Tree diversity effects on species richness and infestation of foliar fungal pathogens in European tree diversity experiments

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“When we try to pick out anything by itself,
we find it hitched to everything else in the universe.”

John Muir

(1838-1914, American environmentalist, naturalist, writer and scientist)

CONTENTS

SUMMARY

Foliar fungal pathogens are responsible for a broad range of damage on trees. The top-down control of foliar fungal pathogens on plant diversity and composition has been extensively investigated, in particular the effects on biomass and yield losses in natural and agricultural ecosystems. Conversely, comprehensive knowledge on whether plant ecosystem diversity and composition control foliar fungal pathogen richness and infestation is still missing. This applies in particular to forest ecosystems. An experimental set-up is required to systematically investigate such bottom-up effects, since plant diversity experiments comprise a plant diversity gradient, as well as controlled environmental conditions.

This thesis is aimed at surveying the influence of tree diversity on richness and infestation of foliar fungal pathogens. The particular experimental design of different European tree diversity experiments offered the potential to consider different aspects of tree diversity. Tree species diversity was assessed at the BIOTREE experimental site at Kaltenborn (BK) and the Kreinitz experiment (K), whereas functional tree diversity was investigated at the BIOTREE experimental site at Bechstedt (BB) and tree clone diversity at the Satakunta experiment (S). Considering further aspects of tree diversity, i.e. tree species and tree clone identity effects (BK, BB, K, S), as well as density effects of host and non-host tree species (K) and tree clones (S) were surveyed.

Pathogen species richness and pathogen load of foliar fungal pathogens were determined in a microscopic and macroscopic approach. Here, four different spatial scales were investigated: The *community level* examined the impact of the whole tree community, including all fungus species across all tree species/tree clones. The local neighbourhood focussed beyond that on the influence of the composition of the nearest neighbour tree individuals on a target tree individual. With this focus, the *individual level* considered all fungus species on all target individuals across all tree species/tree clones, the *tree species/tree clone level* regarded all fungus species on all target individuals of a particular tree species/tree clone and the *fungus species level* focused on a particular fungus species across all target individuals of its tree host.

In the first hypothesis it is assumed that with increasing tree diversity/Shannon diversity of the local neighbourhood foliar fungal pathogen species richness increases and pathogen load decreases (H1). For tree species diversity, effects of tree species richness/Shannon diversity of the local neighbourhood on pathogen species richness as well as on pathogen load were absent at *community level* and *tree species level* (BK). In contrast, there was a negative

relationship at *tree species level* between overall pathogen load of *Tilia cordata* and *Quercus petraea* and Shannon diversity of the local neighbourhood (K). This tree host dilution effect was also found for several fungus species at *fungus species level* (BK, K), in particular for the highly abundant or frequent fungi. Furthermore, raising pathogen species richness has been found with increasing tree clone richness at *community level*, whereas pathogen load of two of the eight birch clones in the Satakunta experiment was reduced by tree clone richness in the local neighbourhood at *tree clone level* (S).

In the second hypothesis it is expected that inter-annual variation of both foliar fungal pathogen species richness and load decreases with increasing Shannon diversity in the local neighbourhood (H2). This assumption could not be confirmed, although pathogen species richness and pathogen load exhibited strong inter-annual variability (K).

In the third hypothesis it is assumed that foliar fungal pathogen richness and load decrease with increasing functional tree diversity (H3). Contrary to this expectation, functional tree diversity did neither affect pathogen species richness nor pathogen load at *community level* (BB).

In the fourth hypothesis it is supposed that with increasing tree host density foliar fungal pathogen species richness decreases and pathogen load increases (H4). Density effects of particular host tree species were generally absent at *tree species level* and *fungus species level* (K). Foliar fungal pathogen load of a particular tree clone was also not affected by the increasing density of the same tree clone in the local neighbourhood (S). These results pointing beyond the evidence of observed tree diversity effects.

In the fifth hypothesis it is presumed for the *community level* that both pathogen species richness and pathogen load increase or decrease with the presence of disease-prone or disease-resistant tree species/tree clones, respectively (H5). Such identity effects were found for several tree species (BK, BB), as well as tree clones (S), being in parts more distinctive than tree diversity effects (BK, BB). Moreover, at *tree species level* and *fungus species level* was observed that increasing proportions of particular non-host tree species within the local neighbourhood either increases or decreases pathogen species richness or load, respectively (K). This indicates facilitation either of foliar fungal pathogens or the tree host by the proportion of a particular non-host tree species in the nearest neighbourhood (K). Furthermore, growing densities of particularly susceptible tree clones raised pathogen richness and load of less susceptible target tree clones at all spatial scales (S). By contrast, increasing density of less susceptible tree clones reduced pathogen species richness and load

of highly susceptible tree clones (S). These results indicate a pathogen reservoir function of susceptible tree clones, raising foliar fungal pathogen transmission among tree hosts, while less susceptible tree clones provide a barrier effect, hindering effective transmission among most compatible tree hosts.

In conclusion, this thesis has highlighted the role of bottom-up effects for host-pathogen interactions, as tree diversity strongly controls foliar fungal pathogen species richness and load at all spatial scales in the experimental forests. In particular, tree species diversity and tree clone diversity, as well as identity and density of particular tree species and tree clones were determined as key predictors for infection by foliar fungal pathogens. The results consequently pointing out the importance of tree diversity and composition within a community and within the local neighbourhood for ecosystem stability, through maintaining low disease levels by foliar fungal pathogens in young forest communities.

ZUSAMMENFASSUNG

Parasitische Blattpilze schädigen weltweit Bäume auf unterschiedlichste Art und Weise. Studien hierzu haben sich bisher vor allem auf die Effekte der parasitischen Blattpilze auf die Pflanzendiversität und -zusammensetzung konzentriert (*top-down* Kontrolle), insbesondere auf Biomasse- und Ernteverluste in natürlichen und Agrarökosystemen. Umgekehrt fehlen bislang umfassende Erkenntnisse darüber, inwieweit die Pflanzendiversität und -zusammensetzung von Ökosystemen die Vielfalt und den Befall von parasitischen Blattpilzen bestimmt. Dies trifft insbesondere auf Waldökosysteme zu. Um solche *bottom-up* Effekte systematisch zu untersuchen, sind Pflanzen-Diversitäts-Experimente notwendig, da diese zum einen über einen Pflanzen-Diversitäts-Gradienten, zum anderen über kontrollierte Umweltbedingungen verfügen.

Die vorliegende Dissertation hatte zum Ziel, den Einfluss der Baum-Diversität auf Vielfalt und Befalls-Intensität parasitischer Blattpilze zu untersuchen. Das spezifische experimentelle Design verschiedener europäischer Baum-Diversitäts-Experimente erlaubte es, unterschiedliche Aspekte der Baum-Diversität zu betrachten. So konnte die Baumarten-Diversität im BIOTREE-Experiment/Standort Kaltenborn (BK) sowie im Kreinitz-Experiment (K), die funktionelle Baum-Diversität im BIOTREE-Experiment/Standort Bechstedt (BB) beziehungsweise die Baumklon-Diversität im Satakunta-Experiment (S) analysiert werden. Als weitere Aspekte der Baum-Diversität wurden die Effekte von Baumart- und Baumklon-Identität (BK, BB, K, S) sowie der Dichte von Wirts- und Nicht-Wirts-Baumarten (K) bzw. -Baumklonen (S) untersucht.

Die Vielfalt und der Befall durch parasitische Blattpilze wurden in einem mikro- und einem makroskopischen Ansatz bestimmt. Dabei wurden vier verschiedene Ebenen betrachtet: Die *Gemeinschafts-Ebene* untersucht den Einfluss der gesamten Baum-Gemeinschaft und schließt hierfür alle Pilzarten an allen Ziel-Individuen aller Baumarten/Baumklone mit ein. Darüber hinaus fokussiert die lokale Nachbarschaft auf den Einfluss durch die Zusammensetzung der direkten Baum-Nachbarn auf ein Ziel-Individuum. Unter diesem Fokus betrachtet die *Individuum-Ebene* alle Pilzarten auf allen Ziel-Individuen aller Baumarten/Baumklone, die *Baumart/Baumklon-Ebene* alle Pilzarten an allen Ziel-Individuen einer Baumart/eines Baumklons und die *Pilzart-Ebene* betrachtet eine Pilzart an allen Ziel-Individuen ihres Baumwirtes.

