive ranges. Ecology Letters 6:1046–1050.
Reinhart, K. O., T. Tytgat, W. H. Van der Putten, and K. Clay. 2010. Virulence of soil-borne pathogens and invasion by Prunus serotina. New Phytologist 186:484–495.
Richardson, D. M., N. Allsopp, C. M. D'antonio, S. J. Milton, and M. Rejmánek. 2000a. Plant invasions — the role of mutualisms. Biological Reviews 75:65–93.
Richardson, D. M., and P. Pyšek. 2006. Plant invasions: Merging the concepts of species invasiveness and community invasibility. Progress in Physical Geography 30:409–431.
Richardson, D. M., P. Pyšek, M. Rejmánek, M. G. Barbour, F. Dane Panetta, and C. J. West. 2000b. Naturalization and invasion of alien plants: Concepts and definitions. Diversity and Distributions 6:93–107.
Ricklefs, R., and J. Travis. 1980. A Morphological Approach to the Study of Avian Community Organization. The Auk 91:321–338.
Ritchie, M. E. 2000. Nitrogen limitation and trophic vs. abiotic influences on insect herbivores in a temperate grassland. Ecology 81:1601–1612.
Rizzo, D. M., and M. Garbelotto. 2003. Sudden Oak Death: Endangering California and Oregon Forest Ecosystems. Frontiers in Ecology and the Environment 1:197–204.
Rodriguez-Moreno, L., M. K. Ebert, M. D. Bolton, and B. P. H. J. Thomma. 2018. Tools of the crook-infection strategies of fungal plant pathogens. Plant Journal 93:664–674.
Rognes, T., T. Flouri, B. Nichols, C. Quince, and F. Mahé. 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584.
Rønsted, N., M. R. E. Symonds, T. Birkholm, S. B. Christensen, A. W. Meerow, M. Molander, P. Mølgaard, G. Petersen, N. Rasmussen, J. Van Staden, G. I. Stafford, and A. K. Jäger. 2012. Can phylogeny predict chemical diversity and potential medicinal activity of plants? A case study of amaryllidaceae. BMC Evolutionary Biology 12:182 (2012).
Root, R. B. 1973. Organization of a Plant-Arthropod Association in Simple and Diverse Habitats: The Fauna of Collards (Brassica Oleracea). Ecological Monographs 43:95–124.
Roth, G. A., W. G. Whitford, and Y. Steinberger. 2009. Small mammal herbivory: Feedbacks that help maintain desertified ecosystems. Journal of Arid Environments 73:62–65.
Rottstock, T., J. Joshi, V. Kummer, and M. Fischer. 2014. Higher plant diversity promotes higher diversity of fungal pathogens, while it decreases pathogen infection per plant. Ecology 95:1907–1917.
Rudgers, J. A., S. Y. Strauss, and J. F. Wendel. 2004. Trade-offs among anti-herbivore resistance traits: Insights from Gossypieae (Malvaceae). American Journal of Botany 91:871–880.
Salvaudon, L., T. Giraud, and J. A. Shykoff. 2008. Genetic diversity in natural populations: a fundamental component of plant-microbe interactions. Current Opinion in Plant Biology 11:135–143.
Santísima-Trinidad, A. B. L., M. del Mar Montiel-Rozas, M. Á. Diéz-Rojo, J. A. Pascual, and M. Ros. 2018. Impact of foliar fungicides on target and non-target soil microbial communities in cucumber crops. Ecotoxicology and Environmental Safety 166:78–85.
SAS Institute. 2015. SAS/IML® 14.1 User's Guide. Page 524. SAS Institute Inc.
Schädler, M., R. Brandl, and J. Haase. 2007. Antagonistic interactions between plant competition and insect herbivory. Ecology 88:1490–1498.
Schädler, M., G. Jung, R. Brandl, and H. Auge. 2004. Secondary succession is influenced by belowground insect herbivory on a productive site. Oecologia 138:242–252.
Schadt, C. W., and A. Rosling. 2015. Comment on "Global diversity and geography of soil fungi." Science 348:1438.
Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G. Thallinger, D. J. Van Horn, and C. F. Weber. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75:7537–7541.
Schmidt, R., H. Auge, H. B. Deising, I. Hensen, S. A. Mangan, M. Schädler, C. Stein, and T. M. Knight. 2020a. Abundance, origin, and phylogeny of plants do not predict community-level patterns of pathogen diversity and infection. Ecology and Evolution 10:5506–5516.
Schmidt, R., H. B. Deising, I. Hensen, M. Schädler, and H. Auge. 2020b. Natural enemies do not contribute to negative frequency-dependence in native and exotic grassland plants. Perspectives in Plant Ecology, Evolution and Systematics 46:125565.
Schmitz, O. J., P. A. Hambäck, and A. P. Beckerman. 2000. Trophic cascades in terrestrial systems: A review

of the effects of carnivore removals on plants. American Naturalist 155:141–153.
Schmitz, O. J., and K. B. Suttle. 2001. Effects of top predator species on direct and indirect interactions in a food web. Ecology 82:2072–2081.
Schnitzer, S. A., J. N. Klironomos, J. HilleRisLambers, L. L. Kinkel, P. B. Reich, K. Xiao, M. C. Rillig, B. A. Sikes, R. M. Callaway, S. A. Mangan, E. H. Van Nes, and M. Scheffer. 2011. Soil microbes drive the classic plant diversity-productivity pattern. Ecology 92:296–303.
Schutzenhofer, M. R., T. J. Valone, and T. M. Knight. 2009. Herbivory and population dynamics of invasive and native lespedeza. Oecologia 161:57–66.
Seabloom, E. W., E. T. Borer, K. Gross, A. E. Kendig, C. Lacroix, C. E. Mitchell, E. A. Mordecai, and A. G. Power. 2015. The community ecology of pathogens: Coinfection, coexistence and community composition. Ecology Letters 18:401–415.
Seastedt, T. R. 1984. The role of microarthropods in decomposition and mineralization processes. Annual review of entomology. Vol. 29 29:25–46.
Semchenko, M., J. W. Leff, Y. M. Lozano, S. Saar, J. Davison, A. Wilkinson, B. G. Jackson, W. J. Pritchard, J. R. De Long, S. Oakley, K. E. Mason, N. J. Ostle, E. M. Baggs, D. Johnson, N. Fierer, and R. D. Bardgett. 2018. Fungal diversity regulates plant-soil feedbacks in temperate grassland. Science Advances 4:eaau4578.
Shaffer, T. L., and D. H. Johnson. 2008. Ways of Learning: Observational Studies Versus Experiments. Journal of Wildlife Management 72:4–13.
Shaw, R. G., and T. Mitchell-Olds. 1993. ANOVA for unbalanced data: an overview. Ecology 74:1638–1645.
Shea, K., and P. Chesson. 2002. Community ecology theory as a framework for biological invasions. Trends in Ecology & Evolution 17:170–176.
Shi, S., L. Tian, S. Xu, L. Ji, F. Nasir, X. Li, Z. Song, and C. Tian. 2019. The rhizomicrobiomes of wild and cultivated crops react differently to fungicides. Archives of Microbiology 201:477–486.
Shurin, J. B. 2001. Interactive effects of predation and dispersal on zooplankton communities. Ecology 82:3404–3416.
Siemann, E., W. P. Carson, W. E. Rogers, and W. W. Weisser. 2004. Reducing Herbivory Using Insecticides. Pages 303–327 Insects and Ecosystem Function. Springer, Berlin and Heidelberg, Germany.
Siepielski, A. M., and M. A. McPeek. 2010. On the evidence for species coexistence: A critique of the coexistence program. Ecology 91:3153–3164.
Sikes, B. A., J. L. Bufford, P. E. Hulme, J. A. Cooper, P. R. Johnston, and R. P. Duncan. 2018. Import volumes and biosecurity interventions shape the arrival rate of fungal pathogens. PLoS Biology 16:e2006025.
Simberloff, D., J. L. Martin, P. Genovesi, V. Maris, D. A. Wardle, J. Aronson, F. Courchamp, B. Galil, E. García-Berthou, M. Pascal, P. Pyšek, R. Sousa, E. Tabacchi, and M. Vilà. 2013. Impacts of biological invasions: What's what and the way forward. Trends in Ecology and Evolution 28:58–66.
Smith, D. L., L. Ericson, and J. J. Burdon. 2011. Co-evolutionary hot and cold spots of selective pressure move in space and time. Journal of Ecology 99:634–641.
Song, C., G. Barabás, and S. Saavedra. 2019. On the consequences of the interdependence of stabilizing and equalizing mechanisms. American Naturalist 194:627–638.
Sonnberger, B., and F. Schuhwerk. 2005. Dianthus giganteus D'Urv. - ein verkannter Neophyt in Bayern? Berichte der Bayerischen Botanischen Gesellschaft 75:184–185.
Spear, E. R., P. D. Coley, and T. A. Kursar. 2015. Do pathogens limit the distributions of tropical trees across a rainfall gradient? Journal of Ecology 103:165–174.
Spear, E. R., and E. A. Mordecai. 2018. Foliar pathogens are unlikely to stabilize coexistence of competing species in a California grassland. Ecology 99:2250–2259.
Springer, Y. P. 2007. Clinal resistance structure and pathogen local adaptation in a serpentine flax-flax rust interaction. Evolution 61:1812–1822.
Steffen, W., J. Grinevald, P. Crutzen, and J. Mcneill. 2011. The anthropocene: Conceptual and historical perspectives.
Stein, C., and S. A. Mangan. 2020. Soil biota increase the likelihood for coexistence among competing plant species. Ecology 101:e03147.
Stein, C., S. S. Unsicker, A. Kahmen, M. Wagner, V. Audorff, H. Auge, D. Prati, and W. W. Weisser. 2010. Impact of invertebrate herbivory in grasslands depends on plant species diversity. Ecology 91:1639–1650.

Steinlein, T., H. Dietz, and I. Ullmann. 1996. Growth patterns of the alien perennial Bunias orientalis L. (Brassicaceae) underlying its rising dominance in some native plant assemblages. Vegetatio 125:73–82.

Strauss, S. Y., J. A. Rudgers, J. A. Lau, and R. E. Irwin. 2002. Direct and ecological costs of resistance to herbivory. Trends in Ecology and Evolution 17:278–285.

Stricker, K. B., P. F. Harmon, E. M. Goss, K. Clay, and S. Luke Flory. 2016. Emergence and accumulation of novel pathogens suppress an invasive species. Ecology Letters 19:469–477.

Stricker, K. B., and P. Stiling. 2014. Release from herbivory does not confer invasion success for Eugenia uniflora in Florida. Oecologia 174:817–826.

Suarez, A. V., and N. D. Tsutsui. 2008. The evolutionary consequences of biological invasions. Molecular Ecology 17:351–360.

Sułowicz, S., M. Cycoń, and Z. Piotrowska-Seget. 2016. Non-target impact of fungicide tetraconazole on microbial communities in soils with different agricultural management. Ecotoxicology 25:1047–1060.

Sweeney, C. J., F. T. de Vries, B. E. van Dongen, and R. D. Bardgett. 2021. Root traits explain rhizosphere fungal community composition among temperate grassland plant species. New Phytologist 229:1492–1507.

Swenson, N. G., and B. J. Enquist. 2007. Ecological and evolutionary determinants of a key plant functional trait: Wood density and its community-wide variation across latitude and elevation. American Journal of Botany 94:451–459.

Tamburini, G., E. Dani, R. Bommarco, and L. Marini. 2018. Effect of insect herbivory on plant community dynamics under contrasting water availability levels. Journal of Ecology 106:819–1828.

Tedersoo, L., M. Bahram, T. Cajthaml, S. Põlme, I. Hiiesalu, S. Anslan, H. Harend, F. Buegger, K. Pritsch, J. Koricheva, and K. Abarenkov. 2016. Tree diversity and species identity effects on soil fungi, protists and animals are context dependent. ISME Journal 10:346–362.

Tedersoo, L., M. Bahram, S. Põlme, S. Anslan, T. Riit, U. Kõljalg, R. H. Nilsson, F. Hildebrand, and K. Abarenkov. 2015. Response to Comment on "Global diversity and geography of soil fungi." Science 349:936.

Tedersoo, L., A. Tooming-Klunderud, and S. Anslan. 2018. PacBio metabarcoding of Fungi and other eukaryotes: errors, biases and perspectives. New Phytologist 217:1370–1385.

Těšitel, J., M. Tahadlová, J. Lepš, and N. Hölzel. 2021. Linking insect herbivory with plant traits: Phylogenetically structured trait syndromes matter. Journal of Vegetation Science 32:e13061.

Thimm, T., A. Hoffmann, H. Borkott, J. C. Munch, and C. C. Tebbe. 1998. The gut of the soil microarthropod Folsomia candida (Collembola) is a frequently changeable but selective habitat and a vector for microorganisms. Applied and Environmental Microbiology 64:2660–2669.

Thrall, P. H., J. J. Burdon, and J. D. Bever. 2002. Local adaptation in the Linum marginale-Melampsora lini host-pathogen interaction. Evolution 56:1340–1351.

Thrall, P. H., M. E. Hochberg, J. J. Burdon, and J. D. Bever. 2007. Coevolution of symbiotic mutualists and parasites in a community context. Trends in Ecology and Evolution 22:120–126.

Tilman, D. 1982. Resource competition and community structure. Page Monographs in population biology. Princeton University Press, Princeton, New Jersey, USA.

Tilman, D. 1985. The resource-ratio hypothesis of plant succession. American Naturalist 125:827–852.

Tilman, D. 1987. The Importance of the Mechanisms of Interspecific Competition. The American Naturalist 129:769–774.

Tilman, D. 1994. Competition and biodiversity in spatially structured habitats. Ecology 75:2–16.

Tilman, D. 2007. Resource competition and plant traits: A response to Craine et al. 2005: Forum. Journal of Ecology 95:231–234.

Tilman, D., P. B. Reich, and J. M. H. Knops. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441:629–632.

Tollenaere, C., B. Pernechele, H. S. Mäkinen, S. R. Parratt, M. Z. Németh, G. M. Kovács, L. Kiss, A. J. M. Tack, and A. L. Laine. 2014. A hyperparasite affects the population dynamics of a wild plant pathogen. Molecular Ecology 23:5877–5887.

Turchin, P. 1989. Population Consequences of Aggregative Movement. Journal of Animal Ecology 58:75–100.

Uddin, M. N., T. Asaeda, A. Sarkar, V. P. Ranawakage, and R. W. Robinson. 2021. Conspecific and heterospecific plant–soil biota interactions of Lonicera japonica in its native and introduced range: implications for invasion success. Plant Ecology 222:1313–1324.

Unsicker, S. B., N. Baer, A. Kahmen, M. Wagner, N. Buchmann, and W. W. Weisser. 2006. Invertebrate herbivory along a gradient of plant species diversity in extensively managed grasslands. Oecologia 150:233–246.

Vacher, C., D. Piou, and M. L. Desprez-Loustau. 2008. Architecture of an antagonistic tree/fungus network: The asymmetric influence of past evolutionary history. PLoS ONE 3:e1740.

Valladares, F., C. C. Bastias, O. Godoy, E. Granda, and A. Escudero. 2015. Species coexistence in a changing world. Frontiers in Plant Science 6:866.

Vargas, J. M. 2004. Management of Turfgrass Diseases. Page Management of Turfgrass Diseases. Wiley.

Vaz, A. B. M., P. L. C. Fonseca, F. Badotti, D. Skaltsas, L. M. R. Tomé, A. C. Silva, M. C. Cunha, M. A. Soares, V. L. Santos, G. Oliveira, P. Chaverri, and A. Góes-Neto. 2018. A multiscale study of fungal endophyte communities of the foliar endosphere of native rubber trees in Eastern Amazon. Scientific Reports 18:16151.

De Vienne, D. M., M. E. Hood, and T. Giraud. 2009. Phylogenetic determinants of potential host shifts in fungal pathogens. Journal of Evolutionary Biology 22:2532–2541.

Viola, D. V., E. A. Mordecai, A. G. Jaramillo, S. A. Sistla, L. K. Albertson, J. S. Gosnell, B. J. Cardinale, and J. M. Levine. 2010. Competition-defense tradeoffs and the maintenance of plant diversity. Proceedings of the National Academy of Sciences 107:17217–17222.

Volkov, I., J. R. Banavar, S. P. Hubbell, and A. Maritan. 2003. Neutral theory and relative species abundance in ecology. Nature 424:1035–1037.

Volterra, V. 1926. Fluctuations in the abundance of a species considered mathematically. Nature 118:558–560.

de Vries, F. T., P. Manning, J. R. B. Tallowin, S. R. Mortimer, E. S. Pilgrim, K. A. Harrison, P. J. Hobbs, H. Quirk, B. Shipley, J. H. C. Cornelissen, J. Kattge, and R. D. Bardgett. 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecology Letters 15:1230–1239.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naıve Bayesian Classifier for Rapid Assignment of rRNA Sequences.pdf. Applied and Environmental Microbiology 73:5261–5267.

Wang, S., and M. Loreau. 2016. Biodiversity and ecosystem stability across scales in metacommunities. Ecology Letters 19:510–518.

Wang, S., M. Loreau, C. de Mazancourt, F. Isbell, C. Beierkuhnlein, J. Connolly, D. H. Deutschman, J. Doležal, N. Eisenhauer, A. Hector, A. Jentsch, J. Kreyling, V. Lanta, J. Lepš, H. W. Polley, P. B. Reich, J. van Ruijven, B. Schmid, D. Tilman, B. Wilsey, and D. Craven. 2021. Biotic homogenization destabilizes ecosystem functioning by decreasing spatial asynchrony. Ecology 102:e03332.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. Van Der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science 304:1629–1633.

Warton, D. I., and F. K. C. Hui. 2011. The arcsine is asinine: The analysis of proportions in ecology. Ecology 92:3–10.

Westheide, W., and G. Rieger. 2013. Spezielle Zoologie. Page (W. Westheide and G. Rieger, Eds.). Third edition. Springer Spektrum.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 *in* T. J. W. M. A. Innis, D.H. Gelfand, J. Sninsky, editor. PCR Protocols. Academic Press, San Diego.