In der ersten Hypothese wurde vermutetet, dass mit zunehmender Baum-Diversität/Shannon-Diversität der lokalen Nachbarschaft die Pilzarten-Vielfalt zunimmt, während der Pilzbefall

abnimmt (H1). Für die Baumarten-Diversität wurde gezeigt, dass die Anzahl an Baumarten/Shannon-Diversität der lokalen Nachbarschaft keinen Einfluss auf die Pilzarten-Vielfalt und den Pilzbefall hat (*Gemeinschafts-Ebene*, *Baumarten-Ebene*, BK). Dagegen wurde auf *Baumarten-Ebene* ein negativer Zusammenhang zwischen dem gesamten Pilzbefall an *Tilia cordata* und *Quercus petraea* und Shannon-Diversität in der lokalen Nachbarschaft gefunden (K). Dieser Wirts-Verdünnungs-Effekt zeigte sich auch für verschiedene Pilzarten (*Pilzarten-Ebene*, BK, K), vor allem für die häufigen Pilzarten oder solche mit starkem Befall. Desweiteren konnte jedoch ein Anstieg der Pilzarten-Vielfalt mit zunehmender Anzahl an Baum-Klonen gefunden werden (*Gemeinschafts-Ebene*), während der Pilzbefall von zwei der acht Birken-Klonen im Satakunta-Experiment mit zunehmender Anzahl an Baumklonen in der lokalen Nachbarschaft abnahm (*Baumklon-Ebene*, S).

In der zweiten Hypothese wurde angenommen, dass die Varianz der Pilzarten-Vielfalt und des Pilzbefalls zwischen den Jahren mit zunehmender Shannon-Diversität in der lokalen Nachbarschaft abnimmt (H2). Diese Annahme konnte nicht bestätigt werden, obwohl sowohl die Pilzarten-Vielfalt als auch der Pilzbefall starke jährliche Schwankungen aufwiesen (K).

In der dritten Hypothese wurde vermutetet, dass sowohl die Pilzarten-Vielfalt als auch der Pilzbefall mit zunehmender funktioneller Baum-Diversität abnehmen (H3). Entgegen dieser Erwartung zeigte die funktionelle Baum-Diversität weder einen Einfluss auf die Pilzarten-Vielfalt noch auf den Pilzbefall (*Gemeinschafts-Ebene*, BB).

In der vierten Hypothese wurde angenommen an, dass mit zunehmender Wirtsdichte die Pilzarten-Vielfalt abnimmt und der Pilzbefall zunimmt (H4). Dichte-Effekte einzelner Wirts-Baumarten wurden weder für die *Baumarten-Ebene* noch für die *Pilzarten-Ebene* gefunden (K). Der parasitische Blattpilz-Befall an einem bestimmten Baumklon wurde ebenfalls nicht durch die zunehmende Dichte des gleichen Baumklons in der lokalen Nachbarschaft beeinflusst (S). Die Ergebnisse weisen somit auf echte Baum-Diversitäts-Effekte hin.

In der fünften Hypothese wurde für die *Gemeinschafts-Ebene* angenommen, dass die Pilzarten-Vielfalt und der Pilzbefall in Abhängigkeit von der Anwesenheit krankheitsanfälliger oder weniger krankheitsanfälliger Baumarten/Baumklone jeweils ansteigen bzw. abnehmen (H5). Solche Identitäts-Effekte konnten für einige Baumarten (BK, BB) und Baumklone (S) beobachtet werden und waren teilweise ausgeprägter als die Baum-Diversitäts-Effekte (BK, BB). Für die *Baumarten-Ebene* und die *Pilzarten-Ebene* konnte gezeigt werden, dass mit zunehmendem Anteil einiger Nichtwirts-Baumarten in der lokalen Nachbarschaft ein Anstieg bzw. eine Reduktion der Pilzarten-Vielfalt oder des Pilzbefall

einherging (K). Das heißt, dass der Anteil einer bestimmten Nicht-Wirtsbaumart in der direkten Umgebung eines Zielindividuums entweder die parasitischen Blattpilze bzw. den Wirtsbaum fördert (K). Außerdem wurde für alle Ebenen beobachtet, dass mit zunehmender Dichte an krankheitsanfälligen Baumklonen die Pilzarten-Vielfalt oder der Pilzbefall an weniger krankheitsanfälligen Baumklonen ansteigt (S). Im Gegensatz dazu nahm mit zunehmender Dichte an weniger krankheitsanfälligen Baumklonen die Pilzarten-Vielfalt oder der Pilzbefall an krankheitsanfälligeren Baumklonen ab (S). Diese Ergebnisse weisen auf die Pilzreservoir-Funktion von krankheitsanfälligen Baumklonen hin, da diese die Verbreitung parasitischer Blattpilze zwischen den Wirtsbäumen fördern, während weniger krankheitsanfällige Baumklone eine physikalischer Barriere darstellen und die effektiver Verbreitung zwischen den krankheitsanfälligsten Wirtsbäumen vermindern.

Insgesamt konnte die vorliegende Dissertation die Bedeutung der *bottom-up* Effekte für Wirt-Pathogen-Interaktionen herausstellen, da sowohl die Vielfalt parasitischer Blattpilze als auch deren Befall durch die Baum-Diversität auf allen untersuchten Ebenen in den experimentellen Wäldern kontrolliert werden. Insbesondere wurden die Baumarten-Diversität, die Baumklon-Diversität sowie Identität und Dichte bestimmter Baumarten und Baumklone als stärkste Prädiktoren für die Infektion durch parasitische Blattpilze identifiziert. Die Ergebnisse weisen somit auf die hohe Bedeutung der Baum-Diversität und -Zusammensetzung in der Gemeinschaft, aber auch der lokalen Nachbarschaft, für die Ökosystem-Stabilität hin, indem sie parasitische Blattpilz-Erkrankungen in jungen Waldgemeinschaften auf einem niedrigen Niveau halten.

1. INTRODUCTION

Biodiversity and ecosystem functioning in forests

Forest ecosystems currently cover approximately one-third of the Earth's land surface and account for over two-thirds of terrestrial net primary production on land (TEEB 2010). Accordingly, forests are of high economic, ecologic and social value, for example by a) providing timber, fire wood and other non-timber forest products, b) sustaining large parts of terrestrial biodiversity, carbon storage and sequestration, c) regulating climate and water flows and d) contributing to recreation and amenity (Pearce 2001; Millennium Ecosystem Assessment 2005). However, in large parts of the world, human intervention is responsible for alterations of structure, species composition and properties of forest ecosystems. In many regions, deforestation rates remain quite constant, thus the absolute forested area is steadily decreasing, in particular in the tropics and subtropics (Vitousek et al. 1997; Hooper et al. 2005; FAO 2012). Hence, transformation of natural and anthropogenic forest ecosystems has dramatically diminished connectivity and availability of forests (Ritchie & Olff 1999; Pimm & Raven 2000; Foley et al. 2005), as well as impoverished forest biodiversity (see Glossary) through loss of species and homogenization of species pools (Hector et al. 1999; Millennium Ecosystem Assessment 2005; Cardinale et al. 2012). The biodiversity loss in forests is supposed to affect ecosystem functioning (see Glossary) and consequently the maintenance of ecosystem services, including provisioning, cultural, supporting and regulating services, with an impact on the well-being of the whole human population (Millennium Ecosystem Assessment 2005, Balvanera et al. 2006; Hector & Bagchi 2007). Correspondingly, the current question arises as "How much biodiversity is necessary for sufficient ecosystem functioning?", in particular with respect to the management of anthropogenic agricultural forests and conservation strategies of natural forests.

In the last two decades, experimental research on biodiversity–ecosystem functioning (BEF hereafter) relationships has emerged as a novel approach in ecological and environmental sciences (Balvanera et al. 2006; Loreau 2010). The main aim of BEF research is to quantify the role of plant biodiversity for ecosystem functioning and ecosystem services (Naeem & Wright 2003). Plant BEF research particularly complements observational studies by allowing the establishment and identification of causal relationships, since plant diversity is manipulated in BEF experiments, while other influencing factors are controlled (Caspersen & Pacala 2001). Several fundamental ecological issues have been addressed, for instance, whether increasing plant biodiversity a) promotes ecosystem productivity (Spehn et al. 2005;

Potvin & Dutilleul 2009), b) supports ecosystem stability (Tilman 1996; McCann 2000; Tilman et al. 2006; Eisenhauer et al. 2011; Proulx et al. 2010), c) decreases invasion risk (Elton 1958; Knops et al. 1999; Fridley et al. 2007) and d) how plant biodiversity affects higher trophic levels regarding species diversity and abundance, as well as trophic network complexity (Hutchinson 1959; Naeem et al. 1994; De Deyn et al. 2004; Cardinale et al. 2006; Duffy et al. 2007).

The research field of plant biodiversity–trophic structure relationships also includes the role of pathogens in ecosystem functioning. Pathogens such as fungi, viruses and bacteria, are powerful evolutionary forces and have strong impacts on their plant hosts, thereby they often induce negative consequences for an agricultural economy and an agricultural ecology (Burdon et al. 2006; de Macedo Leal-Bertioli et al. 2010; Scherber et al. 2010). Plant host-fungal pathogen interactions are commonly parasitic partnerships between plant hosts as living microhabitats, e.g. leaves, stem and roots, and fungal pathogens as harmful biotrophic parasites invading the host tissue through infection structures, such as appressoria, penetration and infection hyphae to obtain the host's nutrients and energy (Heath 1997; Shurtleff & Averre III 1997; Mendgen & Hahn 2002). Fungal pathogens that infect leaf tissue subsequently cause leaf spots, necrosis, chlorosis and shot-holes on leaves, as well as early leaf senescence and abscission (Gilbert 2002; Burdon et al. 2006). Thus, foliar fungal pathogen infection and infestation (see Glossary) reduce the plant host's photosynthetic capacity and leaf longevity, which limit fitness and growth and become visible through diminished above-ground and below-ground biomass production (Mitchell 2003; Hajji et al. 2009; Maron et al. 2011), through declined host fecundity and reproduction (Bradley et al. 2008; Seabloom et al. 2009; Balmelli et al. 2013) and through raised host mortality of seedlings and adults (Gilbert 2002; Burdon et al. 2006). On the other hand, plant hosts have developed an immune system including an array of physical, protein-based, chemical, ecological and indirect defence mechanisms to avoid foliar fungal pathogen infections (Maleck & Dietrich 1999; Bonello et al. 2006; Jones & Dangl 2006; Eyles et al. 2009). Both plant hosts and foliar fungal pathogens have evolved manifold mechanisms either to prevent or to increase the infection success.

At the scale of plant ecosystems, the infection by foliar fungal pathogens affects key processes, such as succession, productivity and species coexistence by regulating the relative abundance and fitness of dominant or *keystone* plant species (top-down control; Burdon et al. 2006; Bradley et al. 2008; Allen et al. 2010). By more strongly affecting abundant plant species, foliar fungal pathogens indirectly support rare plant species (Janzen-Connell effects;

Janzen 1970; Connell 1971; Mordecai 2011, 2013). Conversely, Elton (1958) early postulated that higher plant community diversity may decrease the disease risk and severity (biodiversity–disease hypothesis; see Glossary), indicating the influence of plant community diversity, structure and composition on foliar fungal pathogen transmission, community composition and infestation (bottom-up control; Knops et al. 1999; Burdon et al. 2006; Cobb et al. 2010). Several studies confirmed this hypothesis by employing short-lived experimental grassland communities that are easily manipulated (Knops et al. 1999; Mitchell et al. 2002). In contrast, this BEF relationship has rarely been addressed in long-lived experimental communities, in particular not in complex forest ecosystems, in accordance to the greater time the tree diversity experiments required to develop (Scherer-Lorenzen et al. 2005). However, knowledge if and how tree diversity (see Glossary) affects foliar fungal pathogen richness (see Glossary) and infestation is not only important to increase our understanding of ecosystem functioning, but also to better protect natural forests and to develop forests that are managed in a more ecologically worthwhile manner.

1.1 Tree diversity effects on foliar fungal pathogens

It is stressed that all BEF mechanisms described and examples provided hereafter are focused on the particular case of specialist foliar fungal pathogens (see Glossary) if not otherwise stated. This restriction is well-founded as most foliar fungal pathogens are highly specialized and are confined to one single tree genus, tree species or even tree clone and are prior passive transmitted by wind or rain (Prell 1996). In addition, different BEF mechanisms are particularly considered from the broad to the local spatial scale.

Tree species diversity effects

In BEF experiments tree species diversity (see Glossary) is usually manipulated by varying the number of species in a plot. One aspect of tree species diversity focuses on tree species richness (see Glossary), thus the number of tree species in a community. Another aspect of tree species diversity, Shannon diversity (see Glossary), regards also tree species richness, but additionally considers the relative abundance of tree individuals, both with respect to the local neighbourhood of a target tree (Hooper et al. 2005).

According to the specificity of host-pathogen interactions, tree species diversity is expected to increase foliar fungal pathogen species richness (Figure 1-1 H1) as tree species-rich communities provide larger resource supplies, more complex microhabitats, and hence, more variable microclimatic conditions (Bond & Chase 2002; Hooper et al. 2005; Waldrop et al.

2006). Further positive tree species diversity effects might also be possible if additional tree host species are required for completing the life cycle of a particular foliar fungal pathogen, e.g. for the often heteroecous rust fungi (Cheatham et al. 2009; Mundt et al. 2011). As an example, observational studies have found such positive relationships between host species diversity and fungal diversity in forest communities of the Białowieża National Park in Poland (Faliński & Mułenko 1995, 1996, 1997). Furthermore, tree species diversity is assumed to reduce foliar fungal pathogen species richness and infestation (Figure 1-1 H1), since the abundance of susceptible host individuals (see Glossary) decreases in tree species-rich communities (Keesing et al. 2006; Cheatham et al. 2009; Keesing et al. 2010). In consequence, the passive transmission/encounter rate of foliar fungal pathogens between susceptible tree host individuals becomes less effective due to a dilution by disease-resistant hosts (see Glossary), which has been demonstrated for some generalist fungal pathogens (see Glossary) in forest BEF studies (Pautasso et al. 2005; Haas et al. 2011). In addition, several grassland BEF studies found a reduction in pathogen infestation of generalist foliar pathogens (Roscher et al. 2007; Moore & Borer 2012) and of specialist foliar fungal pathogens with increasing plant species richness (Knops et al. 1999; Mitchell et al. 2002; Mitchell et al. 2003). However, the relationship between plant species diversity and species richness, as well as infestation of specialist foliar fungal pathogens have been rarely addressed in grassland experiments, but have not yet been experimentally demonstrated in forest BEF experiments.

Since several life stages of the foliar fungal pathogens depend on the microclimate, changes in weather conditions are responsible for intra- and inter-annual variability in communities' disease risk and severity (IPCC 2011; Cordier et al. 2012). It is an open question to which degree tree species-rich communities can buffer the resulting variation in disease risk and severity more than tree monocultures (Figure 1-1 H2). In grassland BEF studies, the inter-annual variability of abundance of several trophic groups was lower in species-rich communities than in monocultures (Tilman 1996; Tilman et al. 2006; Eisenhauer et al. 2011). In consequence, ecosystem functioning is also more stable in species-rich communities than in monocultures (insurance hypothesis; Yachi & Loreau 1999; Proulx et al. 2010). It is important to analyse such temporal variability effects also in forest ecosystems as their higher structural complexity might result in larger negative impact of reduced forest biodiversity than has been reported from grassland experiments (Reich et al. 2012).