Williamson, M. 1996. Biological invasions. Chapman & Hall, London.

Wilson, J. B., R. K. Peet, J. Dengler, and M. Pärtel. 2012. Plant species richness: The world records. Journal of Vegetation Science 23:796–802.

Wolfe, L. M. 2002. Why alien invaders succeed: Support for the escape-from-enemy hypothesis. American Naturalist 160:705–711.

Wolfe, L. M., J. A. Elzinga, and A. Biere. 2004. Increased susceptibility to enemies following introduction in the invasive plant Silene latifolia. Ecology Letters 7:813–820.

Woodcock, B. A., J. M. Bullock, R. F. Shore, M. S. Heard, M. G. Pereira, J. Redhead, L. Ridding, H. Dean, D. Sleep, P. Henrys, J. Peyton, S. Hulmes, L. Hulmes, M. Sárospataki, C. Saure, M. Edwards, E. Genersch, S. Knäbe, and R. F. Pywell. 2017. Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. Science 356:1393–1395.

Woodward, J. A., and D. G. Bonett. 1991. Simple main effects in factorial designs. Journal of Applied Statistics 18:255–264.

Wubet, T., S. Christ, I. Schöning, S. Boch, M. Gawlich, B. Schnabel, M. Fischer, and F. Buscot. 2012.

Differences in Soil Fungal Communities between European Beech (Fagus sylvatica L.) Dominated Forests Are Related to Soil and Understory Vegetation. PLoS ONE 7:e47500.

Xu, M., X. Mu, S. Zhang, J. T. A. Dick, B. Zhu, D. Gu, Y. Yang, D. Luo, and Y. Hu. 2021. A global analysis of enemy release and its variation with latitude. Global Ecology and Biogeography 30:277–288.

Yang, L. N., O. Nkurikiyimfura, Z. C. Pan, Y. P. Wang, A. Waheed, R. S. Chen, J. J. Burdon, Q. J. Sui, and J. Zhan. 2021. Plant diversity ameliorates the evolutionary development of fungicide resistance in an agricultural ecosystem. Journal of Applied Ecology 58:2566–2578.

Yi, X., and A. M. Dean. 2013. Bounded population sizes, fluctuating selection and the tempo and mode of coexistence. Proceedings of the National Academy of Sciences 110:16945–16950.

Zaller, J. G., and C. A. Brühl. 2019. Editorial: Non-target Effects of Pesticides on Organisms Inhabiting Agroecosystems. Frontiers in Environmental Science 7:75.

Zanne, A. E., D. C. Tank, W. K. Cornwell, J. M. Eastman, S. A. Smith, R. G. Fitzjohn, D. J. McGlinn, B. C. O'Meara, A. T. Moles, P. B. Reich, D. L. Royer, D. E. Soltis, P. F. Stevens, M. Westoby, I. J. Wright, L. Aarssen, R. I. Bertin, A. Calaminus, R. Govaerts, F. Hemmings, M. R. Leishman, J. Oleksyn, P. S. Soltis, N. G. Swenson, L. Warman, and J. M. Beaulieu. 2014. Three keys to the radiation of angiosperms into freezing environments. Nature 506:89–92.

Zhang, Q. G., A. Buckling, and H. C. J. Godfray. 2009. Quantifying the relative importance of niches and neutrality for coexistence in a model microbial system. Functional Ecology 23:1139–1147.

Zhang, Y., Z. Leng, Y. Wu, H. Jia, C. Yan, X. Wang, G. Ren, G. Wu, and J. Li. 2022. Interaction between Nitrogen, Phosphorus, and Invasive Alien Plants. Sustainability 14:746.

Zheng, Y. L., Y. L. Feng, L. K. Zhang, R. M. Callaway, A. Valiente-Banuet, D. Q. Luo, Z. Y. Liao, Y. B. Lei, G. F. Barclay, and C. Silva-Pereyra. 2015. Integrating novel chemical weapons and evolutionarily increased competitive ability in success of a tropical invader. New Phytologist 205:1350–1359.

Zink, M., L. Samaniego, R. Kumar, S. Thober, J. Mai, D. Schafer, and A. Marx. 2016. The German drought monitor. Environmental Research Letters 11:074002.

Zobel, K., M. Moora, V. K. Brown, P. Niemela, and M. Zobel. 1997. Secondary succession and summer herbivory in a subarctic grassland: Community structure and diversity. Ecography 20:595–604.

Zou, J., W. E. Rogers, and E. Siemann. 2008. Increased competitive ability and herbivory tolerance in the invasive plant Sapium sebiferum. Biological Invasions 10:291–302.

Zubrod, J. P., M. Bundschuh, G. Arts, C. A. Brühl, G. Imfeld, A. Knäbel, S. Payraudeau, J. J. Rasmussen, J. Rohr, A. Scharmüller, K. Smalling, S. Stehle, R. Schulz, and R. B. Schäfer. 2019. Fungicides: An Overlooked Pesticide Class? Environmental Science and Technology 53:3347–3365.

7

Appendices

7.1 Supplemental Information Chapter 2

Appendix 7.1.1 Plant species at the 'Perilla site', along with their origin status (N=native, E=exotic), abundance (average percent cover) and presence or absence of infection by different pathogen groups (X indicates presence). B/V = Bacterial and viral diseases.

				Infection by				
Plant species	**Family**	**Origin**	**Abundance [%]**	**Downy mildews**	**Fungal leaf spot diseases**	**Powdery mildews**	**Rusts**	**B/V diseases**
Ageratina altissima	Asteraceae	N	2.4		X			X
Carex muhlenbergii	Cyperaceae	N	11.4				X	X
Croton monanthogynus	Euphorbiaceae	N	0.1				X	
Daucus carota	Apiaceae	E	0.1		X			X
Desmodium paniculatum	Fabaceae	N	0.1					X
Festuca subverticillata	Poaceae	N	3.7		X	X		X
Galium tinctorium	Rubiaceae	N	0.3		X			X
Geum canadense	Rosaceae	N	3.5		X			X
Muhlenbergia sobolifera	Poaceae	N	0.8		X			X
Oxalis stricta	Oxalidaceae	N	0.1		X			
Parietaria pennsylvanica	Urticaceae	N	11.5					X
Perilla frutescens	Lamiaceae	E	9.8					X
Pilea pumila	Urticaceae	N	0.1					
Plantago lanceolata	Plantaginaceae	E	0.1					X
Plantago rugelli	Plantaginaceae	N	0.2					X
Polygonum pennsylvanicum	Polygonaceae	N	0.9					
Torilis arvensis	Apiaceae	E	0.1		X			X
Viola sororia	Violaceae	N	0.6					X

Appendix 7.1.2 Plant species at the 'Carduus site', along with their origin status (N=native, E=exotic), abundance (average percent cover) and presence or absence of infection by different pathogen groups (X indicates presence). B/V = Bacterial and viral diseases.

				Infection by				
Plant species	**Family**	**Origin**	**Abundance [%]**	**Downy mildews**	**Fungal leaf spot diseases**	**Powdery mildews**	**Rusts**	**B/V diseases**
Calystegia sepium	Convolvulaceae	N	1.6				X	X
Cirsium discolor	Asteraceae	N	0.2		X			
Conyza canadensis	Asteraceae	N	6.1					X
Croton monanthogynus	Euphorbiaceae	N	3.3				X	X
Daucus carota	Apiaceae	E	1.1			X		X
Desmodium canadense	Fabaceae	N	0.6					X
Elephantopus caroliniana	Asteraceae	N	2.3		X			
Eupatorium altissimum	Asteraceae	N	0.2		X			
Eupatorium serotinum	Asteraceae	N	0.1					
Geum canadense	Rosaceae	N	0.1					
Helianthus pauciflorus	Asteraceae	N	1.6					X
Lespedeza cuneata	Fabaceae	E	7.0					X
Monarda fistulosa	Lamiaceae	N	0.2		X			
Oxalis stricta	Oxalidaceae	N	0.8					X
Phyla lanceolata	Verbenaceae	N	1.6		X			X
Plantago rugelli	Plantaginaceae	N	0.1		X			X
Polygonum aviculare	Polygonaceae	E	0.3					
Solanum carolinense	Solanaceae	N	7.7		X			X
Taraxacum officinale	Asteraceae	E	2.4					
Teucrium canadense	Lamiaceae	N	2.0		X			
Verbena urticifolia	Verbenaceae	N	0.3		X			X
Verbesina alternifolia	Asteraceae	N	4.0		X			X
Vernonia baldwinii	Asteraceae	N	0.5				X	X

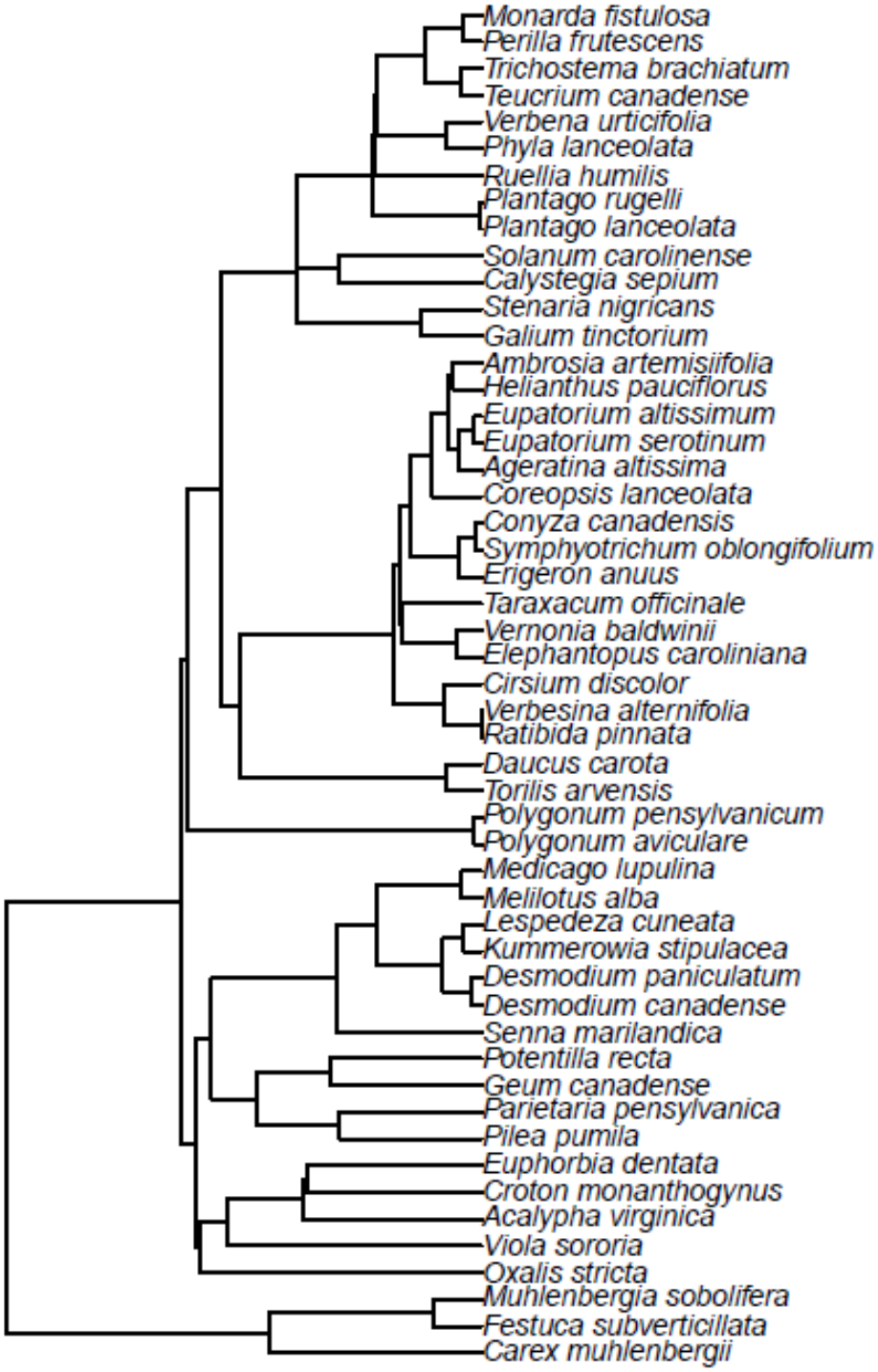
Appendix 7.1.3 Plant species at the 'Potentilla site', along with their origin status (N=native, E=exotic), abundance (average percent cover) and presence or absence of infection by different pathogen groups (X indicates presence). B/V = Bacterial and viral diseases.

				Infection by				
Plant species	**Family**	**Origin**	**Abundance [%]**	**Downy mildews**	**Fungal leaf spot diseases**	**Powdery mildews**	**Rusts**	**B/V diseases**
Acalypha virginica	Euphorbiaceae	N	0.2		X			
Ambrosia artemisiifolia	Asteraceae	N	0.3					X
Coreopsis lanceolata	Asteraceae	N	0.1					X
Croton monanthogynus	Euphorbiaceae	N	4.1				X	
Erigeron annuus	Asteraceae	N	6.6		X		X	X
Eupatorium altissimum	Asteraceae	N	0.1		X			
Euphorbia dentata	Euphorbiaceae	N	0.5					X
Festuca subverticillata	Poaceae	N	11.4	X				X
Kummerowia stipulacea	Fabaceae	E	0.5		X			X
Lespedeza cuneata	Fabaceae	E	0.4					X
Medicago lupulina	Fabaceae	E	2.3		X	X		X
Melilotus albus	Fabaceae	E	0.3		X		X	X
Potentilla recta	Rosaceae	E	6.6		X			
Ratibida pinnata	Asteraceae	N	0.3					X
Ruellia humilis	Acanthaceae	N	4.6				X	
Senna marilandica	Fabaceae	N	0.2		X			
Stenaria nigricans	Rubiaceae	N	0.9		X			
Symphyotrichum oblongifolium	Asteraceae	N	1.0					X
Teucrium canadense	Lamiaceae	N	2.2					X
Trichostema brachiata	Lamiaceae	N	0.1		X			

Appendix 7.1.4 Geographic distribution, global host ranges and infected (bold) and known, but not infected host species for each pathogen species at the three study sites. Asterisks (*****) indicate pathogen-host combinations not yet reported for the US (according to the USDA Fungus-Host distribution database). PM = Powdery mildews. NA = North America, EU = Europe, AS = Asia, SA = South America, AF = Africa, AU = Australia and Oceania.

	Pathogen (Global host range in number of genera and families)	**Geographic distribution**	**Host species per site** Perilla site	Carduus site	Potentilla site
Fungal leaf spot diseases	*Ascochyta caulicola* (5; 1)	NA, EU, AS			***M. albus***
	Boeremia exigua (275; 83)	cosmopolitan	*P. lanceolata*	***S. carolinense***, ***T. canadense***, *D. carota*, *G. canadense*, *M. fistulosa*, *P. rugelli*, *T. officinale*	
	Cercospora apii (241; 79)	cosmopolitan	***T. arvensis****, *D. carota*, *P. lanceolata*	*D. carota*	*P. recta*
	Cercospora elephantopi (2; 1)	NA, SA, AF		***E. carolinianus***	
	Cercospora galii (1; 1)	NA, EU, AS	***G. tinctorium***		
	Cercospora houstoniae (2; 1)	NA			***S. nigricans***
	Cercospora isanthi (1; 1)	NA			***T. brachiata***
	Cercospora medicaginis (6; 3)	cosmopolitan			***M. lupulina***, *M. albus*
	Colletotrichum dematium (206; 76)	cosmopolitan	***O. stricta****, *P. pennsylvanicum*	*P. aviculare*	
	Leptosphaeria muehlenbergiae (1; 1)	NA	***M. sobolifera***		
	Phyllachora lespedezae (4; 1)	NA			***K. stipulacea***
	Septoria cf. *mississippiensis* (1; 1)	NA	***M. sobolifera****		
	Septoria erigerontis (3; 1)	NA, EU, AS, AU, SA		*C. canadensis*	***E. annuus***
	Septoria gei (1; 1)	NA, EU, AS	***G. canadense***		
	Septoria verbenae (1; 1)	NA, SA, EU, AU		***V. urticifolia***	

PM	*Erysiphe heraclei* (91; 7)	cosmopolitan	***D. carota***, *T. arvensis*		
	Erysiphe pisi (293; 75)	cosmopolitan		*P. aviculare*	***M. lupulina***, *L. cuneata*, *K. stipulacea*, *M. albus*
Rusts	*Coleosporium vernoniae* (6; 2)	NA, SA, AS		***V. baldwinii***, *E. carolinianus*	
	Phakopsora crotonis (3; 1)	NA, SA	***C. monanthogynus***	***C. monanthogynus***	***C. monanthogynus***
	Puccinia convolvuli (6; 1)	cosmopolitan		***C. sepium***	
	Puccinia dioicae (40; 9)	NA, EU, AS, SA	***C. muehlenbergii***		*E. annuus*
	Puccinia extensicola (43; 8)	NA, EU, AS, SA	*C. muehlenbergii*		***E. annuus***
	Puccinia lateripes (9; 2)	NA, SA			***R. humilis***
	Uromyces striatus (17; 2)	cosmopolitan			***M. albus***, *M. lupulina*



Appendix 7.1.5 Phylogenetic relationships among the 51 focal plant species recorded in three communities, created by using a dated molecular phylogeny of vascular plants (Zanne et al. 2014).

7.2 Supplemental Information Chapter 3

Appendix 7.2.1. Plant species used in the experiment, along with their respective family affiliation, species origin (N = native, E = exotic), mode of introduction for exotic species (F = fodder plant for livestock, S = seed mixtures for horticulture or grasslands, C = contaminant in seed mixtures, Klotz et al. 2002, Frank and John 2007) and designated usage, including assigned blocks for matrix species in brackets. All exotic species, with the exception of *P. peregrina*, are naturalized exotic species in Central European grasslands. *P. peregrina* is a casual exotic species (Floraweb.de, Bundesamt für Naturschutz 2019). *B. orientalis* and *D. giganteus* are considered potentially invasive species in Germany (Bundesamt für Naturschutz 2019b). The other exotic species are considered non-invasive.