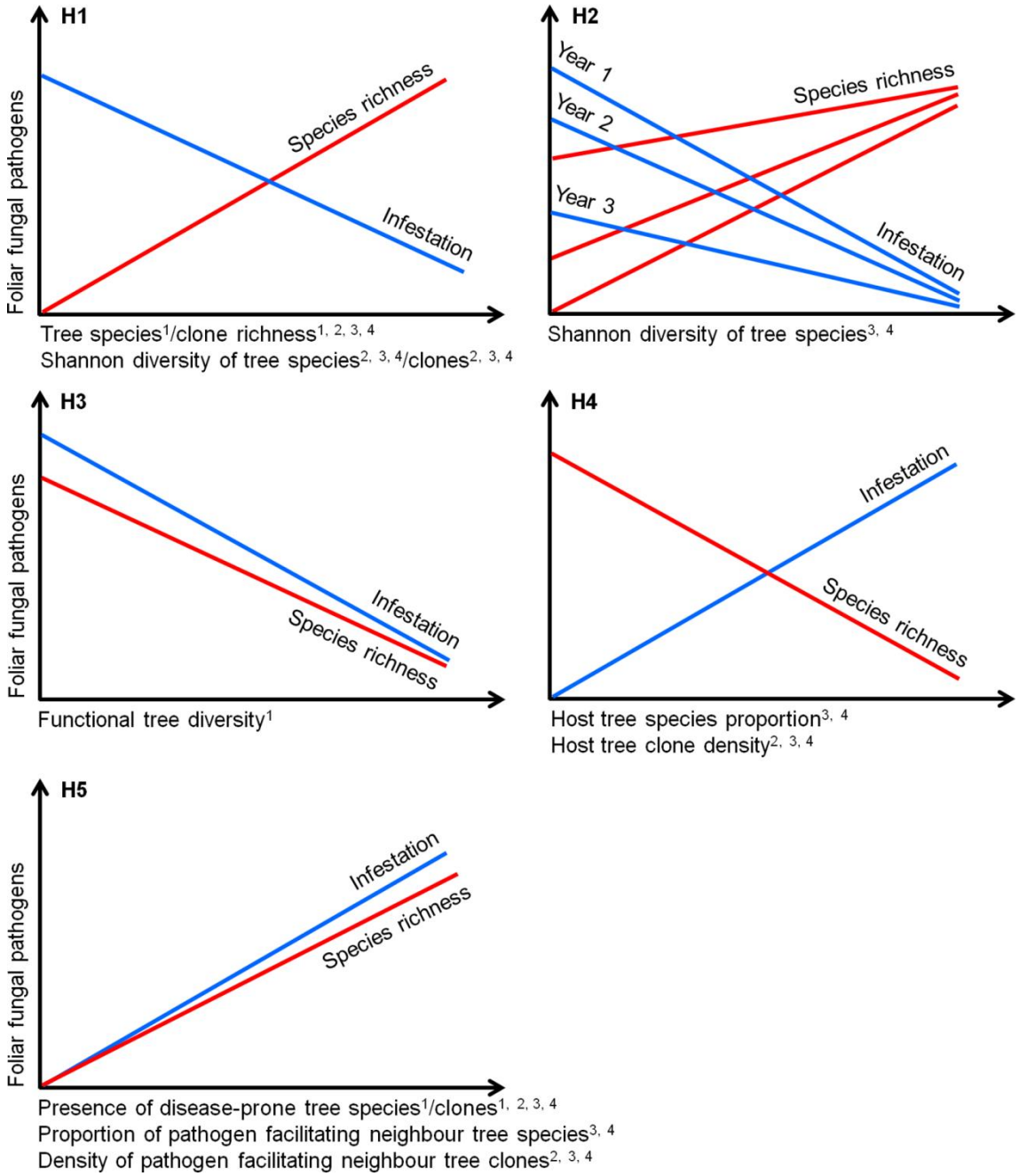


Figure 1-1 Expected relationships between different aspects of tree diversity and foliar fungal pathogen species richness and infestation reflecting the main hypotheses H1-H5, as well as providing the considered spatial scales: 1 = *community level*, 2 = *individual level*, 3 = *tree species/clone level* and 4 = *fungus species level*. Definitions of spatial scales are provided in Chapter 1.2.

Experimental and observational studies demonstrated that negative plant diversity effects could be brought about by lower host species density within species-rich plant communities, indicating a reduction in fungal pathogen infestation (Figure 1-1 H4) and transmission (Keesing et al. 2006), both for forests (Pautasso et al. 2005; Norghauer et al. 2010) and grasslands (Knops et al. 1999; Mitchell et al. 2002, 2003; Mundt et al. 2011; Latz et al. 2012). Tree species diversity effects might be caused by density-dependent host species effects, since

susceptible tree hosts were diluted within tree species-rich communities, and thus, escaped from specialist pathogens (Janzen 1970; Connell 1971; Keesing et al. 2010; Mordecai 2011). Moreover, changes in host density (see Glossary) alter competition between tree individuals and accordingly the tree individual's size, shape and growth rate (Burdon & Chilvers 1982). When weighting tree host species density by the individual's performance within the local neighbourhood, then it results in tree host species proportions (see Glossary). Such effects of host species proportion have already been demonstrated for grass rust fungi in grassland communities where infestation decreases with host species proportion (Roscher et al. 2007).

In addition to plant host density effects, strong non-host plant species identity and density effects (see Glossary) might mask plant species diversity effects (Hooper et al. 2005). Such non-host plant species identity effects have been recorded both from forests (Nadrowski et al. 2010; Rajala et al. 2013) and grasslands (Mouillot et al. 2011; Moore & Borer 2012). In BEF research plant identity effects are known as *sampling effect*, which describe that the probability to include a particularly well (or poorly) performing plant species in a plant community increases with plant species richness (Loreau & Hector 2001). Thus, the performance of tree species-rich forest communities tends to be close to that of the most influencing tree species (Cardinale et al. 2006). Correspondingly, the presence of particular disease-prone (see Glossary) or disease-resistant tree species might amplify or buffer foliar fungal pathogen transmission, respectively, as well as foliar fungal pathogen richness and infestation (Figure 1-1 H5; Keesing et al. 2010). So far, such non-host plant species identity effects for foliar fungal pathogen infestation have only be reported from grassland communities (Mitchell et al. 2002), but not yet from forests. When focusing on the local neighbourhood, the presence, density or proportion of a particular non-host tree species is supposed to facilitate or hinder infection by particular foliar fungal pathogens (Figure 1-1 H5), since non-host neighbours affect the target tree individual's growth, and thus, alter the stand structure in the neighbourhood (Potvin & Dutilleul 2009). Structural differences might result in differences in insolation and microclimatic conditions, such as air temperature or humidity (Bourke 1970; Bahnweg et al. 2008). This in consequence might indirectly influence germination, development, reproduction and transmission success of foliar fungal pathogens (associational resistance hypothesis; Tahvanainen & Root 1972; Tainter & Baker 1996).

To sum up, tree species diversity effects can be brought about by an increasing number of tree species that are directly involved as hosts, but also by differences in host proportions, as well as by non-host identity and proportions in the local neighbourhood. Moreover, inter-annual

variances in weather conditions might determine the BEF relationships of Shannon diversity and foliar fungal pathogen richness, as well as infestation.

Functional tree diversity effects

Functional tree diversity (see Glossary) describes the inter-specific variance of one or several tree species' functional traits in a forest community (Hooper et al. 2005; Petchey & Gaston 2006). Many studies suggest that the variance of trait values is more crucial for the maintenance of ecosystem functioning than the community's mean trait values (Petchey 2004; Petchey & Gaston 2006; Reiss et al. 2009; Mouillot et al. 2011). Moreover, functional tree diversity can be partitioned into a) functional richness, i.e. the functional niche space which is occupied by the present tree species, b) functional evenness, indicating the regularity of tree species abundances within this functional niche space and c) functional divergence which describes the degree of divergence in trait values (Villéger et al. 2008; Schleuter et al. 2010; Böhnke et al. 2013). The latter also corresponds to functional dispersion (Laliberté & Legendre 2010) and Rao's (1982) quadratic entropy (FDQ) (Botta-Dukát 2005; Pavoine & Dolédec 2005).

For host-pathogen interactions in forest communities, high functional tree diversity is supposed to reduce pathogen species richness and infestation of foliar fungal pathogens (Figure 1-1 H3). This assumption is based on the high tree host-specificity of foliar fungal pathogens (Prell 1996), which is responsible for the tree host susceptibility to a particular pathogen, as well as for the resistance to other pathogen species. Hence, highly susceptible tree host species positively contributed to the overall communities' pathogen richness and load, while highly resistant tree host species have a negative influence on both response variables (Figure 1-1 H5). These tree species identity effects point to the importance of the tree species community composition for foliar fungal pathogen infections. As tree host defence is induced by gene expression, high specificity of host-pathogen interactions is an ecological consequence of highly specific molecular mechanisms between hosts and pathogens (Keen 1990; Chisholm et al. 2006). According to the Red Queen hypothesis (Van Valen 1973) foliar fungal pathogens have co-evolved with their tree hosts in a genetic *arms race* and become mainly specialized on common tree host genotypes (Clay & Kover 1996b; McDonald & Linde 2002, Parker & Gilbert 2004, Thines & Kamoun 2010). Although the Red Queen hypothesis primarily focuses on the micro-evolutionary scope and aims to explain the origin of genetic variation in tree hosts and foliar fungal pathogen species, it can be beyond that extend to the macro-evolutionary scope when focussing on the biochemical and physical

arms race of tree hosts and foliar fungal pathogens. Since some of the tree host defence mechanisms, such as secondary metabolites, have probably developed in close co-evolution with the evolvement of foliar fungal pathogens disease effectors, which modulate host cell physiology and immunity, both partners stimulated each other (McDonald & Linde 2002; Shanmugam et al. 2010; Thines & Kamoun 2010). Hence, for specialized host-pathogen interactions certain physical and chemical leaf traits might be particularly important, either from the tree host's perspective as a defence against infection and infestation of foliar fungal pathogens or from the pathogen's perspective as providing nutrient-rich and easily accessible substrates (Mendgen 1981; Manners 1987). So far, it is not clear, whether phylogenetically closely related tree species suffer from a similar risk of infection by closely related foliar fungal pathogen species (Gilbert & Webb 2007). However, this might be expected if the defence traits are phylogenetically conserved, but phylogenetic conservation patterns of tree species, their leaf defence traits and foliar fungal pathogen richness and infestation have not yet been addressed in a functional tree diversity experiment.

Tree clone diversity effects

Tree clone diversity (see Glossary) comprises the intra-specific genetic and phenotypic variance among tree clone individuals (Hughes et al. 2008). Genetic variance is particularly important for the fitness and adaptability of plant communities as, for instance, genetically diverse plant populations have been demonstrated to be less vulnerable to diseases (Burdon 1987; Schmid 1994; Cadotte et al. 2012). However, human-managed agricultural and forest ecosystems comprises often of only one plant species or even single genotypes which strongly select foliar fungal pathogens to overcome these single resistance genes (Stukenbrock & McDonald 2008). Furthermore, several studies discovered genotype-specificity of host resistance and pathogen virulence, indicating that the infection success might depend on the compatibility between a particular tree host genotype and a particular foliar fungal pathogen genotype (Garrett & Mundt 1999; Zhu et al. 2005; Burdon et al. 2006; Roscher et al. 2007; Miranda et al. 2013). As a consequence, communities with high genotype diversity, e.g. tree clones richness and Shannon diversity of the local neighbourhood, are expected to reflect high foliar fungal pathogen species richness (Figure 1-1 H1; Hudson et al. 2006; Bálint et al. 2013). Conversely, foliar fungal pathogen infestation should be lower in tree clone-rich communities because most compatible host tree clones are diluted by less compatible clones, hence reducing the effectiveness of pathogen transmission and the infection success of susceptible tissue by specialist pathogen species in mixtures (Figure 1-1 H1; Leonard 1969; Keesing et al. 2010; Haas et al. 2011).

In accordance, high density of susceptible tree host clones is supposed to facilitate pathogen transmission and to increase foliar fungal pathogen infestation (Figure 1-1 H4; Mundt & Browning 1985) as has been already reported from observations and experiments in grasslands and agriculture (Zhu et al. 2000; Valério et al. 2004), as well as from observational studies in forests (Pautasso et al. 2005).

If tree clones differ in their susceptibility against specialist foliar fungal pathogens, the presence and density of particular host tree clones in a mixture will determine the dynamics of pathogen communities and disease severity (Garrett & Mundt 1999; Zhan et al. 2002). Within a community, the presence of high susceptible tree clones might enhance foliar fungal pathogen richness and infestation, whereas the presence of less susceptible tree clones might decrease the response variables (Figure 1-1 H5). Within the local neighbourhood, high density of susceptible tree clones is assumed to increase foliar fungal pathogen richness and infestation on less susceptible clones through higher pathogen transmission, while less susceptible tree clones hamper pathogen transmission as they achieve a physical barrier (Burdon 1978; Wolfe 1985; Garrett & Mundt 1999). Identity and density effects have been already demonstrated for tree species (Nadrowski et al. 2010), but whether and to which degree they might be of importance for foliar fungal pathogen infection on tree clones remains still open.

1.2 Foliar fungal pathogen analyses in tree diversity experiments

Tree diversity experiments

To disentangle underlying mechanisms of tree diversity-foliar fungal pathogen richness and infestation relationships, different aspects of tree diversity have to be examined. Thus, different tree diversity experiments were chosen which were feasible to specifically investigate effects of tree species diversity, functional tree diversity and tree clone diversity (Table 1-1). All of those experiments are part of the experimental platform of the common framework of FunDivEUROPE (Figure 1-2). As all trees have approximately the same age in these experiments, they represent a kind of common garden situation which allows controlling for confounding variables which render analyses in natural forests difficult and error-prone. For this purpose, field data was sampled in the years 2010-2012 within the German BIOTREE experimental sites at Kaltenborn (BK) and at Bechstedt (BB) in Thuringia, the German Kreinitz experiment (K) in Saxony, as well as the Satakunta birch-clone experiment (S) in

southwest Finland. Main characteristics regarding the experimental design of all four experimental sites are provided in Table 1-1.

Foliar fungal pathogen analyses

The analyses focused on two main types of response variables: pathogen species richness and pathogen load (see Glossary) of foliar fungal pathogens. The leaf and needle sampling from tree individuals and the foliar fungal pathogen analysis protocol was standardized, as much as possible, across these four sites. In a microscopic approach, all visible biotrophic fungus species on the leaf and needle surface were recorded and determined, while all visible saprophytic phylloplane and endophytic fungi were excluded since they contribute different ecological functions (e.g. Rudgers et al. 2004; Rodriguez et al. 2009). In a macroscopic approach, pathogen load of each fungus species was visually estimated as damaged area per leaf, using a percentage class system (see Schuldt et al. 2012). Macroscopic images of the encountered foliar fungal pathogens (0.65 x/2 x/4 x magnification of each pathogen species) are provided on pages 23–27.

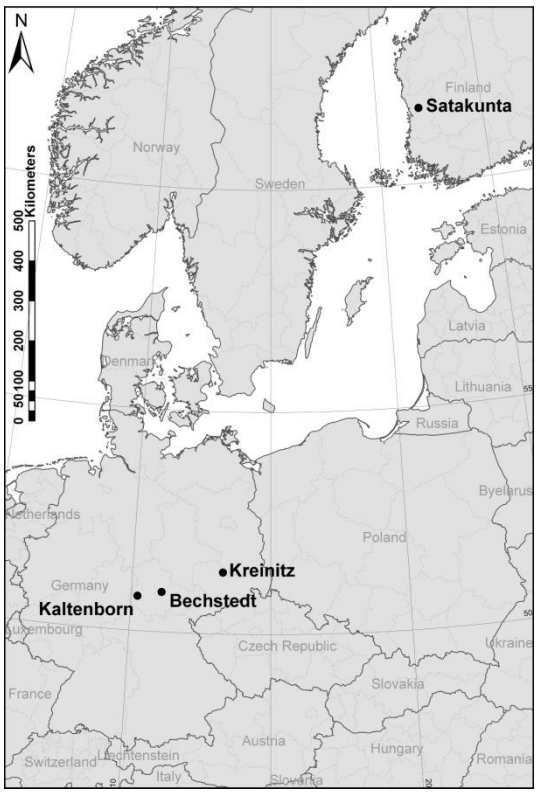


Figure 1-2 Locations of the German and Finnish tree diversity experiments (© Erik Welk).

Table 1-1 European tree diversity experiments of the FunDivEurope experimental platform included in this thesis. In addition, the main characteristics of the particular experimental design, as well as information concerning the analysed spatial scales (see Chapter 1.2) and the tested main hypotheses (see Chapter 1.3) are provided.

Experiment and the year of establishment	**BIOTREE – Kaltenborn (BK) 2003/2004**	**Kreinitz (K) 2005**	**BIOTREE – Bechstedt (BB) 2003/2004**	**Satakunta (S) 1999**
Type of tree diversity gradient	Tree species diversity	Tree species diversity	Functional tree diversity	Tree clone diversity
Number of plots	16	96	25	49
Tree mixtures	monocultures, 2-, 3-, 4- species mixtures	monocultures, 2-, 3-, 5-, 6-species mixtures	4- species mixtures different in their functional diversity	monocultures, 2-, 4-, 8-clone mixtures
Tree species	*Fagus sylvatica* *Quercus petraea* *Picea abies* *Pseudotsuga menziesii*	*Fagus sylvatica* *Fraxinus excelsior* *Quercus petraea* *Picea abies* *Pinus sylvestris* *Tilia cordata*	*Acer campestre* *Acer platanoides* *Acer pseudoplatanus* *Betula pendula* *Carpinus betulus* *Fagus sylvatica* *Fraxinus excelsior* *Larix decidua* *Quercus petraea* *Pinus sylvestris* *Populus tremula* *Prunus avium* *Sorbus aucuparia* *Sorbus torminalis* *Tilia cordata* *Ulmus glabra*	*Betula pendula* 8 different clones (Blue, Green, Orange, Pink, Red, Violet, White, Yellow)
Spatial scales	*Community level* *Individual level* *Tree species level* *Fungus species level*	*Tree species level* *Fungus species level*	*Community level* *Tree species level*	*Community level* *Individual level* *Tree clone level* *Fungus species level*
Hypotheses	H1, H5.1, H5.2	H1, H3, H4, H5.2	H3, H5.1	H1, H4, H5.1, H5.2