Plant species	Family	Origin	Mode of introduction	Usage
Arrhenatherum elatius	Poaceae	N		Focal
Lotus corniculatus	Fabaceae	N		Focal
Medicago falcata	Fabaceae	N		Focal
Tragopogon pratensis	Asteraceae	N		Focal
Hypericum perforatum	Hypericaceae	N		Focal
Falcaria vulgaris	Apiaceae	N		Focal
Lolium multiflorum	Poaceae	E	F	Focal
Onobrychis viciifolia	Fabaceae	E	F, S	Focal
Medicago x *varia*	Fabaceae	E	F, S	Focal
Dianthus giganteus	Caryophyllaceae	E	S	Focal
Pimpinella peregrina	Apiaceae	E	S	Focal
Bunias orientalis	Brassicaceae	E	C	Focal
Agrimonia eupatoria	Rosaceae	N		Matrix (1; 4)
Dactylis glomerata	Poaceae	N		Matrix (4)
Daucus carota	Apiaceae	N		Matrix (1; 2)
Festuca rubra	Poaceae	N		Matrix (1; 3)
Knautia arvensis	Dipsacaceae	N		Matrix (2; 4)
Leucanthemum vulgare	Asteraceae	N		Matrix (3; 5)
Picris hieracioides	Asteraceae	N		Matrix (2; 3)
Pimpinella saxifraga	Apiaceae	N		Matrix (3; 4; 5)
Poa angustifolia	Poaceae	N		Matrix (2; 5)
Silene latifolia	Caryophyllaceae	N		Matrix (1; 5)

Appendix 7.2.2 Schematic overview of the treatment structure of the field experiment. Each framed square represents one combination of dominant species (abbreviated with two letters) and pesticide treatment (F+ = fungicide application; F- = no fungicide application; I+ = insecticide application; I- = no insecticide application; denoted by different colors). Along with each dominant species, the remaining five species of each origin were sown at low frequency. Note that these 48 combinations were replicated five times according to a randomized block design in the field, resulting in 240 plots in total. *Ae* = *Arrhenatherum elatius*, *Lc* = *Lotus corniculatus*, *Mf* = *Medicago falcata*, *Tp* = *Tragopogon pratensis*, *Hp* = *Hypericum perforatum*, *Fv* = *Falcaria vulgaris*, *Lm* = *Lolium multiforum*, *Ov* = *Onobrychis viciifolia*, *Mv* = *Medicago* x *varia*, *Dg* = *Dianthus giganteus*, *Pp* = *Pimpinella peregrina*, *Bo* = *Bunias orientalis*.

Treatment	Native focal species						Exotic focal species					
F+ I+	*Ae*	*Lc*	*Mf*	*Tp*	*Hp*	*Fv*	*Lm*	*Ov*	*Mv*	*Dg*	*Pp*	*Bo*
F+ I-	*Ae*	*Lc*	*Mf*	*Tp*	*Hp*	*Fv*	*Lm*	*Ov*	*Mv*	*Dg*	*Pp*	*Bo*
F- I+	*Ae*	*Lc*	*Mf*	*Tp*	*Hp*	*Fv*	*Lm*	*Ov*	*Mv*	*Dg*	*Pp*	*Bo*
F- I-	*Ae*	*Lc*	*Mf*	*Tp*	*Hp*	*Fv*	*Lm*	*Ov*	*Mv*	*Dg*	*Pp*	*Bo*

Appendix 7.2.3A *Methods used for evaluation of fungicide efficacy*

We evaluated fungicide efficacy by quantifying diversity and infection of foliar fungal pathogens on two selected focal species, native *A. elatius* and exotic *M.* x *varia*, in October 2016, after three years of fungicide application. Species were selected to represent both species origins and different functional and taxonomic affiliations. For each species, we sampled pathogens on up to ten individuals per plot on five plots with fungicide application (and no insecticide application) and five plots with no fungicide application (and no insecticide application), each plot representing a high frequency plot for the particular species. For each individual, pathogen infection apparent on the leaf surface was assessed visually using a percentage-based nine-level rating scheme, according to Oberforster (2001), ranging from 1 = 0% infection to 9 ≥ 70% infection. We assigned all pathogens observed on individual plants to broad pathogen groups following Rottstock et al. (2014): rusts, powdery mildews and fungal leaf spot diseases. We recorded rust infection as presence of sporulation structures, powdery mildews as presence of mycelium and fungal leaf spot diseases as presence of necrotic leaf lesions. Despite being major pathogen groups, we did not find any downy mildews and smut fungi on our plants. For each of the two plant species, we took one sample of every occurring sign of pathogen infection, which we dried and pressed, to preserve them for further determination via digital microscopy (VHX-2000, Keyence Corp., Osaka, Japan). Determination of pathogens to species followed Brandenburger (1985) and Klenke and Scholler (2015).

Pathogen diversity was measured as the number of pathogen species per plot, and per individual, respectively. Pathogen infection was measured as incidence (the mean percentage of infected individuals per plant species and plot), severity (the mean percentage of infected plant tissue per plant species, individual and plot) and the product of both, overall infection. These three metrics of pathogen infection were calculated across all pathogen groups and for each group separately.

Statistical analysis on the effect of fungicide application on pathogen diversity and infection were performed using generalized linear mixed models in SAS® Release 9.4 (procedure GLIMMIX). We analyzed the number of pathogen species per plot using a model with Poisson error distribution and a logarithmic link-function, and the number of pathogen species per individual using a model with Gaussian error distribution and an identity link-function. For pathogen incidence we used a model with binomial error distribution and a logit link-function. The percentage data for pathogen severity and overall infection was logit-transformed (Warton and Hui 2011) and analyzed using models with Gaussian error distribution and identity link-function. In each analysis, 'block' was considered as a random factor and 'fungicide application' was treated as a fixed factor.

Appendix 7.2.3B *Results of evaluation of fungicide efficacy*

We identified ten different pathogen species, none of them appearing on both plant species. *A. elatius* hosted three species of rust and three leaf spot diseases, while three leaf spot diseases and one powdery mildew species infected *M.* x *varia* (Appendix 7.2.3C). However, the powdery mildew was not considered for separate analysis because it only occurred on one individual of *M.* x *varia.* Application of fungicide did not influence the number of pathogen species per plot for either species, but there were significantly less pathogen species per individual on *A. elatius* when fungicide was applied, compared to individuals on control plots (Appendix 7.2.3D). Fungicide application had no effect on the number of pathogen species on *M.* x *varia* individuals (Appendix 7.2.3D). Pathogen incidence was significantly lower on plots treated with fungicide of *M.* x *varia*, while there was no such difference for *A. elatius*, neither for a particular group of pathogens nor across all groups (Appendix 7.2.3D). Severity of infection with rusts and with leaf spot diseases, as well as across both pathogens groups, was significantly lower on plots treated with fungicide for *A. elatius*, while fungicide application showed no such effect on pathogen severity for *M.* x *varia* (Appendix 7.2.3D). The product of both metrics, overall infection, was significantly lower when fungicide was applied for both species, when considering each pathogen group separately and across all pathogen groups (Appendix 7.2.3D).

Appendix 7.2.3C Pathogen species, along with the corresponding pathogen group and taxonomic affiliation, found on one native (*Arrhenatherum elatius*) and one exotic (*Medicago* x *varia*) focal species in October 2016.

A. elatius	**Pathogen group**	**Order**
Alternaria sp.	Leaf spot diseases	Pleosporales
Cercosporidium graminis	Leaf spot diseases	Capnodiales
Colletotrichum graminicola	Leaf spot diseases	Glomerellales
Puccinia arrhenatheri	Rusts	Pucciniales
Puccinia coronata	Rusts	Pucciniales
Puccinia graminis	Rusts	Pucciniales
M.* x *varia		
Alternaria sp.	Leaf spot diseases	Pleosporales
Cercospora medicaginis	Leaf spot diseases	Capnodiales
Stagonospora meliloti	Leaf spot diseases	Pleosporales
Erysiphe pisi var. *pisi*	Powdery mildews	Erysiphales

Appendix 7.2.3D Results of generalized linear mixed models for the effect of fungicide application on pathogen diversity, as measured by the number of pathogen species per plot, and per individual, respectively, and on pathogen infection, measured as pathogen incidence, severity and overall infection of rusts, leaf spot diseases and across pathogen groups for one native (*Arrhenatherum elatius*) and one exotic (*Medicago* x *varia*) focal species treated with fungicide and the respective control. Values given are least square means ± standard errors for pathogen incidence (backtransformed using the /ilink function of proc GLIMMIX in SAS® Release 9.4), and means from raw data ± standard error for the other response variables. Note that there was no rust infection on *M.* x *varia*. (* $P < 0.05$, ** $P < 0.01$)

		A. elatius			***M.* x *varia***		
	d.f.	With fungicide	Without fungicide	*F-values*	With fungicide	Without fungicide	*F-values*
Pathogen diversity							
Pathogen species per plot	1, 4	1.20 ± 0.49	2.00 ± 0.63	0.98	1.00 ± 0.45	1.20 ± 0.49	0.09
Pathogen species per individual	1, 4	0.54 ± 0.13	0.94 ± 0.13	13.33*****	0.32 ± 0.14	0.80 ± 0.14	7.32
Pathogen infection							
Pathogen incidence [%]							
Rusts	1, 4	1.99 ± 1.99	23.97 ± 6.40	6.64	-	-	-
Leaf spot diseases diseases	1, 4	52.22 ± 11.04	71.29 ± 9.43	3.57	30.87 ± 9.72	73.65 ± 10.05	13.15*****
All pathogen groups	1, 4	54.20 ± 10.20	76.98 ± 7.92	5.40	30.87 ± 9.72	73.65 ± 10.05	13.15*****
Pathogen severity [%]							
Rusts	1, 4	0.20 ± 0.20	3.18 ± 1.26	37.71******	-	-	-
Leaf spot diseases diseases	1, 4	2.07 ± 0.64	7.20 ± 1.80	19.63*****	1.63 ± 0.65	3.74 ± 0.82	5.58
All pathogen groups	1, 4	2.06 ± 0.64	7.39 ± 1.58	18.23*****	1.63 ± 0.65	3.88 ± 0.95	5.59
Overall infection [%]							
Rusts	1, 4	0.02 ± 0.02	0.86 ± 0.39	18.45*****	-	-	-
Leaf spot diseases diseases	1, 4	1.01 ± 0.31	4.74 ± 1.24	17.35*****	0.43 ± 0.13	2.94 ± 1.06	11.83*****
All pathogen groups	1, 4	1.03 ± 0.31	5.60 ± 1.34	23.72******	0.43 ± 0.13	3.07 ± 1.19	11.67*****

Appendix 7.2.4A. *Methods used for evaluation of insecticide efficacy*

We evaluated insecticide efficacy by quantifying diversity and abundance of soil arthropods on additional 1 m^2 plots, which were established in March 2015 at the same site as the main experiment. Vegetation on these plots was left in its natural state after plowing (see methods) and no seeds were added. On these plots, the four pesticide treatments were randomly applied in the same way as in the main experiment and replicated five times, resulting in 20 plots in total. In November 2015, one core with a diameter of 20 cm was taken from the upper 10 cm of soil from the center of each plot. Soil meso- and macrofauna from these cores was extracted by heat, using a Kempson apparatus (Kempson et al. 1963) with propylene glycol as a collection fluid. Collected fauna was transferred to 70% ethanol and subsequently identified to order level, and further when possible, using a dissecting microscope. Determination followed Dunger and Fiedler (1999) and Brohmer (2016).

Diversity of soil arthropods was measured as the number of hexapod orders per plot. Abundance of soil arthropods was measured as the total number of all arthropods found on each plot, and the number of several arthropod orders. Here, we present the results for the abundances of mites (Acari), myriapods (Myriapoda), hexapods (Hexapoda), springtails (Collembola) and insects (Insecta s. str.), as they were the most abundant taxa found in our samples. Taxonomy followed Westheide and Rieger (2013).

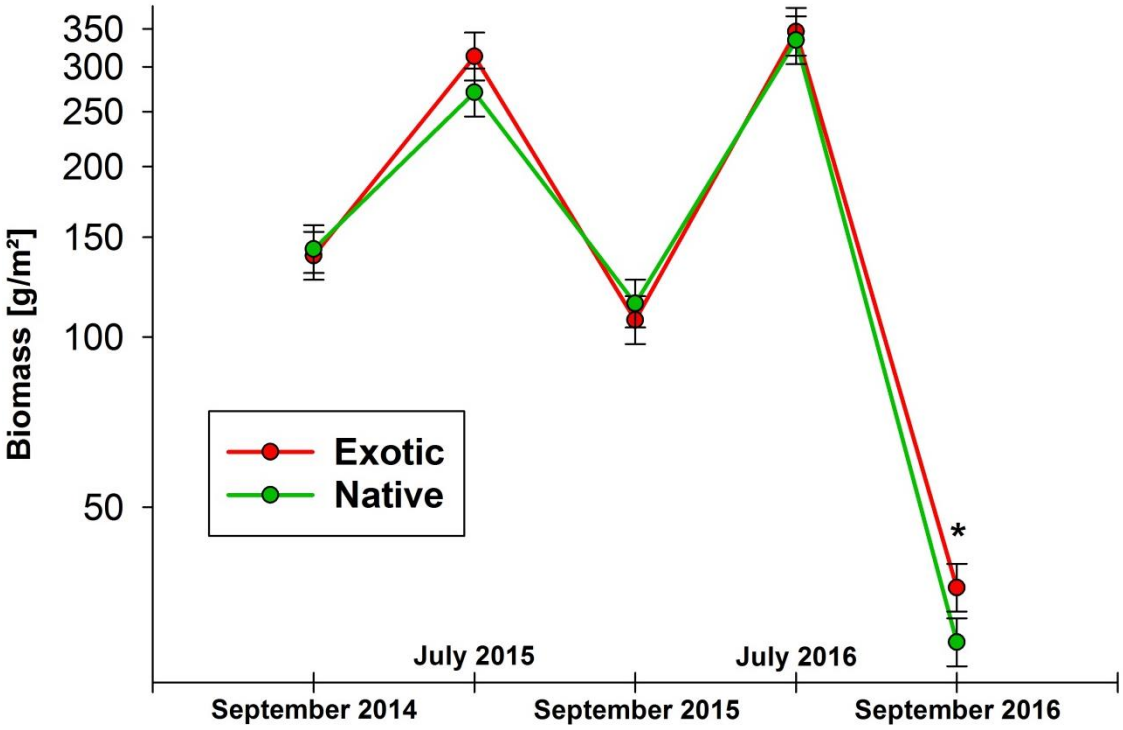
Statistical analysis on the effect of insecticide application on soil arthropod diversity and abundance were performed using generalized linear mixed models in SAS® Release 9.4 (procedure GLIMMIX). We analyzed arthropod diversity per plot using a model with Poisson error distribution and a logarithmic link-function, and the abundances of all arthropods found on each plot, and the number of mites, myriapods, hexapods, springtails and insects (Insecta s. str.) per plot using a model with negative binomial error distribution and a logarithmic link-function. In each analysis, 'fungicide application' and 'insecticide application', as well as their interaction, were treated as fixed factors. Due to an unusually high number of mealybugs (family Pseudococcidae in the superfamily Coccoidea) we excluded one sample (combined fungicide and insecticide application) as a potential outlier when analysing the abundance of insects (s. str.).

Appendix 7.2.4B *Results of evaluation of insecticide efficacy*

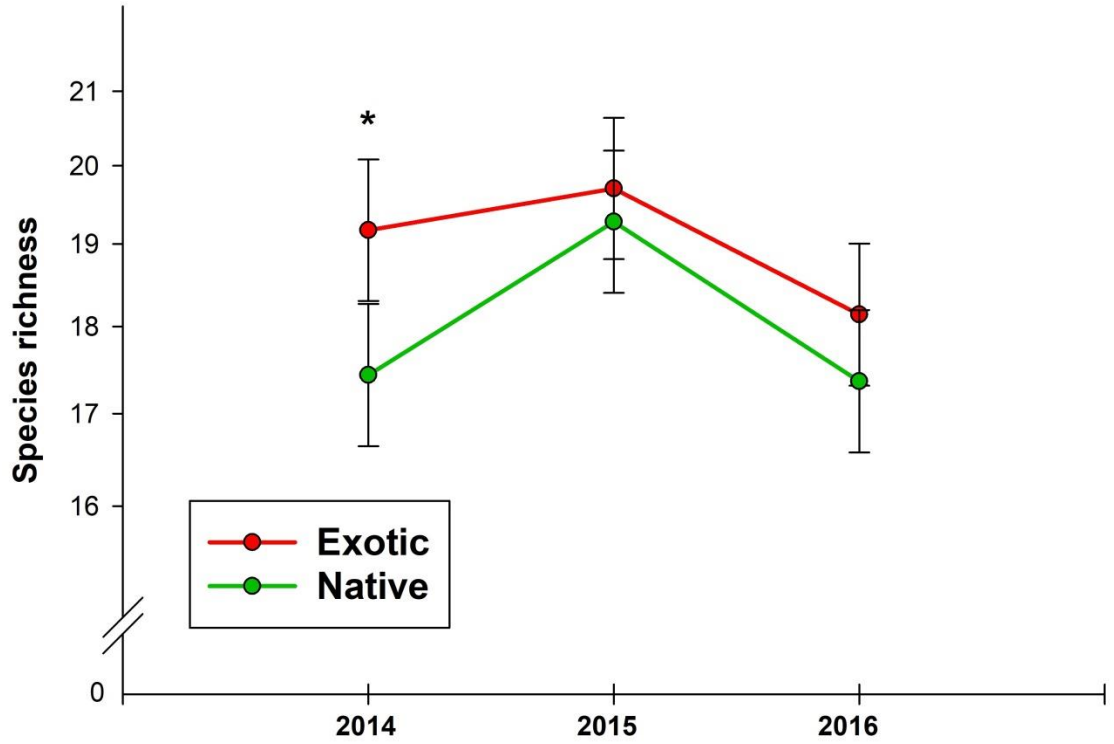
We found a total of 15415 soil arthropods in our samples, with mites constituting the most abundant group (total of 12390 individuals). Arthropod diversity was significantly higher on control plots, compared to plots treated with insecticide (5.8 ± 0.6 vs. 4.0 ± 0.5 hexapod orders, Appendix 7.2.3C). The insecticide treatment reduced the total abundance of all arthropods by more than 50% with an average of 497 ± 106 specimens on plots treated with insecticide and 1024 ± 217 specimens on control plots, indicating an effective application of insecticide (Appendix 7.2.4C). This effect was mainly driven by a strong decrease in springtail abundance (232 ± 44 vs. 16 ± 3 individuals, Appendix 7.2.4C). Since springtails represented the majority of hexapods, the abundance of hexapods showed a similar response to insecticide application (255 ± 52 vs. 26 ± 6, Appendix 7.2.4C). Insect (s.str.) abundances were also affected by the insecticide treatment, but only when one outlier sample was excluded from analysis (see Appendices 7.2.4A and 7.2.4C). When included, the effect of insecticide application on the number of insect (s. str.) individuals was barely non-significant ($F = 4.34$, $p = 0.0537$, see Appendix 7.2.4C). All other effects did not change qualitatively when this sample was excluded and, thus, are not shown. Insecticide application had no effect on mite and myriapod abundances (Appendix 7.2.4C). Fungicide application had no effect on arthropod diversity and abundances with the following exception: when including the outlier sample in analysis fungicide application had a significantly positive effect on insect (s. str.) abundance ($P < 0.05$). Fungicides are known to have no harmful effect on, or may even be beneficial for insect metabolism and populations (Moreby et al. 1997, Andrade et al. 2010). Furthermore, it is possible that insects capitalized on the absence of fungi and increased in abundance on plots treated with fungicide. There was no interaction effect among fungicide and insecticide application.