Investigation levels

The response variables foliar fungal pathogen species richness and pathogen load were investigated at four different investigation levels (Figure 1-3, Table 1-1). The *community level* (synonym: *plot level*) employs leaf mean values of the response variables of all target tree individuals across all tree species/tree clones averaged for each plot's tree community; including all foliar fungal pathogen species (n_{plots}). The *individual level* regards leaf mean values of the response variables averaged for all target tree individuals across all tree species/tree clones; including all foliar fungal pathogen species ($n_{all\ target\ individuals}$). Leaf mean values of the response variables averaged for all target tree individuals of a particular tree species/tree clone; including all foliar fungal pathogen species are considered by the *tree species/tree clone level* ($n_{target\ individuals\ per\ tree\ species}$), while the *fungus species level* includes

each foliar fungal pathogen species separately ($n_{\text{target individuals per tree species}}$). At the *community level*, plot means of pathogen species richness and pathogen load were used in linear models. In contrast, as several trees were sampled per plot, analyses of *individual level*, *tree species/tree clone level* and *fungus species level* were based on the local neighbourhood of the target trees and tree individuals' means were run in linear mixed effects models. For this purpose, the local neighbourhood considered the tree species/tree genotype identity of the nearest neighbour tree individuals (Figure 1-3) and allowed beyond that the calculation of the neighbour tree species proportions (weighted for tree individual's basal area; K) and tree clone density (S). Predictors for pathogen species richness and load were different at these four levels and with respect to the particular experimental design (see Figure 1-1 and Chapter 1.3).

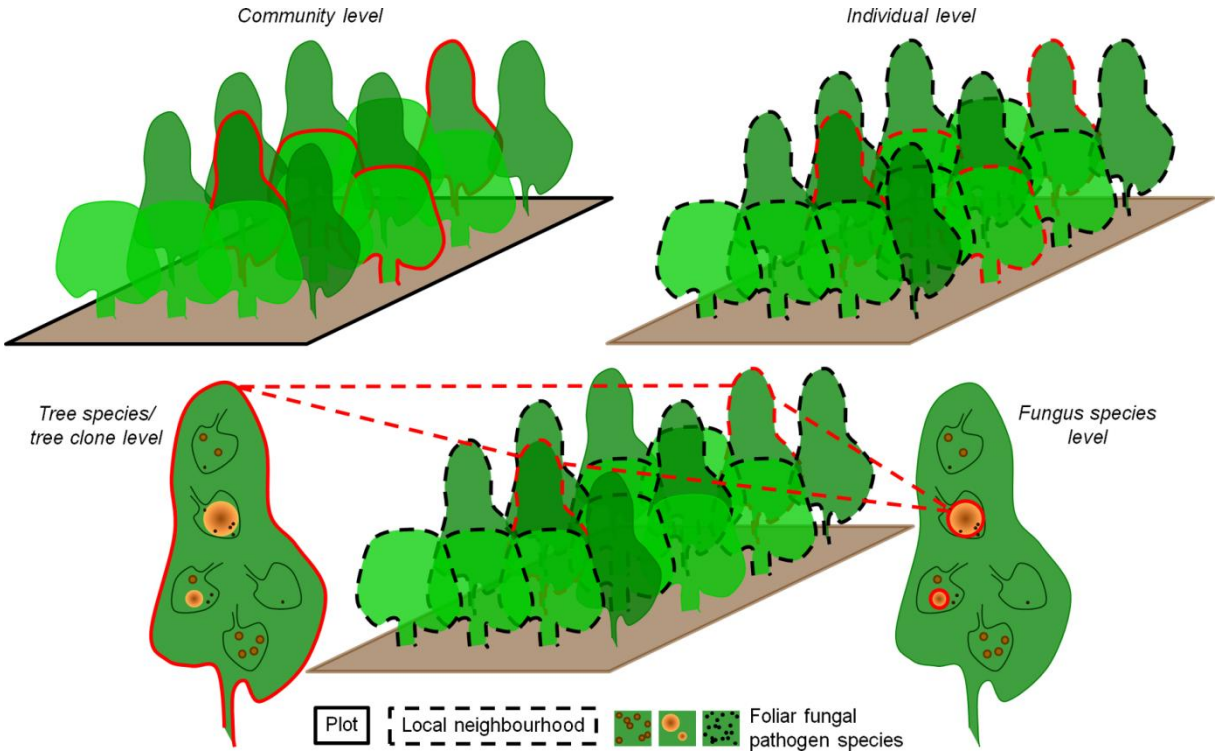
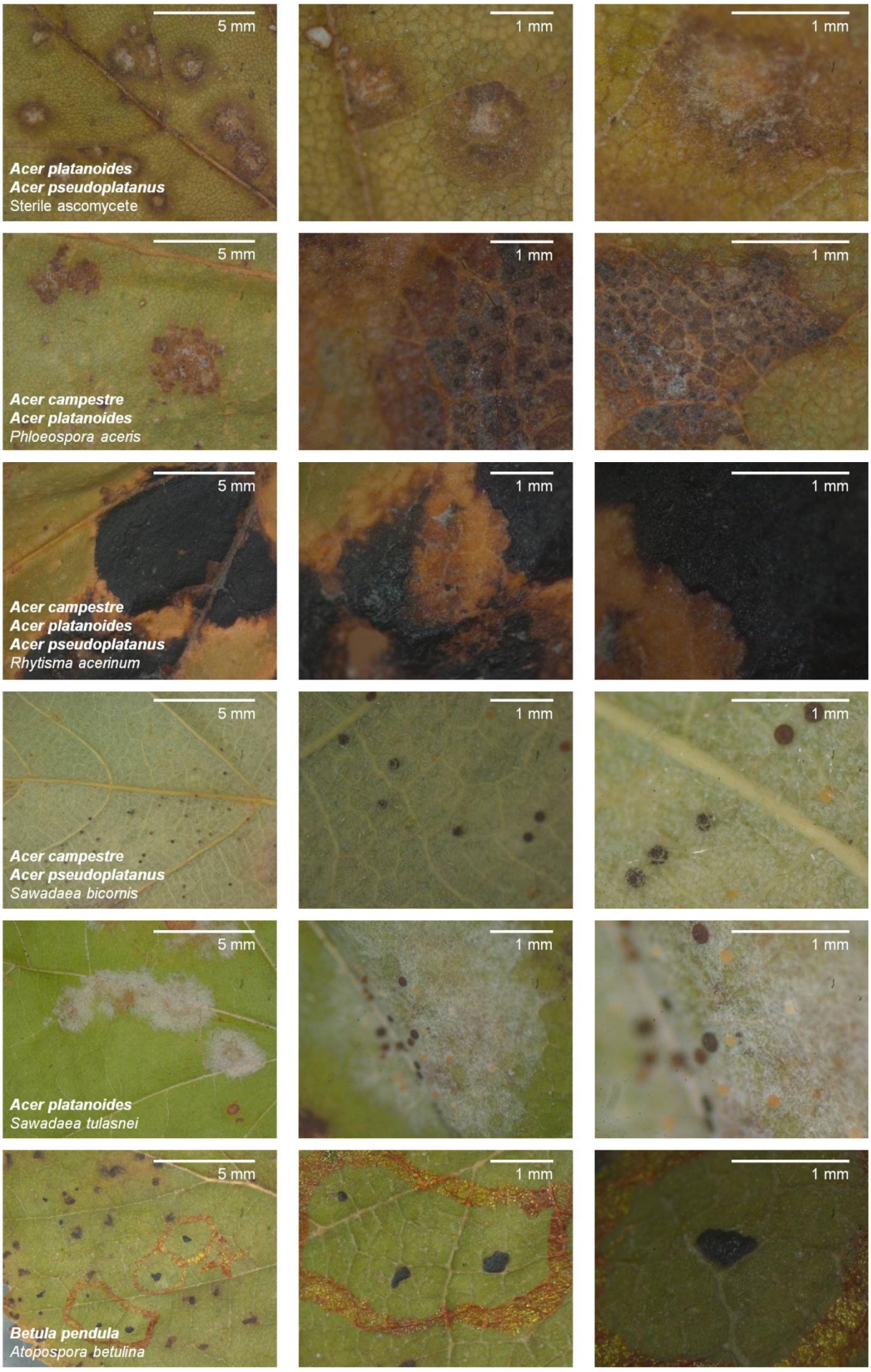