Appendix 7.2.4C Results of generalized linear mixed models for the effect of insecticide application on arthropod diversity, as measured by the number of hexapod orders per plot and arthropod abundance, as measured by the total number of all arthropods found on each plot, and the number of mites (Acari), myriapods (Myriapoda), hexapods (Hexapoda), springtails (Collembola) and insects (Insecta s. str.) per plot when treated with insecticide and the respective control. Values given are least square means ± standard errors. Insect abundance was analyzed including and excluding one sample with an unsually high number of mealybugs (family Pseudococcidae in the superfamily Coccoidea, see Appendices 7.2.4A and 7.2.4B for detailed information). Note that hexapods include springtails and insects (Insecta s. str.) and that arthropods include all mentioned groups. For taxonomy see Westheide and Rieger (2013). For the effect of fungicide application see Appendix 7.2.4B. The interaction of fungicide and insecticide application had no effect on arthropod diversity and abundance. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

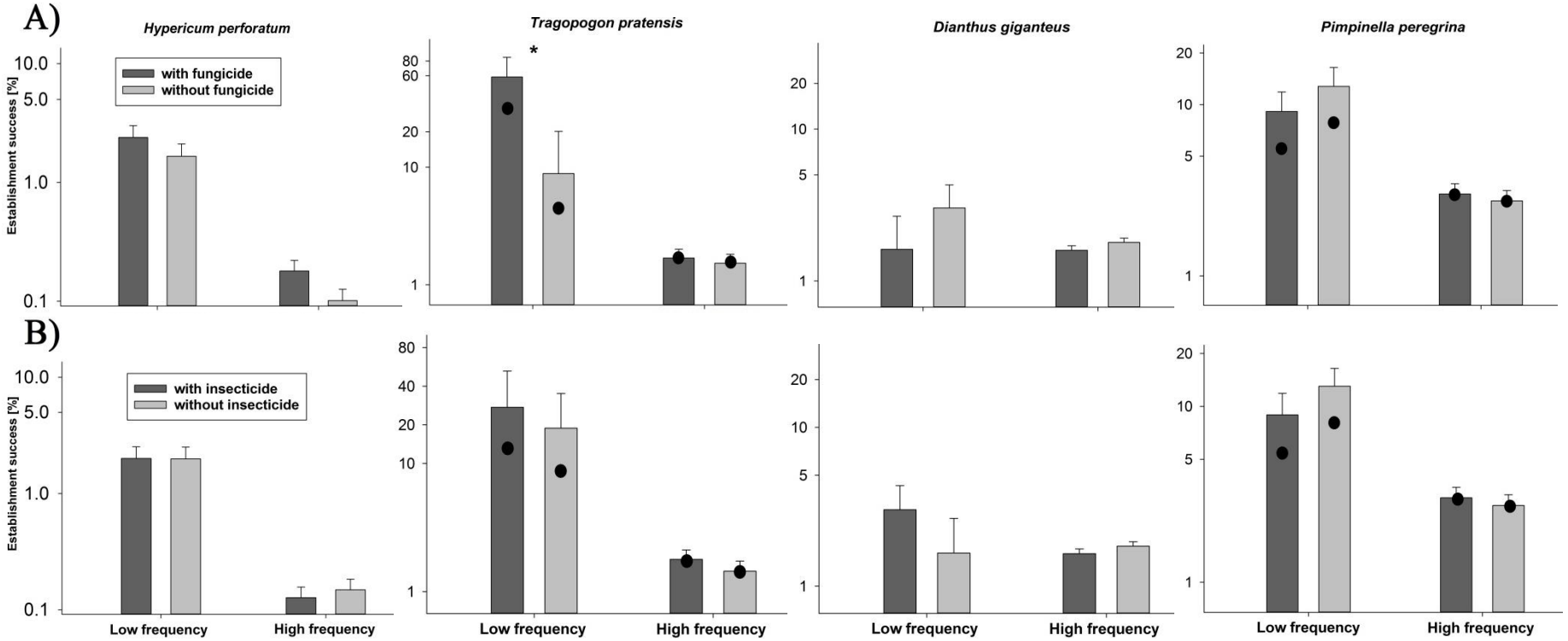
	d.f.	With insecticide	Without insecticide	*F* values
Arthropod diversity				
Hexapod orders	1, 16	4.0 ± 0.5	5.8 ± 0.6	5.29*
Arthropod abundance				
All arthropods	1, 16	497 ± 106	1024 ± 217	5.79*
Mites (Acari)	1, 16	464 ± 102	759 ± 166	2.53
Myriapods (Myriapoda)	1, 16	7 ± 2	7 ± 2	0.03
Hexapods (Hexapoda)	1, 16	26 ± 6	255 ± 52	59.02***
Springtails (Collembola)	1, 16	16 ± 3	232 ± 44	89.97***
Insects (Insecta s. str.) excl. outlier	1, 16	5 ± 1	19 ± 4	18.58**
Insects (Insecta s. str.) incl. outlier	1, 16	8 ± 2	19 ± 5	4.34



Appendix 7.2.5 Aboveground biomasses (least square means and standard errors) of native and exotic-dominated communities for each harvest. Asterisks indicate significant differences in productivity among the two species origins. Note the logarithmic scale of the y-axis. (* $P < 0.05$)



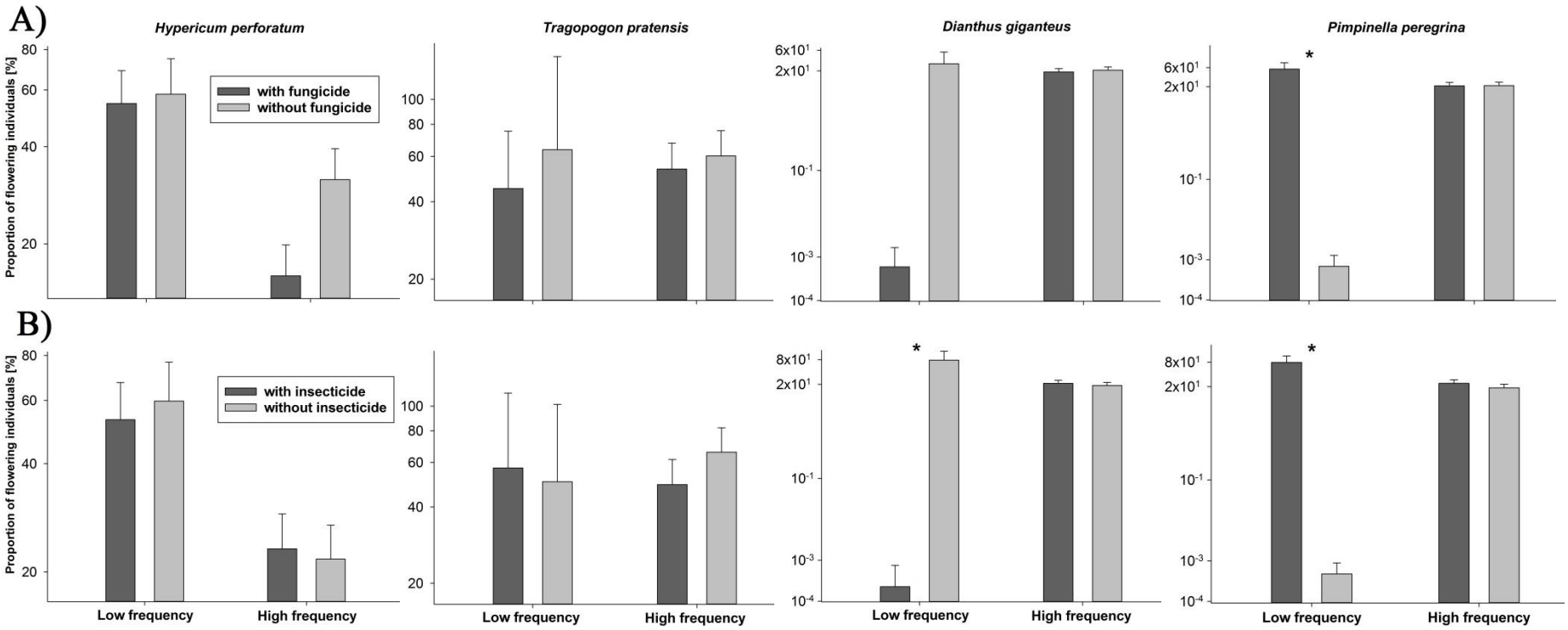
Appendix 7.2.6 Species richness (least square means and standard errors) of native and exotic-dominated communities for each year. Asterisks indicate significant differences in species richness among the two species origins. (* $P < 0.05$)



Appendix 7.2.7A Establishment success (least square means and standard errors of the mean), as estimated by the total number of individuals in the second year after sowing relative to the number of sown seeds, for two native (*Hypericum perforatum* and *Tragopogon pratensis*) and two exotic (*Dianthus giganteus* and *Pimpinella peregrina*) focal species on low and high frequency plots treated with A) fungicide and B) insecticide and the respective controls. Note that for *T. pratensis* and *P. peregrina*, columns and error bars represent means and standard errors adjusted for excessive zeros. Black dots indicate mean values including excessive zeros (i.e. plots with zero values exceeding the number of plots expected by the underlying distribution, see methods for detailed information). The interaction effect of fungicide and insecticide application is not shown, because it was not significant (see Appendix 7.2.7B). Asterisks indicate significant effects of insecticide or fungicide application on establishment success at the respective level of relative frequency (* $P < 0.05$). Note the logarithmic scale of the y-axis.

Appendix 7.2.7B Results of generalized linear models on the effects of a species' relative frequency in the seed mixture *(hypothesis 3)*, fungicide application *(hypothesis 4)*, and insecticide application *(hypothesis 4)*, as well as their interactions, and the effect of block on the estimates of establishment success, as estimated by the total number of individuals in the second year after sowing relative to the number of sown seeds, for two native (*Hypericum perforatum*, *Tragopogon pratensis*) and two exotic (*Dianthus giganteus*, *Pimpinella peregrina*) focal species. Note that block had to be excluded for *T. pratensis* because of zero values for all replicates of a certain treatment combination, and that zero inflation was only present in the datasets of *T. pratensis* and *P. peregrina*. See method section for information on model structure. Abbreviations for factors are given in brackets. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

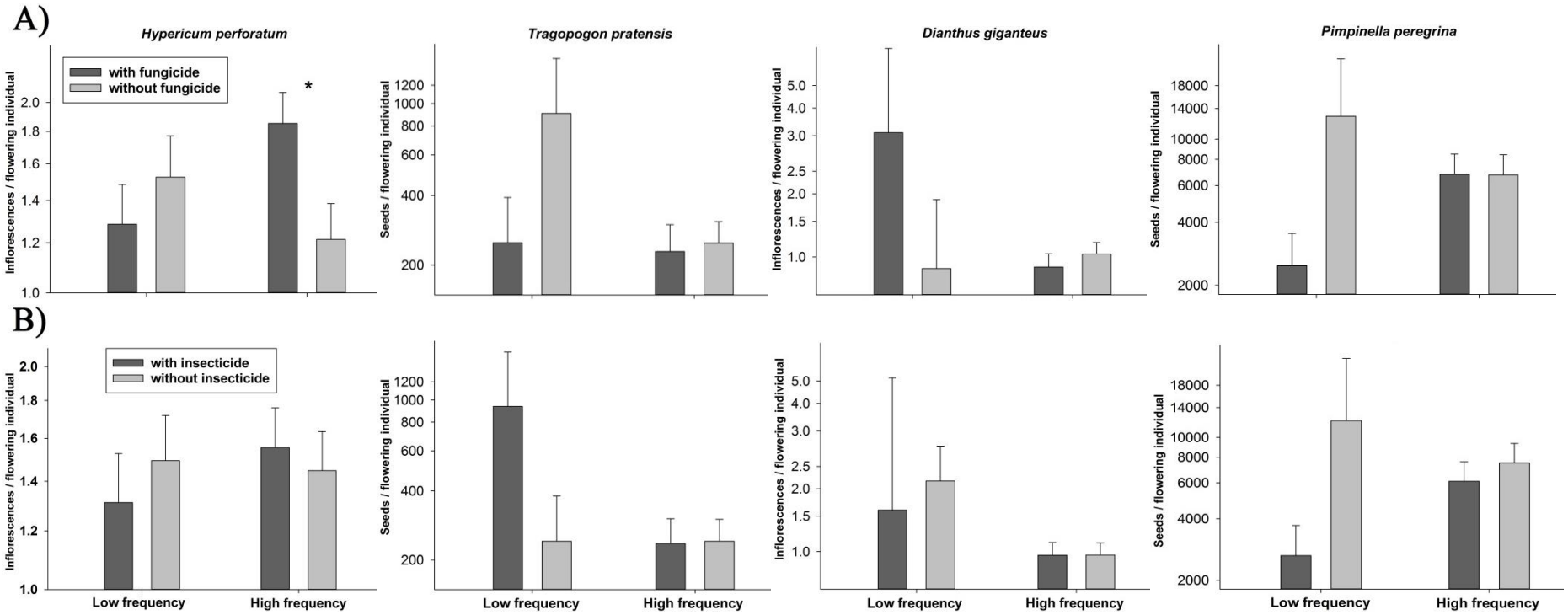
Source		***H. perforatum***	***T. pratensis***	***D. giganteus***	***P. peregrina***
	d.f.		χ^2 values		
Relative frequency [RF]	1	50.82***	15.36***	0.68	26.69***
Relative frequency – Zero inflation	1	-	6.02*	-	12.54***
Fungicide application [F]	1	3.79	4.94*	1.56	0.35
Insecticide application [I]	1	0.11	0.42	0.75	0.44
RF x F	1	0.15	3.98*	0.7	1.06
RF x I	1	0.14	0.03	1.51	1.24
F x I	1	0.53	1.47	0.11	0.01
RF x F x I	1	0.29	0.00	0.38	0.19
Block	4	11.21*	-	13.88**	1.99



Appendix 7.2.8A Proportion of flowering individuals (least square means and standard errors of the mean), as estimated by the number of flowering individuals relative to the total number of individuals present in the second year after sowing, for two native (*Hypericum perforatum* and *Tragopogon pratensis*) and two exotic (*Dianthus giganteus* and *Pimpinella peregrina*) focal species on low and high frequency plots treated with A) fungicide and B) insecticide and the respective controls. Although there is one significant three-way interaction (*P. peregrina*: RF × F × I, see Appendix 7.2.8B), it is not shown because of consistency. Asterisks indicate significant effects of insecticide or fungicide application on proportion of flowering individuals at the respective level of relative frequency (* $P < 0.05$). Note the logarithmic scale of the y-axis.