Figure 1-3 Investigation levels of tree diversity effects on foliar fungal pathogens. The *community level* (synonym: *plot level*) observed the influence of the whole plot and considers all pathogen species on all target individuals across all tree species/tree clones. The *individual level* regards all pathogen species on all target individuals across all tree species/tree clones. The *tree species/tree clone level* considers all pathogen species on all target individuals of a particular tree species/tree clone. The *fungus species level* focuses separately on every foliar fungal pathogen species on a particular host tree species. In contrast to *community level* analyses, *individual level*, *tree species/tree clone level* and *fungus species level* analyses were related to the local tree neighbourhood.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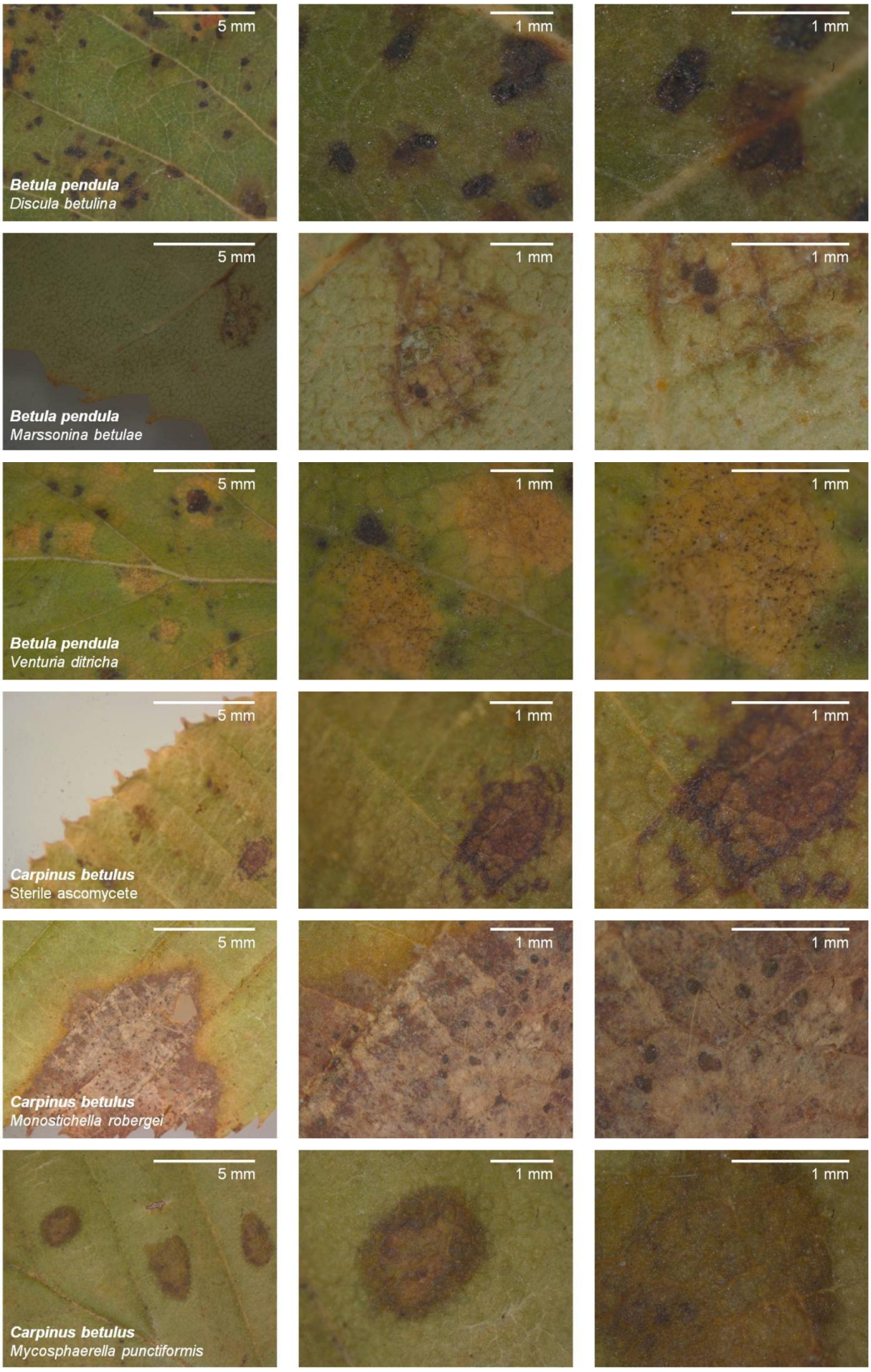
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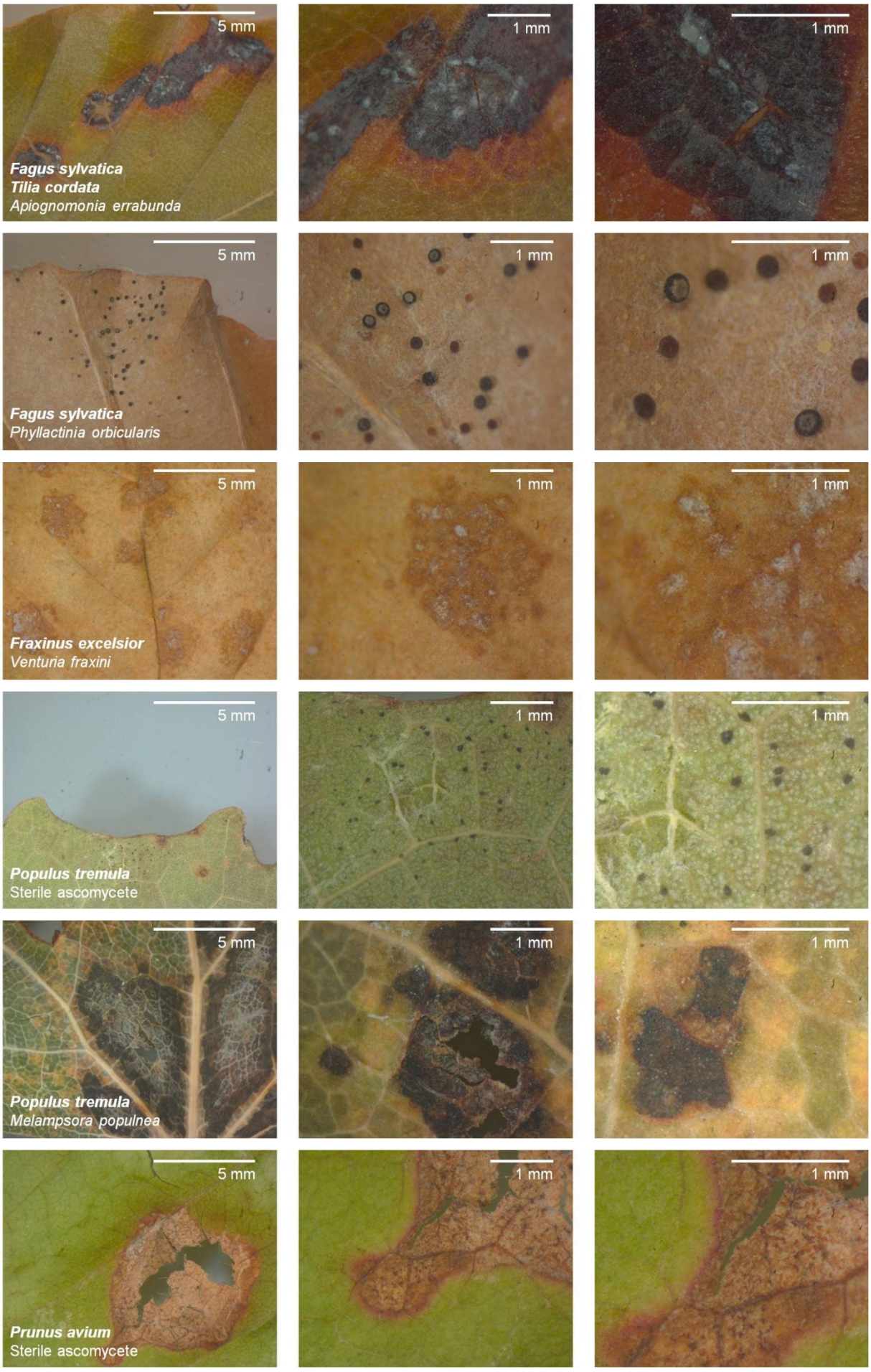
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Fraxinus excelsior
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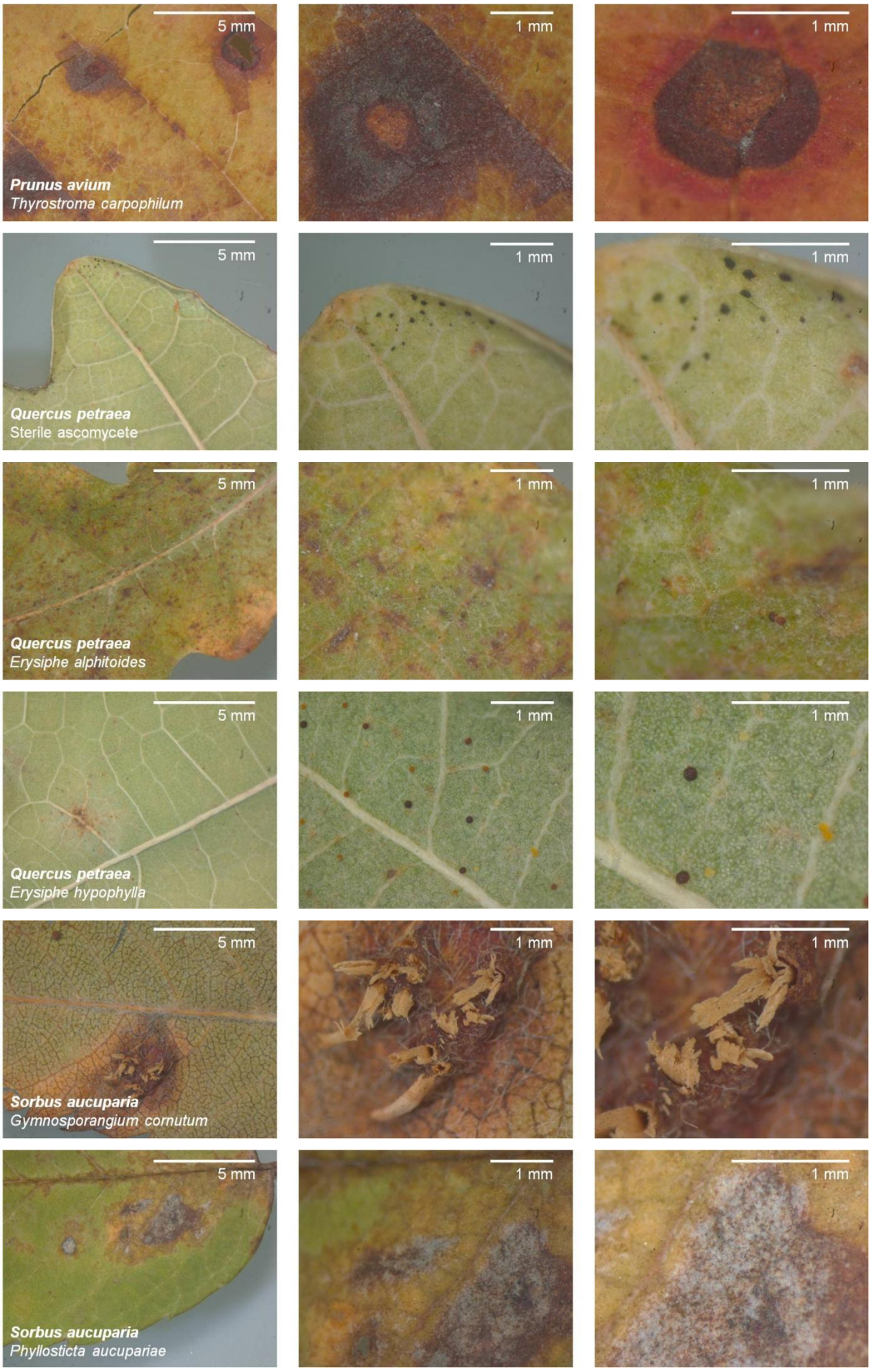
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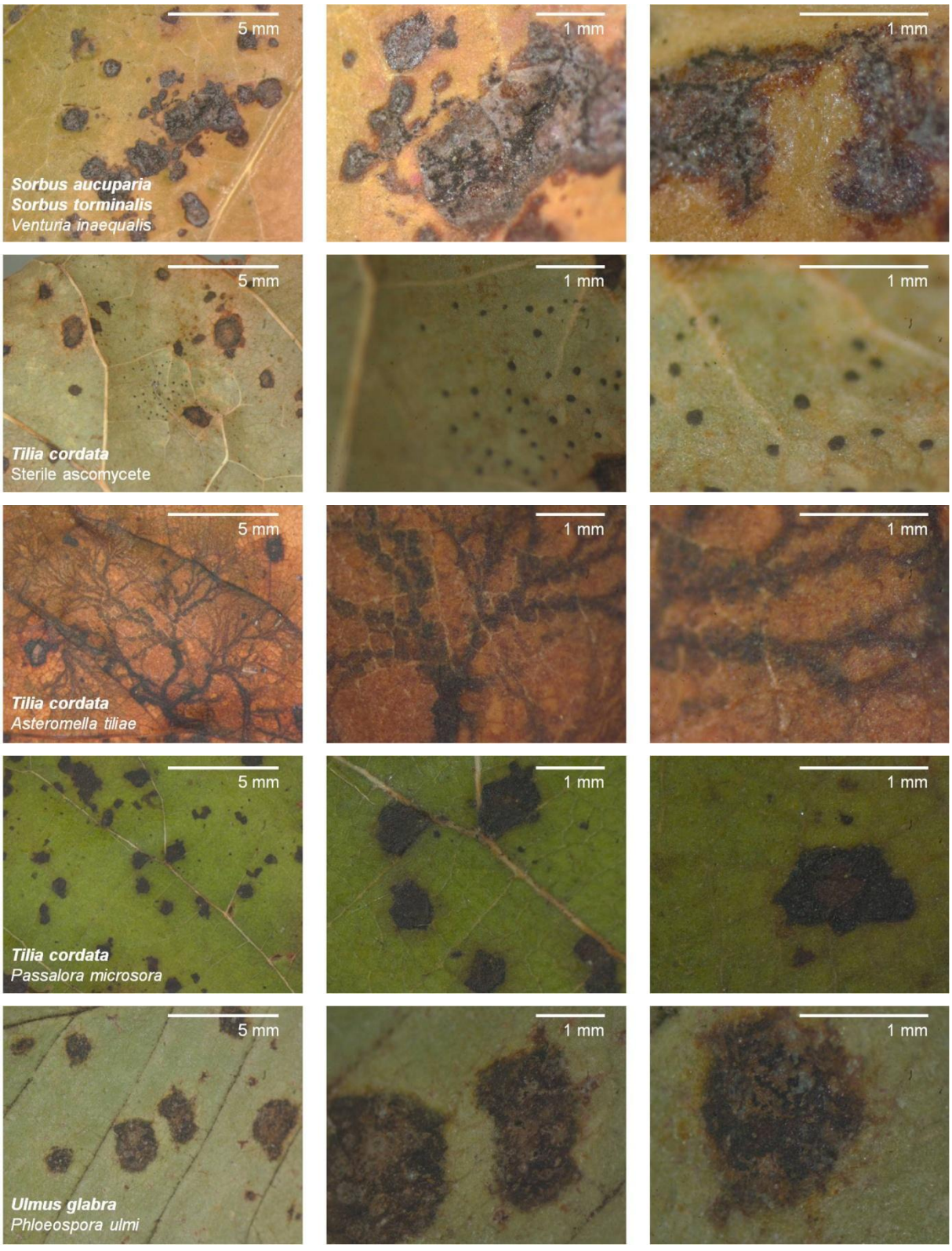
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Tilia cordata
Asteromella tiliae
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Tilia cordata
Passalora microsora
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1 mm
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Ulmus glabra
Phloeospora ulmi