Appendix 7.2.8B. Results of generalized linear models on the effects of relative frequency *(hypothesis 3)*, fungicide application *(hypothesis 4)*, and insecticide application *(hypothesis 4)*, as well as their interactions, and the effect of block on the estimates of proportion of flowering individuals, as estimated by the number of flowering individuals relative to the total number of individuals present in the second year after sowing, for two native (*Hypericum perforatum* and *Tragopogon pratensis*) and two exotic (*Dianthus giganteus* and *Pimpinella peregrina*) focal species. Note that block had to be excluded for *D. giganteus* because of zero values for all replicates of a certain treatment combination. See method section for information on model structure. Abbreviations for factors are given in brackets. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

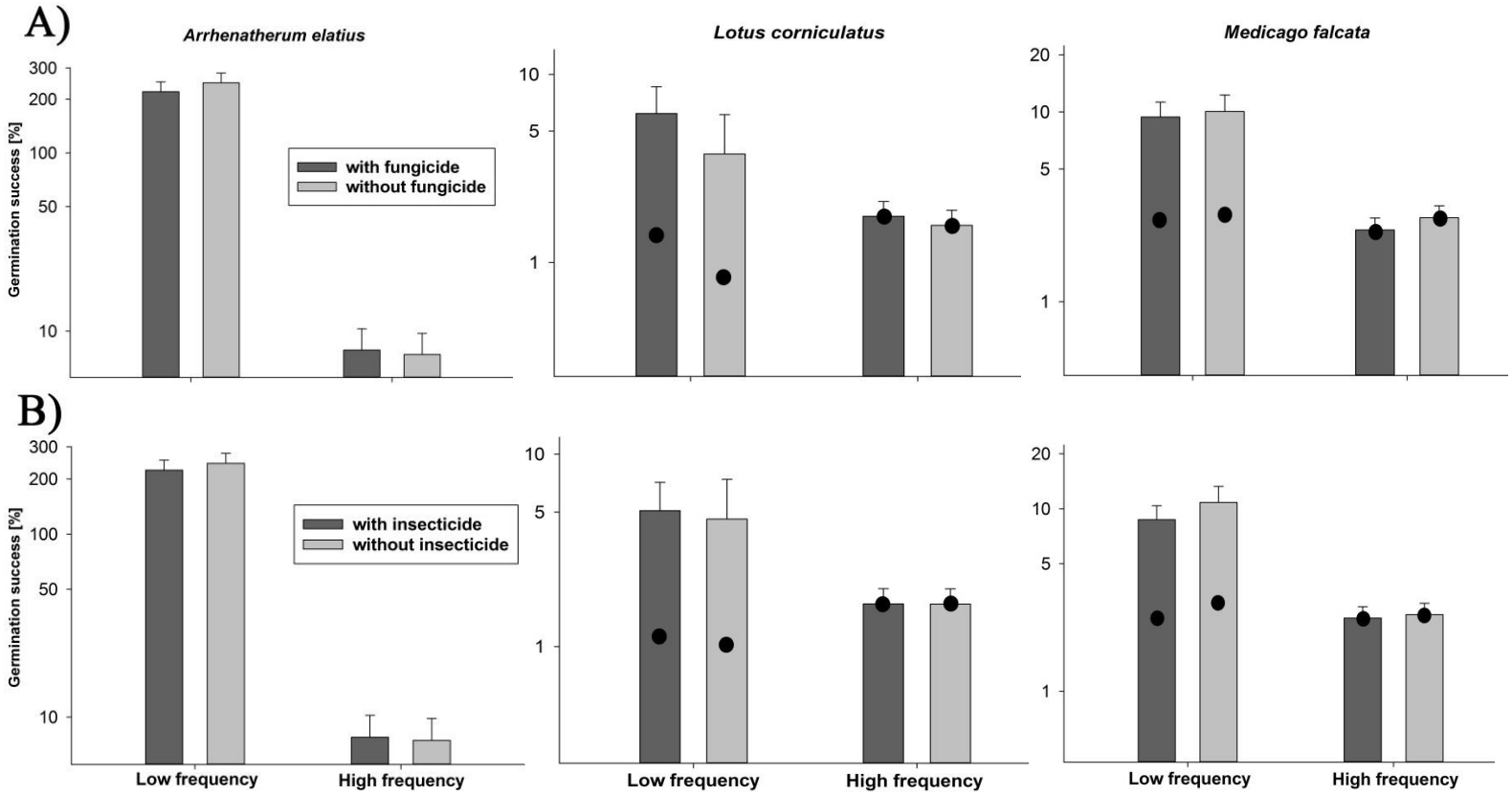
Source		***H. perforatum***	***T. pratensis***	***D. giganteus***	***P. peregrina***
	d.f.		χ^2 values		
Relative frequency [RF]	1	11.45***	0.01	0.16	0.24
Fungicide application [F]	1	2.46	0.21	0.67	4.24*
Insecticide application [I]	1	0.01	0.03	5.17*	10.41**
RF × F	1	1.77	0.05	0.50	4.21*
RF × I	1	0.16	0.18	6.26*	6.50*
F × I	1	1.93	0.02	1.19	2.75
RF × F × I	1	0.07	0.16	1.19	4.71*
Block	4	6.11	4.66	-	3.20



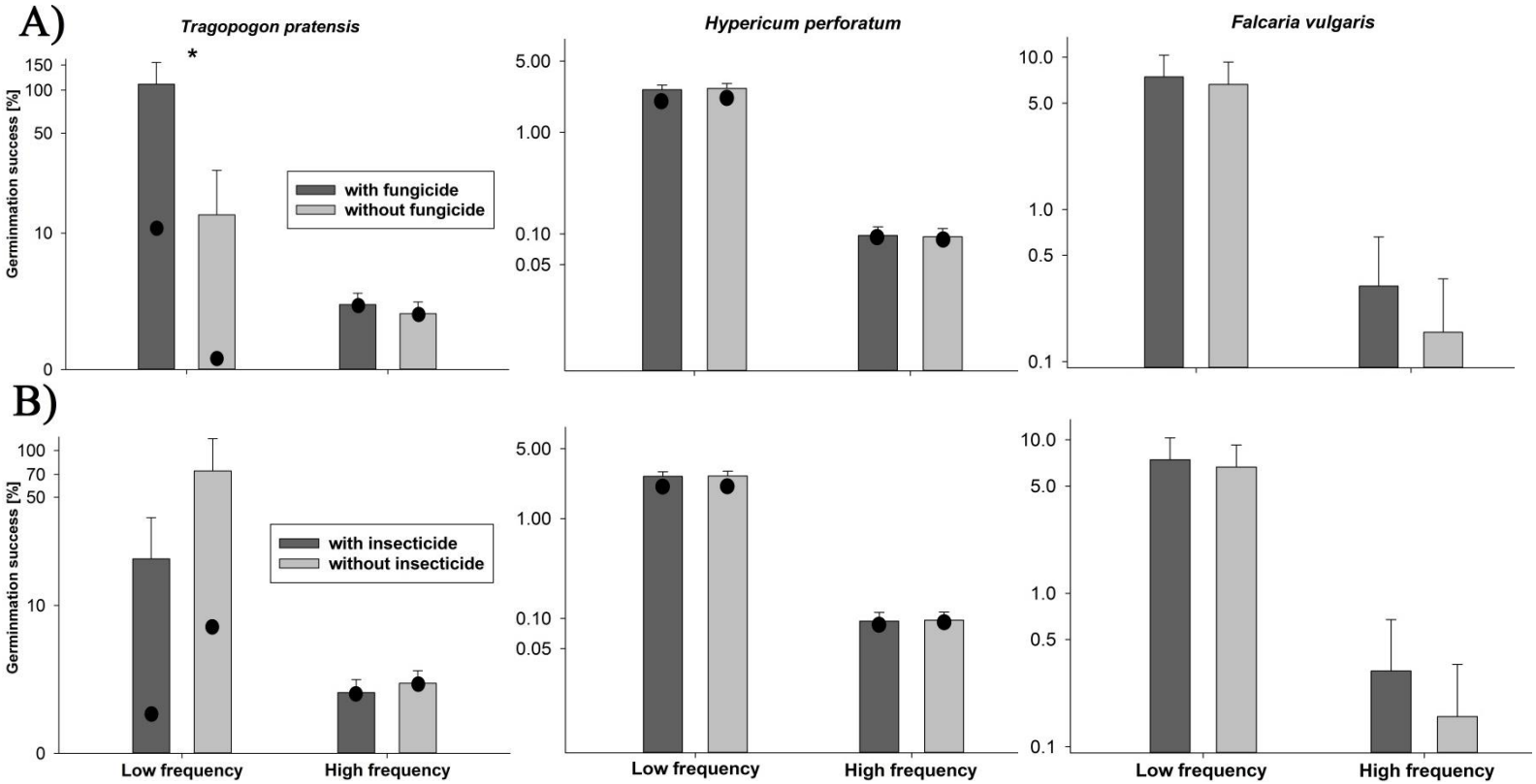
Appendix 7.2.9A Fecundity (least square means and standard errors of the mean), as estimated by the number of seeds, or the number of inflorescences, produced per flowering individual, in the second year after sowing, for two native (*Hypericum perforatum* and *Tragopogon pratensis*) and two exotic (*Dianthus giganteus* and *Pimpinella peregrina*) focal species on low and high frequency plots treated with A) fungicide and B) insecticide and the respective controls. The interaction effect of fungicide and insecticide application is not shown, because it was not significant (see Appendix 7.2.9B). Asterisks indicate significant effects of insecticide or fungicide application on fecundity at the respective level of relative frequency (* $P < 0.05$). Although there is a significant RF × F interaction for *D. giganteus* (see Appendix 7.2.9B), the effect of fungicide application is not significant at any level of relative frequency. Note the logarithmic scale of the y-axis.

Appendix 7.2.9B Results of generalized linear models on the effects of a species' relative frequency in the seed mixture *(hypothesis 3)*, fungicide application *(hypothesis 4)*, and insecticide application *(hypothesis 4)*, as well as their interactions, and the effect of block on the estimates of fecundity, as estimated by the number of seeds, or the number of inflorescences, produced per flowering individual in the second year after sowing, for two native (*Hypericum perforatum* and *Tragopogon pratensis*) and two exotic (*Dianthus giganteus* and *Pimpinella peregrina*) focal species. Note that the three-way interaction had to be excluded for *D. giganteus* and *P. peregrina* because of zero values for all replicates of a certain treatment combination. See method section for information on model structure. Abbreviations for factors are given in brackets. (* $P < 0.05$, *** $P < 0.001$)

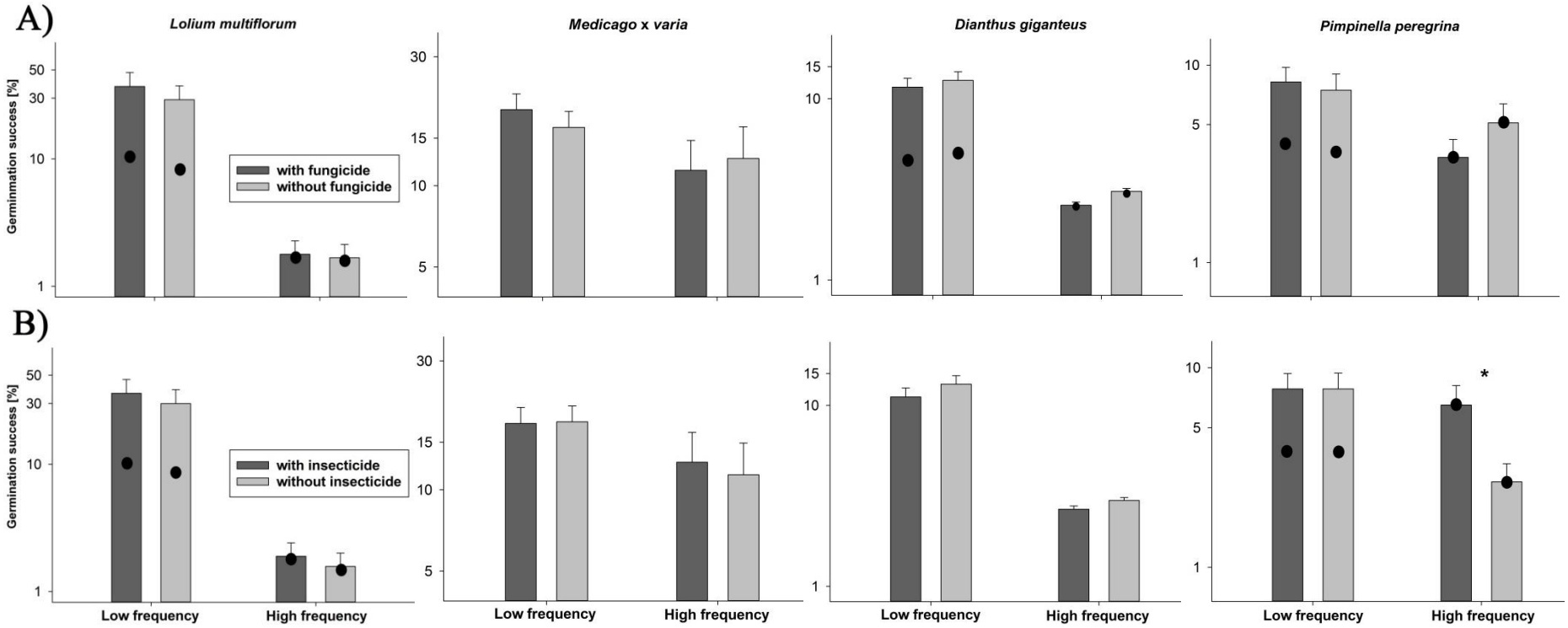
Source		***H. perforatum***	***T. pratensis***	***D. giganteus***	***P. peregrina***
	d.f.		χ^2 values		
Relative frequency [RF]	1	0.27	2.67	0.60	0.20
Fungicide application [F]	1	0.87	3.18	2.93	3.51
Insecticide application [I]	1	0.05	2.95	0.05	3.31
RF × F	1	4.94*	2.37	4.19*	3.77
RF × I	1	0.58	3.23	0.04	2.10
F × I	1	0.03	0.92	0.02	0.31
RF × F × I	1	0.69	1.12	-	-
Block	4	9.17	10.48*	23.88***	1.98



Appendix 7.2.10A Germination success (least square means and standard errors of the mean), as estimated by the total number of individuals present in the first year after sowing relative to the number of sown seeds, for three out of six native focal species (for the three other native species see Appendix 7.2.10B) on low and high frequency plots treated with A) fungicide and B) insecticide and the respective controls. Note that for *L. corniculatus* and *M. falcata*, columns and error bars represent means and standard errors adjusted for excessive zeros. Black dots indicate mean values including excessive zeros (i.e. plots with zero values exceeding the number of plots expected by the underlying distribution, see methods for detailed information). The interaction effect of fungicide and insecticide application is not shown, because it was not significant (see Appendix 7.2.10D). Note the logarithmic scale of the y-axis.



Appendix 7.2.10B Germination success (least square means and standard errors of the mean), as estimated by the total number of individuals present in the first year after sowing relative to the number of sown seeds, for three out of six native focal species (for the three other native species see Appendix 7.2.10A) on low and high frequency plots treated with A) fungicide and B) insecticide and the respective controls. Note that for *T. pratensis* and *H. perforatum*, columns and error bars represent means and standard errors adjusted for excessive zeros. Black dots indicate mean values including excessive zeros (i.e. plots with zero values exceeding the number of plots expected by the underlying distribution, see methods for detailed information). The interaction effect of fungicide and insecticide application is not shown, because it was not significant (see Appendix 7.2.10D). Asterisks indicate significant effects of insecticide or fungicide application on germination success at the respective level of relative frequency (* $P < 0.05$). Note the logarithmic scale of the y-axis.



Appendix 7.2.10C Germination success (least square means and standard error of the mean), as estimated by the total number of individuals present in the first year after sowing relative to the number of sown seeds, for four exotic focal species on low and high frequency plots treated with A) fungicide and B) insecticide and the respective controls. Note that for *L. multiflorum, P. peregrina* and *D. giganteus*, columns and error bars represent means and standard errors adjusted for excessive zeros. Black dots indicate mean values including excessive zeros (i.e. plots with zero values exceeding the number of plots expected by the underlying distribution, see methods for detailed information). Although there is a significant interaction effect (F × I for *D. giganteus*, see Appendix 7.2.10E), it is not shown because of consistency. Asterisks indicate significant effects of insecticide or fungicide application on germination success at the respective level of relative frequency (* $P < 0.05$). Note the logarithmic scale of the y-axis.

Appendix 7.2.10D Results of generalized linear models on the effects of a species' relative frequency in the seed mixture *(hypothesis 3)*, fungicide application *(hypothesis 4)*, and insecticide application *(hypothesis 4)*, as well as their interactions, and the effect of block on the estimates of germination success, as estimated by the number of seedlings in the year after sowing, relative to the number of seeds sown, for six native focal species. Note that zero inflation was not present in the datasets of *A. elatius* and *F. vulgaris*. See method section for further information on model structure. Abbreviations for factors are given in brackets. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Source		***A. elatius***	***L. corniculatus***	***M. falcata***	***T. pratensis***	***H. perforatum***	***F. vulgaris***
	d.f.			χ^2 values			
Relative frequency [RF]	1	87.74 ***	7.86 **	35.18 ***	16.79 ***	96.33 ***	15.29 ***
Relative frequency – Zero inflation	1	-	37.03 ***	41.79 ***	48.44 ***	4.09 *	-
Fungicide application [F]	1	0.02	1.08	0.41	7.22 **	0.00	0.42
Insecticide application [I]	1	0.01	0.03	0.62	3.40	0.01	0.42
RF x F	1	0.16	0.43	0.06	5.78 *	0.03	0.22
RF x I	1	0.09	0.03	0.26	2.06	0.00	0.22
F x I	1	0.58	2.97	1.90	1.47	1.09	0.62
RF x F x I	1	0.86	1.40	2.66	1.26	0.04	1.76
Block	4	11.81 *	16.48 **	3.43	15.29 **	27.46 ***	5.88

Appendix 7.2.10E Results of generalized linear models on the effects of a species' relative frequency in the seed mixture *(hypothesis 3)*, fungicide application *(hypothesis 4)*, and insecticide application *(hypothesis 4)*, as well as their interactions, and the effect of block on the estimates of germination success, as estimated by the number of seedlings in the year after sowing, relative to the number of seeds sown, for four exotic focal species. Note that zero inflation was not present in the datasets of *M. x varia* and that two species (*O. viciifolia, B. orientalis*) had to be omitted because of poor germination. See method section for further information on model structure. Abbreviations for factors are given in brackets. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Source		***L. multiflorum***	***M.* x *varia***	***P. peregrina***	***D. giganteus***
	d.f.		χ^2 values		
Relative frequency [RF]	1	40.10 ***	3.13	7.18 **	195.60 ***
Relative frequency – Zero inflation	1	32.35 ***	-	24.84 ***	32.50 ***
Fungicide application [F]	1	0.33	0.01	0.62	2.47
Insecticide application [I]	1	0.60	0.05	4.82 *	2.68
RF x F	1	0.15	0.37	1.60	0.29
RF x I	1	0.00	0.08	4.97 *	0.09
F x I	1	3.81	0.19	2.27	4.91 *
RF x F x I	1	0.41	0.11	0.93	0.80
Block	4	18.36 ***	2.80	6.03	37.07 ***

Appendix 7.2.11 Results of generalized linear models on the effects of a species' relative frequency in the seed mixture *(hypothesis 3)*, fungicide application *(hypothesis 4)*, and insecticide application *(hypothesis 4)*, as well as their interactions, and the effect of block on the estimates of per capita rate of increase, as estimated by the number of seeds, or the number of inflorescences, relative to realized abundance after two years, as estimated by the number of individuals in that year, for two native (*Hypericum perforatum* and *Tragopogon pratensis*) and two exotic (*Dianthus giganteus* and *Pimpinella peregrina*) focal species. Note that that zero inflation was only present in the dataset of *P. peregrina*, and that the three-way interaction had to be excluded for this species because of zero values for all replicates of a certain treatment combination. See method section for further information on model structure. Abbreviations for factors are given in brackets. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Source	***H. perforatum***	***T. pratensis***	***D. giganteus***	***P. peregrina***
	d.f.	χ^2 values		
Relative frequency [RF]	1	11.53 ***	0.84	0.05	7.78 **
Relative frequency – Zero inflation	1	-	-	-	11.44 ***
Fungicide application [F]	1	1.98	2.51	0.61	2.69
Insecticide application [I]	1	0.01	1.04	16.28 ***	2.69
RF x F	1	0.02	0.77	0.30	3.49
RF x I	1	1.05	1.60	16.08 ***	3.07
F x I	1	2.74	0.79	4.65 *	0.23
RF x F x I	1	0.56	0.07	4.88 *	-
Block	4	1.24	5.92	34.82 ***	2.24

Appendix 7.2.12 Abundance, cover and aboveground biomass of focal species. Values given are means ± standard deviation. (N) = Native species, (E) = Exotic species. LF = Low-frequency plots; HF = High-frequency plots; F+ = Fungicide application; F- = No fungicide application; I+ = Insecticide application; I- = No insecticide application.

	Mean no. of individuals per m² 2014	Mean cover per m² 2015 [%]	Mean cover per m² 2016 [%]	Mean biomass 2015 [g/m²]
(N) *Arrhenatherum elatius*				
LF F- I-	28.7 ± 23.3	11.5 ± 8.7	9.7 ± 8.0	105.6 ± 135.5
LF F- I+	26.9 ± 20.8	11.0 ± 8.2	11.3 ± 9.2	92.8 ± 134.8
LF F+ I-	28.9 ± 34.5	14.7 ± 10.0	13.6 ± 7.8	137.5 ± 114.9
LF F+ I+	23.8 ± 20.5	14.0 ± 11.4	18.6 ± 10.8	95.5 ± 118.8
HF F- I-	56.8 ± 23.9	32.0 ± 6.7	20.0 ± 5.3	135.4 ± 80.0
HF F- I+	42.4 ± 19.3	25.0 ± 7.2	23.6 ± 6.8	182.9 ± 58.0
HF F+ I-	42.4 ± 15.1	32.2 ± 15.1	35.6 ± 15.2	173.1 ± 113.2
HF F+ I+	59.2 ± 29.0	26.4 ± 11.3	28.0 ± 9.6	169.9 ± 130.4
(N) *Lotus corniculatus*				
LF F- I-	0.2 ± 0.8	1.5 ± 3.7	3.7 ± 5.7	0.8 ± 33.2
LF F- I+	0.8 ± 1.6	0.8 ± 1.5	3.1 ± 4.1	2.2 ± 64.3
LF F+ I-	1.3 ± 2.5	1.1 ± 1.6	2.1 ± 3.5	1.9 ± 71.7
LF F+ I+	0.4 ± 1.2	1.1 ± 3.6	2.9 ± 4.1	2.2 ± 10.8
HF F- I-	32.8 ± 22.5	51.0 ± 21.9	64.6 ± 8.4	58.8 ± 73.6
HF F- I+	44.8 ± 30.8	45.4 ± 25.7	53.0 ± 29.4	41.9 ± 84.4
HF F+ I-	61.6 ± 67.9	24.4 ± 13.4	60.8 ± 11.0	31.5 ± 25.4
HF F+ I+	41.6 ± 24.4	36.8 ± 29.8	60.0 ± 29.6	50.0 ± 54.5
(N) *Medicago falcata*				
LF F- I-	1.3 ± 2.8	0.8 ± 1.5	7.2 ± 11.4	3.2 ± 15.1
LF F- I+	1.0 ± 1.7	1.0 ± 1.4	14.2 ± 17.9	0.0 ± 0.0
LF F+ I-	1.0 ± 1.7	1.1 ± 1.8	11.4 ± 17.1	0.3 ± 1.5
LF F+ I+	1.8 ± 2.6	0.5 ± 1.0	6.3 ± 7.5	0.9 ± 2.2
HF F- I-	77.6 ± 43.7	20.6 ± 32.4	60.8 ± 17.0	39.8 ± 85.9
HF F- I+	77.6 ± 34.8	40.6 ± 26.8	65.2 ± 27.5	42.2 ± 29.6
HF F+ I-	69.6 ± 31.1	31.2 ± 22.4	66.8 ± 29.0	33.6 ± 37.7
HF F+ I+	63.2 ± 37.4	20.8 ± 24.7	43.0 ± 40.9	27.9 ± 30.5
(N) *Tragopogon pratensis*				
LF F- I-	0.2 ± 8.2	0.4 ± 0.8	0.2 ± 0.3	2.8 ± 13.7
LF F- I+	0.1 ± 0.2	0.4 ± 1.2	0.1 ± 0.3	0.0 ± 0.0
LF F+ I-	0.3 ± 1.1	0.2 ± 0.5	0.3 ± 0.7	0.2 ± 0.86
LF F+ I+	0.5 ± 1.3	0.1 ± 0.3	0.0 ± 0.1	10.1 ± 5.1
HF F- I-	9.6 ± 6.1	5.0 ± 2.9	1.0 ± 1.7	10.2 ± 14.1
HF F- I+	9.8 ± 10.2	5.2 ± 3.2	0.6 ± 0.9	20.7 ± 36.9
HF F+ I-	10.6 ± 8.0	5.2 ± 1.6	1.0 ± 1.7	43.2 ± 86.6
HF F+ I+	11.6 ± 11.9	3.4 ± 2.9	2.1 ± 2.2	39.5 ± 48.1

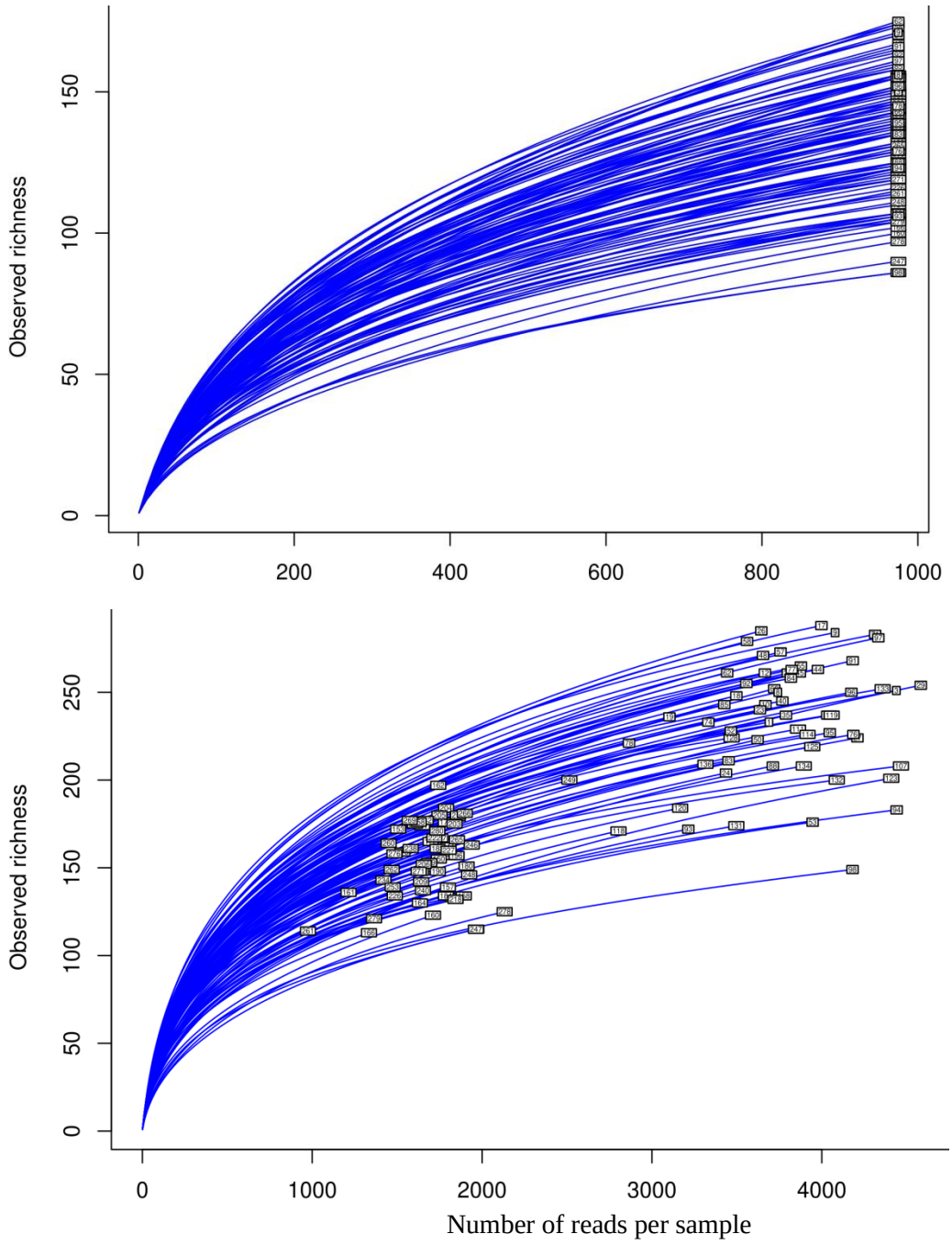
	Mean no. of individuals per m² 2014	Mean cover per m² 2015 [%]	Mean cover per m² 2016 [%]	Mean biomass 2015 [g/m²]
(N) *Hypericum perforatum*				
LF F- I-	9.4 ± 10.9	3.2 ± 2.3	2.9 ± 2.6	11.1 ± 20.9
LF F- I+	9.2 ± 8.6	2.7 ± 2.6	2.4 ± 2.4	12.9 ± 29.7
LF F+ I-	9.5 ± 11.7	3.4 ± 2.8	4.7 ± 5.1	18.9 ± 56.4
LF F+ I+	10.9 ± 9.5	3.8 ± 3.4	5.6 ± 3.1	14.7 ± 23.5
HF F- I-	29.6 ± 24.4	5.2 ± 2.0	6.6 ± 4.4	13.3 ± 22.5
HF F- I+	24.8 ± 20.1	2.8 ± 1.6	6.8 ± 4.1	5.9 ± 5.7
HF F+ I-	21.6 ± 13.4	7.2 ± 3.6	18.8 ± 11.9	17.8 ± 16.6
HF F+ I+	21.6 ± 17.3	11.6 ± 8.6	13.8 ± 8.3	68.5 ± 76.9
(E) *Lolium multiflorum*				
LF F- I-	0.9 ± 2.3	0.2 ± 0.6	0.6 ± 0.9	0.6 ± 1.2
LF F- I+	2.9 ± 8.1	0.3 ± 1.2	0.7 ± 1.5	0.7 ± 1.6
LF F+ I-	3.0 ± 6.6	0.3 ± 0.7	0.7 ± 1.1	0.5 ± 1.7
LF F+ I+	1.7 ± 3.8	0.3 ± 0.6	0.8 ± 1.1	1.6 ± 5.4
HF F- I-	12.4 ± 7.8	5.8 ± 5.6	12.8 ± 7.7	47.4 ± 58.3
HF F- I+	19.2 ± 15.1	6.8 ± 8.0	10.6 ± 6.5	29.7 ± 51.6
HF F+ I-	16.0 ± 13.9	1.5 ± 1.6	8.4 ± 4.5	8.1 ± 14.5
HF F+ I+	12.8 ± 11.5	2.9 ± 2.5	9.0 ± 7.8	14.6 ± 24.9
(E) *Onobrychis viciifolia*				
LF F- I-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
LF F- I+	0.0 ± 0.0	0.7 ± 3.6	0.7 ± 2.8	0.0 ± 0.0
LF F+ I-	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.6	0.0 ± 0.0
LF F+ I+	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 1.7	0.0 ± 0.0
HF F- I-	2.6 ± 3.4	5.2 ± 9.5	13.2 ± 28.4	23.1 ± 51.7
HF F- I+	3.4 ± 3.1	9.4 ± 10.5	21.8 ± 25.0	35.5 ± 79.4
HF F+ I-	0.8 ± 1.8	6.2 ± 13.3	8.4 ± 16.6	12.9 ± 28.9
HF F+ I+	2.0 ± 2.0	9.6 ± 14.7	19.4 ± 27.7	0.0 ± 0.0
(E) *Medicago* x *varia*				
LF F- I-	3.4 ± 3.4	3.5 ± 5.6	17.1 ± 16.0	14.0 ± 63.8
LF F- I+	3.7 ± 4.0	4.7 ± 6.4	18.6 ± 16.4	19.3 ± 51.3
LF F+ I-	4.1 ± 3.6	7.2 ± 8.3	19.4 ± 21.2	35.0 ± 119.6
LF F+ I+	3.9 ± 3.3	8.3 ± 10.5	27.2 ± 17.9	29.9 ± 80.3
HF F- I-	149.0 ± 87.7	30.3 ± 39.0	44.0 ± 34.8	83.7 ± 162.3
HF F- I+	172.8 ± 39.8	23.1 ± 20.0	42.8 ± 28.6	43.7 ± 54.4
HF F+ I-	143.2 ± 26.1	28.6 ± 27.3	53.2 ± 22.7	30.7 ± 29.5
HF F+ I+	139.2 ± 52.3	45.6 ± 36.0	56.6 ± 33.2	103.8 ± 100.7
(E) *Dianthus giganteus*				
LF F- I-	1.7 ± 3.7	0.3 ± 0.6	0.3 ± 0.8	0.2 ± 0.9
LF F- I+	1.9 ± 2.5	0.4 ± 0.6	1.2 ± 1.5	0.2 ± 0.9
LF F+ I-	2.0 ± 2.6	0.4 ± 0.7	1.4 ± 2.3	6.7 ± 33.5
LF F+ I+	1.4 ± 2.3	0.3 ± 0.5	0.7 ± 1.1	0.2 ± 1.2
HF F- I-	80.0 ± 43.7	6.4 ± 2.4	10.8 ± 3.3	10.9 ± 10.2
HF F- I+	64.0 ± 7.5	8.4 ± 4.3	15.2 ± 6.4	45.1 ± 58.0
HF F+ I-	60.0 ± 29.4	8.2 ± 2.5	11.8 ± 4.0	13.4 ± 13.3
HF F+ I+	60.0 ± 11.7	6.4 ± 1.5	13.0 ± 4.4	13.7 ± 15.6

	Mean no. of individuals per m² 2014	Mean cover per m² 2015 [%]	Mean cover per m² 2016 [%]	Mean biomass 2015 [g/m²]
(E) *Pimpinella peregrina*				
LF F- I-	2.3 ± 3.9	5.4 ± 7.3	3.8 ± 3.2	18.3 ± 40.5
LF F- I+	3.5 ± 4.8	5.6 ± 7.4	3.7 ± 5.6	30.5 ± 61.2
LF F+ I-	4.2 ± 5.8	4.7 ± 5.6	3.4 ± 4.1	8.3 ± 19.6
LF F+ I+	2.6 ± 4.7	5.3 ± 10.4	2.5 ± 2.7	0.2 ± 0.37
HF F- I-	124.8 ± 56.4	52.4 ± 17.0	35.8 ± 18.8	203.1 ± 169.9
HF F- I+	462.0 ± 671.1	45.8 ± 21.3	20.0 ± 8.6	199.9 ± 263.5
HF F+ I-	140.0 ± 94.2	37.0 ± 15.7	25.8 ± 29.9	105.3 ± 143.2
HF F+ I+	167.8 ± 79.0	62.0 ± 17.5	33.6 ± 29.5	415.9 ± 282.5
(E) *Bunias orientalis*				
LF F- I-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
LF F- I+	0.2 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
LF F+ I-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
LF F+ I+	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
HF F- I-	0.0 ± 0.0	0.6 ± 1.3	0.0 ± 0.0	0.0 ± 0.0
HF F- I+	0.0 ± 0.0	0.4 ± 0.9	0.1 ± 1.7	0.0 ± 0.0
HF F+ I-	1.6 ± 3.6	4.5 ± 9.8	2.2 ± 4.4	27.2 ± 60.8
HF F+ I+	0.8 ± 1.8	0.8 ± 1.8	1.2 ± 2.7	0.0 ± 0.0

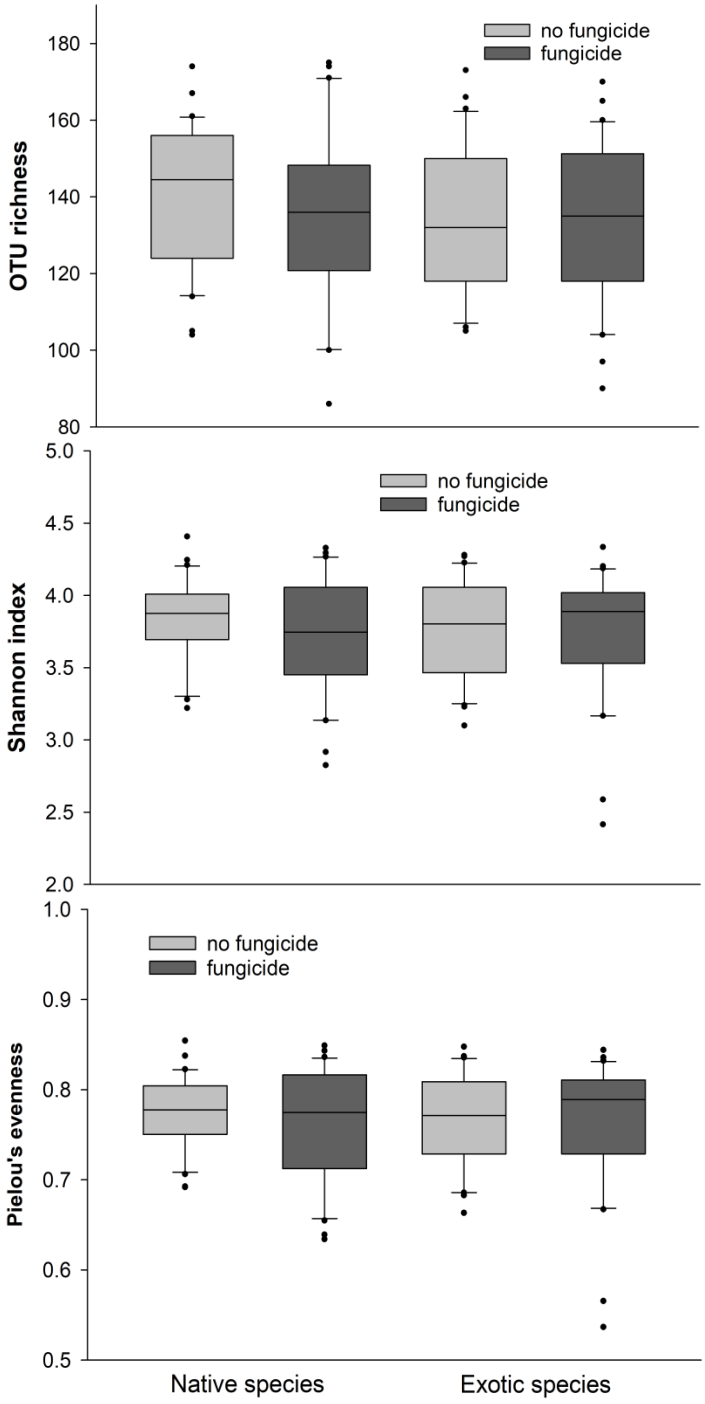
Appendix 7.2.13 Realized frequency for each native and exotic focal species in May 2014, measured as proportional number of individuals per plot (low frequency vs. high frequency plots) relative to the total number of individuals of all species per plot, and proportional biomasses per plot (low frequency vs. high frequency plots) for each native and exotic focal species of the July 2015 harvest, relative to the total biomass of all species per plot. Realized frequency and total proportional biomass for all native and exotic focal species were also calculated across all 120 plots per origin. Values given are means ± standard deviation.

	Proportional number of individuals per plot 2014 [%]	Proportional biomass per plot 2015 [%]
(N) *Arrhenatherum elatius*		
Low frequency	11.8 ± 10.0	3.6 ± 6.5
High frequency	25.4 ± 8.4	6.6 ± 2.8
(N) *Lotus corniculatus*		
Low frequency	0.4 ± 0.9	0.1 ± 0.2
High frequency	18.5 ± 13.8	1.8 ± 2.3
(N) *Medicago falcata*		
Low frequency	0.6 ± 1.2	0.1 ± 0.4
High frequency	26.7 ± 12.1	1.5 ± 2.3
(N) *Tragopogon pratensis*		
Low frequency	0.2 ± 0.6	0.1 ± 0.1
High frequency	5.4 ± 4.4	0.9 ± 1.3
(N) *Hypericum perforatum*		
Low frequency	4.1 ± 3.8	0.5 ± 1.1
High frequency	10.4 ± 6.1	0.9 ± 1.2
(N) *Falcaria vulgaris*		
Low frequency	1.0 ± 2.0	0.1 ± 0.2
High frequency	2.4 ± 5.3	0.1 ± 0.1
Native focal species	29.7 ± 14.5	48.8 ± 29.8
(E) *Lolium multiflorum*		
Low frequency	0.7 ± 1.7	0.1 ± 0.1
High frequency	6.8 ± 4.5	0.8 ± 1.3
(E) *Onobrychis viciifolia*		
Low frequency	0.0 ± 0.0	0.0 ± 0.0
High frequency	1.3 ± 1.8	0.4 ± 1.0
(E) *Medicago* x *varia*		
Low frequency	1.7 ± 1.8	0.5 ± 1.4
High frequency	40.3 ± 16.0	2.0 ± 3.1
(E) *Dianthus giganteus*		
Low frequency	0.8 ± 1.4	0.1 ± 0.3
High frequency	24.3 ± 8.2	0.6 ± 0.7
(E) *Pimpinella peregrina*		
Low frequency	1.3 ± 2.0	0.4 ± 0.9
High frequency	42.5 ± 17.3	4.6 ± 3.7
(E) *Bunias orientalis*		
Low frequency	0.1 ± 0.1	0.0 ± 0.0
High frequency	0.3 ± 1.0	0.3 ± 1.1
Exotic focal species	22.9 ± 19.4	23.9 ± 33.5

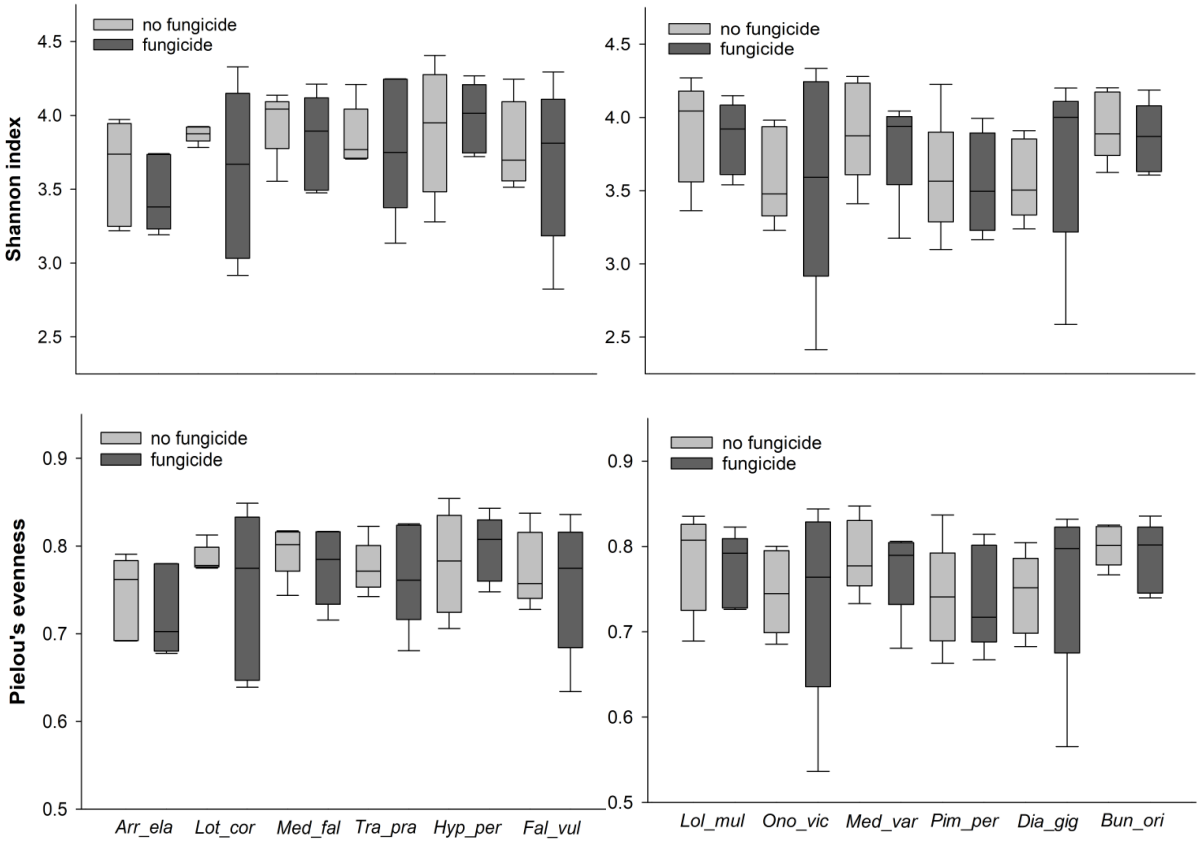
7.3 Supplemental Information Chapter 4



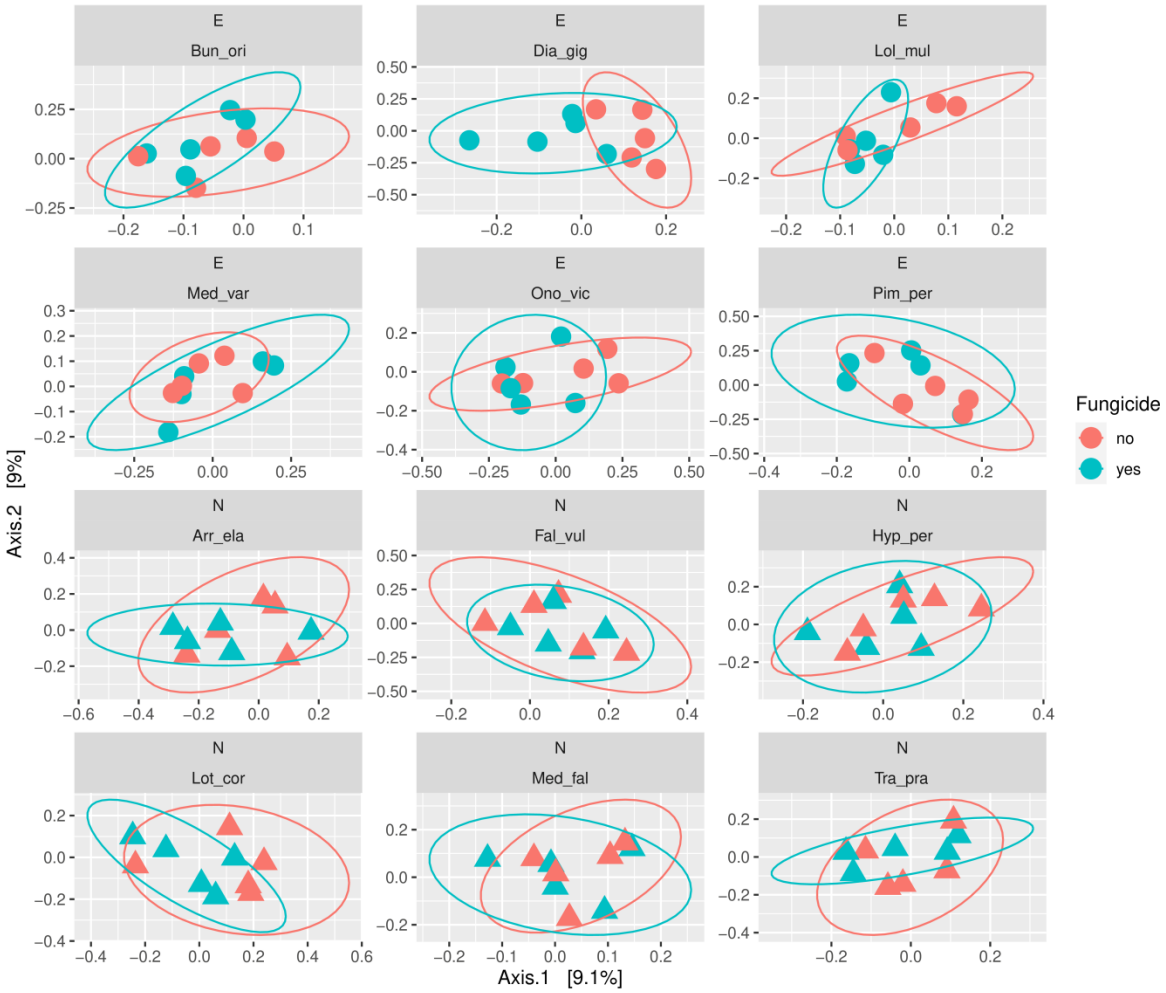
Appendix 7.3.1 Rarefaction curves of the rarefied (upper panel) and unrarefied (lower panel) dataset. Per sample read count was normalized to the sample with the lowest read counts using the 'rarefy_even_depth' function of the phyloseq package (McMurdie and Holmes 2013) in R version 3.6.2 (R Development Core Team 2019).



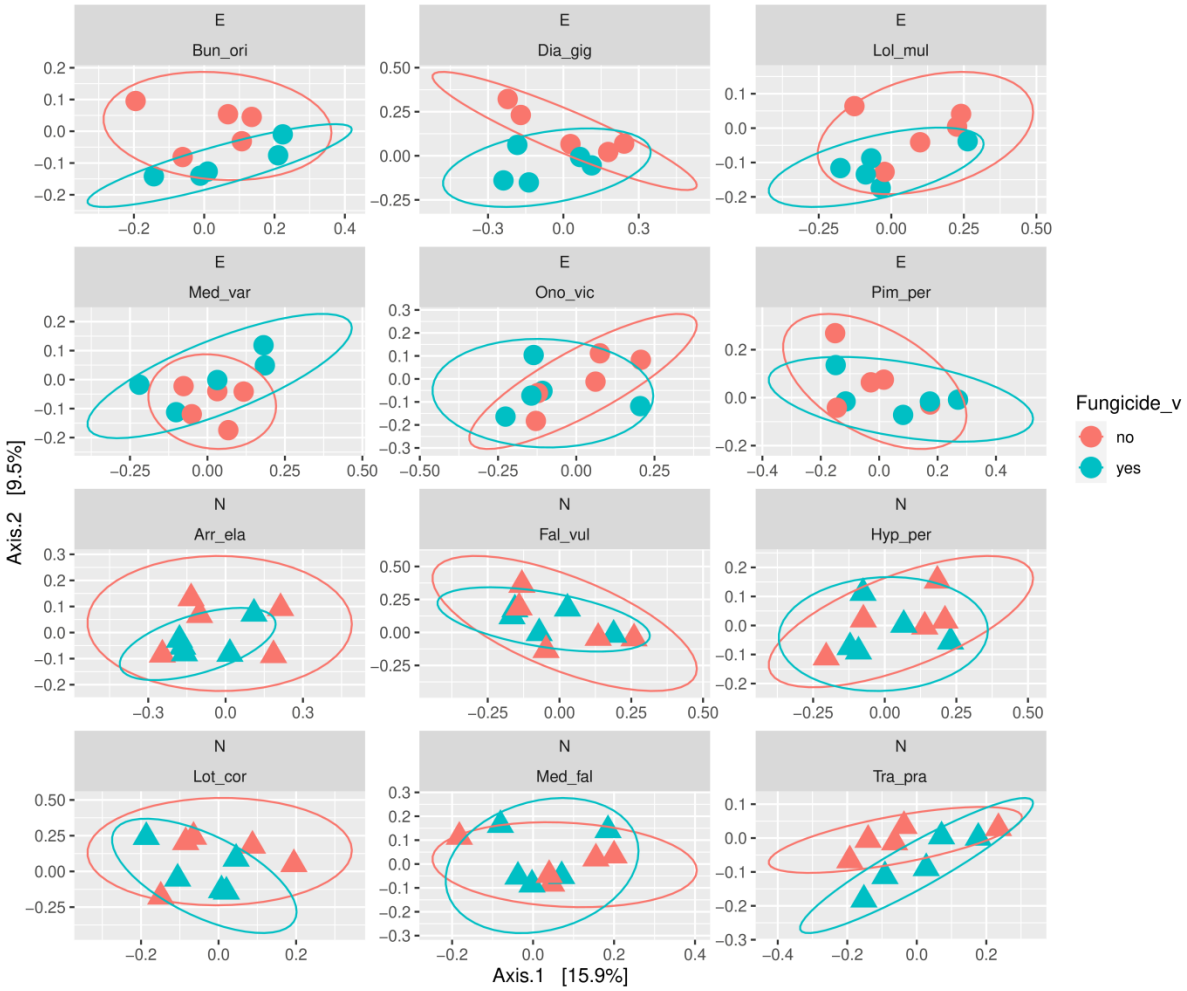
Appendix 7.3.2 Effects of the interaction of fungicide application and plant species origin on the α-diversity measures OTU richness, Shannon diversity index and Pielou's evenness of soil fungal communities. Boxes represent 25th and 75th percentiles, error bars show 10th and 90th percentiles. The median is indicated by the vertical line in each box. Each outlier is shown. N for each analyzed group (Native species with no fungicide, Native species with fungicide, Exotic species with no fungicide, Exotic species with fungicide) is 30. There were no significant effects.



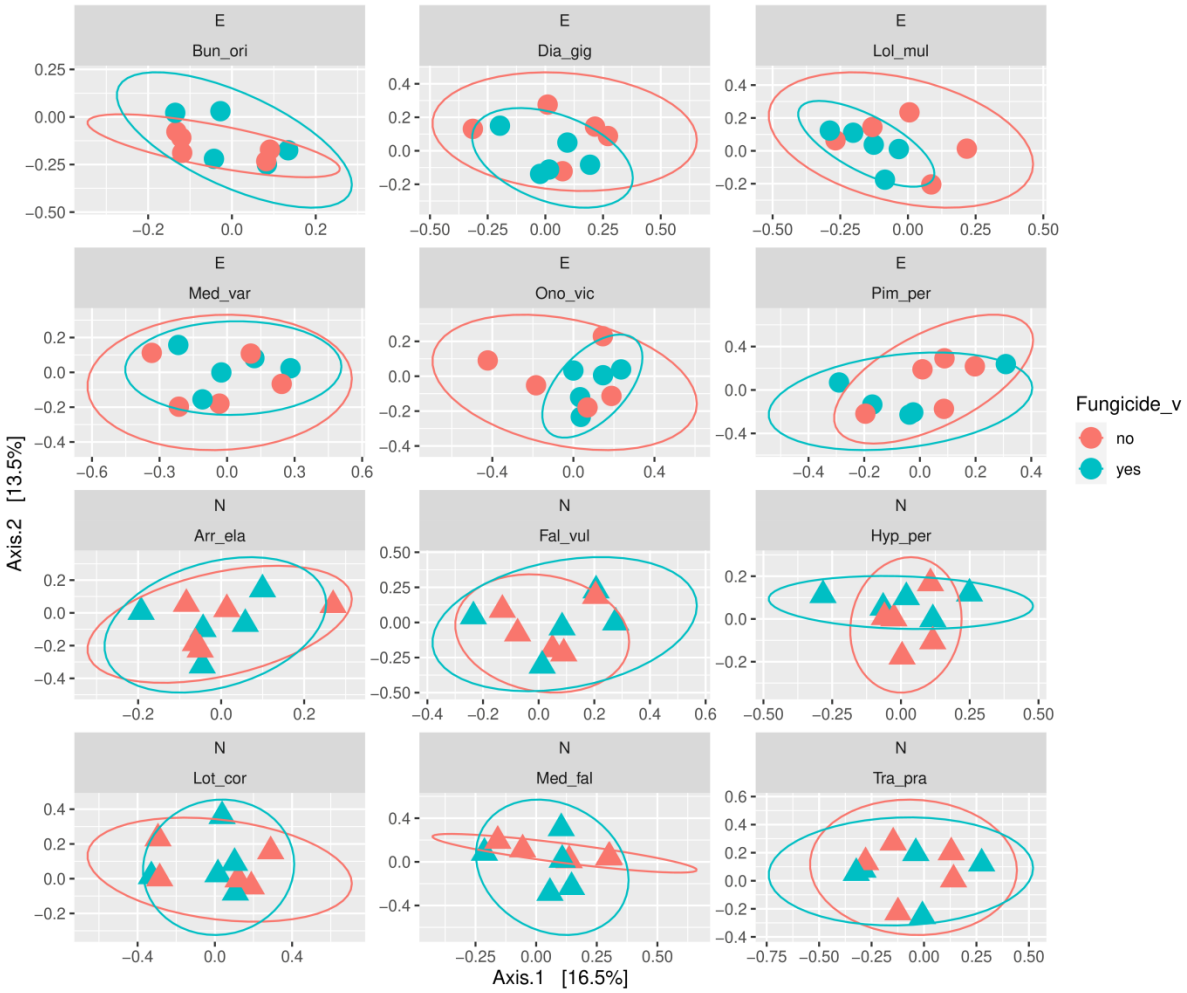
Appendix 7.3.3 Effects of fungicide application on the Shannon index and Pielou's evenness of soil fungal communities depending on the dominant plant species. Boxes represent 25th and 75th percentiles, error bars show 10th and 90th percentiles. The median is indicated by the vertical line in each box. Each outlier is shown. Results of within-species comparisons using t-tests are not shown because all of these comparisons yielded non-significant results (with $\alpha = 0.05$). N for each species is 10. There were no significant effects. Because of the low sample size we also conducted a non-parametric Mann-Whitney U test, which yielded a non-significant result as well.



Appendix 7.3.4A Results of Principal Coordinate Analysis (PCoA) for the effects of fungicide application on fungal OTU composition of the whole fungal community for each of the six exotic and six native plant species when sown as a dominant species. Each symbol represents the data from one plot. The colored ellipses indicate 90% confidence. Note the differences in scale among the graphs. E = exotic plant species, N = native plant species. Arr_ela = *Arrhenaterum elatius*, Lot_cor = *Lotus corniculatus*, Med_fal = *Medicago falcata*, Tra_pra = *Tragopogon pratensis*, Hyp_per = *Hypericum perforatum*, Fal_vul = *Falcaria vulgaris*, Lol_mul = *Lolium multiforum*, Ono_vic = *Onobrychis viciifolia*, Med_var = *Medicago* x *varia*, Dia_gig = *Dianthus giganteus*, Pim_per = *Pimpinella peregrina*, Bun_ori = *Bunias orientalis*.



Appendix 7.3.4B Results of Principal Coordinate Analysis (PCoA) for the effects of fungicide application on fungal OTU composition of the saprotrophic community for each of the six exotic and six native plant species when sown as a dominant species. Each symbol represents the data from one plot. The colored ellipses indicate 90% confidence. Note the differences in scale among the graphs. E = exotic plant species, N = native plant species. Arr_ela = *Arrhenaterum elatius*, Lot_cor = *Lotus corniculatus*, Med_fal = *Medicago falcata*, Tra_pra = *Tragopogon pratensis*, Hyp_per = *Hypericum perforatum*, Fal_vul = *Falcaria vulgaris*, Lol_mul = *Lolium multiforum*, Ono_vic = *Onobrychis viciifolia*, Med_var = *Medicago* x *varia*, Dia_gig = *Dianthus giganteus*, Pim_per = *Pimpinella peregrina*, Bun_ori = *Bunias orientalis*.



Appendix 7.3.4C Results of Principal Coordinate Analysis (PCoA) for the effects of fungicide application on fungal OTU composition of the pathotrophic community for each of the six exotic and six native plant species when sown as a dominant species. Each symbol represents the data from one plot. The colored ellipses indicate 90% confidence. Note the differences in scale among the graphs. E = exotic plant species, N = native plant species. Arr_ela = *Arrhenaterum elatius*, Lot_cor = *Lotus corniculatus*, Med_fal = *Medicago falcata*, Tra_pra = *Tragopogon pratensis*, Hyp_per = *Hypericum perforatum*, Fal_vul = *Falcaria vulgaris*, Lol_mul = *Lolium multiforum*, Ono_vic = *Onobrychis viciifolia*, Med_var = *Medicago* x *varia*, Dia_gig = *Dianthus giganteus*, Pim_per = *Pimpinella peregrina*, Bun_ori = *Bunias orientalis*.

Appendix 7.3.5A Results of indicator species analysis to identify OTUs specific to soil fungal communities of exotic plant species subjected to fungicide treatment using the *indicspecies* package (ver. 1.7.9), where OTUs with P < 0.05 were considered as indicator taxa. 0 and 1 indicate absence and presence, respectively, of the OTU in exotic plant communities with or without fungicide treatment. Species abbreviations: *Lolium multiflorum*, *Onobrychis viciifolia*, *Medicago* x *varia*, *Pimpinella peregrina*, *Dianthus giganteus*, *Bunias orientalis*. F-: no fungicide application, F+: fungicide application, P: Pathotroph, SA: Saprotroph, SY: Symbiotroph, na: information not available.

OTU Nr.	Taxonomy	Functional group	Lm F+	Lm F-	Ov F+	Ov F-	Mv F+	Mv F-	Pp F+	Pp F-	Dg F+	Dg F-	Bo F+	Bo F-	Stat	p-value
0007	Amphisphaeriaceae	SA	0	0	0	0	0	0	0	0	0	1	0	0	0.5230	0.020
0047	Sordariomycetes	na	0	1	1	0	0	0	0	0	0	0	1	1	0.5646	0.007
0073	Sebacinales	na	0	0	0	0	0	1	0	0	0	0	1	0	0.5256	0.037
0083	Pleosporales	na	0	0	0	0	0	0	0	0	0	1	0	0	0.5350	0.031
0101	*Cladorrhinum flexuosum*	P-SA-SY	0	0	0	0	0	0	0	1	0	0	0	0	0.4717	0.008
0102	Pyronemataceae	na	1	0	0	0	0	0	0	0	0	0	0	0	0.5584	0.025
0130	*Chrysosporium chiropterorum*	na	1	0	0	0	0	1	0	0	0	0	1	0	0.6086	0.002
0133	*Fusarium oxysporum*	P-SA-SY	0	1	0	0	1	0	0	0	0	1	0	0	0.4958	0.046
0167	GS02	na	0	0	0	1	0	0	0	0	0	0	0	0	0.5681	0.025
0168	Pleosporales	na	0	0	0	0	0	1	0	0	0	0	0	0	0.6871	0.003
0193	*Rhizophlyctis rosea*	SA	0	0	0	0	0	0	0	1	0	0	0	0	0.5762	0.027
0207	*Peziza pseudoviolacea*	SY	0	0	0	0	0	0	0	0	0	0	1	0	0.4873	0.016
0231	*Gymnoascus reessii*	SA	0	0	0	0	1	0	0	0	0	0	0	0	0.5366	0.023
0242	Unclassified fungi	na	0	0	0	1	0	0	0	0	0	0	0	0	0.4980	0.047
0252	*Mortierella alpina*	SA	0	1	1	0	0	0	0	0	0	0	0	0	0.5636	0.025
0258	Pezizaceae	na	0	1	0	1	0	0	0	0	0	0	1	0	0.5544	0.015
0369	Agaricomycetes	na	0	1	0	0	0	0	0	0	0	0	0	0	0.5579	0.029
0399	*Melanocarpus albomyces*	na	1	0	1	0	0	0	0	0	0	0	0	0	0.5247	0.038
0413	*Rhodotorula ferulica*	P-SA	0	1	0	0	0	0	0	0	0	0	0	0	0.5971	0.021
0417	*Funneliformis geosporum*	SY	0	0	0	0	0	1	0	0	0	0	0	0	0.5628	0.041
0447	*Funneliformis mosseae*	SY	0	0	0	0	0	0	0	0	0	1	0	0	0.5636	0.035
0508	Unclassified fungi	na	0	1	0	0	0	0	0	0	0	0	0	0	0.5579	0.038

Appendix 7.3.5B Results of indicator species analysis to identify OTUs specific to soil fungal communities of native plant species subjected to fungicide treatment using the *indicspecies* package (ver. 1.7.9), where OTUs with P < 0.05 were considered as indicator taxa. 0 and 1 indicate absence and presence, respectively, of the OTU in native plant communities with or without fungicide treatment. Species abbreviations: *Arrhenatherum elatius*, *Lotus corniculatus*, *Medicago falcata*, *Tragopogon pratensis*, *Hypericum perforatum*, *Falcaria vulgaris*. F-: no fungicide application, F+: fungicide application, P: Pathotroph, SA: Saprotroph, SY: Symbiotroph, na: information not available.

OTU Nr.	Taxonomy	Functional group	Ae F+	Ae F-	Lc F+	Lc F-	Mf F+	Mf F-	Tp F+	Tp F-	Hp F+	Hp F-	Fv F+	Fv F-	Stat	p-value
0011	*Mortierella* sp.	SA	0	0	0	0	1	0	0	0	0	0	1	1	0.4857	0.047
0056	*Tetracladium marchalianum*	SA	0	0	0	0	0	0	1	0	0	0	0	0	0.5748	0.002
0057	*Scutellinia scutellata*	SA	0	0	0	0	0	0	0	1	0	0	0	0	0.5449	0.038
0111	Agaricomycetes	na	0	0	1	0	0	0	0	0	0	0	0	0	0.5012	0.045
0208	*Metacordyceps chlamydosporia*	P	1	0	0	0	0	0	0	1	1	1	1	1	0.5285	0.025
0280	Lophiostomataceae	na	0	0	0	1	1	1	0	0	1	0	0	1	0.5233	0.035
0304	Montagnulaceae	na	0	0	0	0	0	0	0	0	0	1	0	0	0.6390	0.006
0322	*Acremonium spinosum*	P-SA-SY	0	0	0	0	0	0	0	0	1	0	0	0	0.5715	0.013
0376	*Arthropsis hispanica*	P	0	0	0	0	0	0	0	0	1	0	0	0	0.5636	0.033
0443	*Macrocystidia cucumis*	SA	0	0	0	0	0	1	1	0	0	1	0	0	0.5410	0.032
0516	*Cadophora luteo-olivacea*	SY	0	0	0	0	0	0	0	0	0	0	1	0	0.7153	0.003
0539	*Clonostachys* sp.	P	0	0	0	0	0	0	1	0	0	0	0	0	0.7609	0.004
0566	*Cylindrocarpon* sp.	P	0	1	0	0	0	0	0	0	0	0	1	0	0.5340	0.047

7.4 Personal contributions to the manuscripts

Chapter 2: Schmidt R, Auge H, Deising HB, Hensen I, Mangan SA, Schädler M, Stein C, Knight TM (2020): Abundance, origin, and phylogeny of plants do not predict community-level patterns of pathogen diversity and infection. *Ecology and Evolution* 10: 5506–5516. DOI: 10.1002/ece3.6292

Field work	Robin Schmidt (75%), Scott A. Mangan, Claudia Stein, Tiffany M. Knight, field technicians, student helpers (together 25%)
Lab work	Robin Schmidt (90%), lab technicians (10%)
Analysis	Robin Schmidt (70%), Tiffany M. Knight (20%), Harald Auge (10%)
Writing	Robin Schmidt (75%), corrections by Tiffany M. Knight, Harald Auge (together 15%), Holger B. Deising, Isabell Hensen, Scott A. Mangan, Martin Schädler, Claudia Stein (together 10%)

Chapter 3: Schmidt R, Deising HB, Hensen I, Schädler M, Auge H (2020): Natural enemies do not contribute to negative frequency-dependence in native and exotic grassland plants. *Perspectives in Plant Ecology, Evolution and Systematics* 46: 125565. DOI: 10.1016/j.ppees.2020.125565

Field work	Robin Schmidt (70%), various student helpers and interns, field technicians, friends (together 20%) Harald Auge, Martin Schädler (together 10%)
Lab work	Robin Schmidt (80%), Martin Schädler, student helpers and interns (together 20%)
Analysis	Robin Schmidt (75%), Harald Auge (25%)
Writing	Robin Schmidt (70%), corrections by Harald Auge (20%), Holger B. Deising, Isabell Hensen, Martin Schädler (together 10%)

Chapter 4: Schmidt R, Auge H, Deising HB, Hensen I, Lentendu G, Schädler M, Wirsel S, Wubet T: Impact of fungicide application, plant species origin and identity on soil fungi community composition

Field work	Robin Schmidt (70%), Harald Auge, Martin Schädler, numerous field technicians, student helpers, friends (together 30%)
Lab work	Robin Schmidt (65%), lab technicians (35%)
Analysis	Robin Schmidt (60%), Tesfaye Wubet (30%), Guillaume Lentendu (10%)

Writing	Robin Schmidt (70%), corrections by Tesfaye Wubet (20%), Harald Auge, Holger B. Deising, Isabell Hensen, Martin Schädler, Stefan Wirsel (together 10%)

7.5 Curriculum vitae

Personal Data

Name	Robin Schmidt
Birthday/-place	18.01.1986, Magdeburg, Germany
E-Mail	schmidt.robin@gmx.de

Education

September 2013 – April 2023	Martin-Luther-University Halle-Wittenberg, German Centre for Integrative Biodiversity Research, Leipzig, Helmholtz Centre for Environmental Research (UFZ), Department of Community Ecology, Halle (Saale): PhD Studies – Experimental interaction ecology
November 2006 – July 2013	Martin-Luther-University Halle-Wittenberg: Diploma studies in Biology (1.3), Diploma thesis: *The impact of exotic versus native plant species origin and gastropod herbivory on productivity of experimental grassland communities* Major subjects: Plant ecology, Nature conservation, Plant Physiology, Geology/Paleontology

Employments

August 2022 – present	Enviromental protection agency Saxony-Anhalt: Conceptional work on German impact mitigation regulation, FFH impact assessment; statements on project impacts of nature conservation issues;
May 2020 – July 2022	IHB GmbH: Environmental planning, mapping and monitoring
September 2019 – April 2020	Stiftung Kulturlandschaft Sachsen-Anhalt: Project position (Conservation of endangered agricultural weeds)
December 2018 – June 2019	Martin-Luther-University Halle-Wittenberg: PhD Project position
January 2018 – December 2018	Korina (UfU e.V.): Intern and project position (management of invasive plants in Saxony-Anhalt)
March 2017 – December 2017	UFZ: Scientific assistance
September 2013 – February 2017	iDiv: PhD Project position

7.6 Publications and conference contributions

List of publications

Korell, L.*****, **Schmidt, R.***, Bruelheide, H., Hensen, I. and Auge, H. (2016) Mechanisms driving diversity–productivity relationships differ between exotic and native communities and are affected by gastropod herbivory. *Oecologia* 180, 1025–1036. https://doi.org/10.1007/s00442-015-3395-2, *** shared first-authorship**

Rosche, C., Hensen, I., Schaar, A., Zehra, U., Jasieniuk, M., Callaway, R. M., Khasa, D. P., Al-Gharaibeh, M. M., Lekberg, Y., Nagy, D. U., Pal, R. W., Okada, M., Schrieber, K., Turner, K. G., Lachmuth, S., Erst, A., Tsunoda, T., Sheng, M., **Schmidt, R.**, Peng, Y., Luo, W., Jäschke, Y., Reshi, Z. A., and Shah, M. A. (2019) Climate outweighs native vs. nonnative range-effects for genetics and common garden performance of a cosmopolitan weed. *Ecological Monographs* 89 (4):e01386. https://doi.org/10.1002/ecm.1386

Schmidt, R., Auge, H., Deising, H. B., Hensen I., Mangan S. A., Stein, C. and Knight, T. M. (2020) Abundance, origin, and phylogeny of plants do not predict community-level patterns of pathogen diversity and infection. *Ecology and Evolution* 10: 5506–5516. https://doi.org/10.1002/ece3.6292

Schmidt, R., Deising, H. B., Hensen, I., Schädler, M. and Auge, H. (2020) Natural enemies do not contribute to negative frequency-dependence in native and exotic grassland plants. *Perspectives in Plant Ecology, Evolution and Systematics* 46:125565. https://doi.org/10.1016/j.ppees.2020.125565

Schmidt, R., Auge, H., Deising, H. B., Hensen, I. Lentendu, G., Schädler, M., Wirsel, S., and Wubet, T. Impact of fungicide application, plant species origin and identity on soil fungi community composition (*in prep.*)

List of conference contributions

Schmidt, R. *The importance of antagonistic interactions on plant species coexistence and ecosystem functioning*. 1st annual iDiv symposium, September 2013, Leipzig, Germany. Oral presentation

Schmidt, R. *Seedling emergence of grassland plants – The effect of species and antagonist exclusion*. 2nd annual iDiv symposium, October 2014, Leipzig, Germany. Poster presentation

Schmidt, R., Auge, H., Schädler, M., Stein, C., Mangan, S. A., Hensen, I., Deising, H. B. and Knight, T. M. *What predicts disease pressure in plant communities: host abundance, phylogeny or species origin?* 29th Conference of the Plant Population Biology Section of the Ecological Society of Germany, Austria and Switzerland (GfÖ), May 2016, Třeboň, Czech Republic. Oral presentation

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7.8 Declaration of Originality / Eigenständigkeitserklärung

Hiermit erkläre ich, dass ich die vorliegende Doktorarbeit mit dem Titel „The abundance, origin and phylogeny of plants: effects on natural enemies and implications for plant coexistence in grasslands“ eigenständig und ohne fremde Hilfe verfasst sowie keine anderen als die im Text angegebenen Quellen und Hilfsmittel verwendet habe.

Textstellen, welche aus verwendeten Werken wörtlich oder inhaltlich übernommen wurden, wurden von mir als solche kenntlich gemacht. Ich erkläre weiterhin, dass ich mich bisher noch nie um einen Doktorgrad beworben habe. Die vorliegende Doktorarbeit wurde bis zu diesem Zeitpunkt weder bei der Naturwissenschaftlichen Fakultät I – Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt.

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Robin Schmidt, Halle (Saale), 19.05.2022