1.3 Objectives of the thesis

The thesis was conducted in order to study the role of tree diversity on foliar fungal pathogens in young experimental forest stands employing four European tree diversity experiments. Thus, this thesis closes a gap in the functional biodiversity research, as most effort was carried out in order to investigate the top-down control by pathogens in host-pathogen interactions at the community scale, but the bottom-up control by host community composition and structure was far less observed. Beyond that nearly all of the current knowledge of such bottom-up effects arose from observational studies and grassland or agricultural ecosystems so far. Thus, there is an urgent request to study this BEF relationship, also in experimental forest communities as they are far more complex than grasslands. Regarding the experimental design of the four sites, these addressed the effects of inter-specific tree species diversity (BK, K) and functional tree diversity (BB), as well as the effects of intra-specific tree clone diversity (S) (see Table 1-1). In accordance to the four experimental sites, this thesis is structured into four main chapters (Chapter 2 to 5).

The objective of Chapter 2 was to determine tree species diversity effects on foliar fungal pathogens of four common forest tree species in the BIOTREE experimental site at Kaltenborn (BK). This experiment allowed assessing primary effects of tree species richness at *community level* and of Shannon diversity of the local tree neighbourhood at *individual level*, *tree species level* and *fungus species level*. Tree species identity effects were tested as secondary predictors at all spatial scales.

Chapter 3 presents tree diversity effects on foliar fungal pathogens of the two disease-prone tree species *Quercus petraea* and *Tilia cordata* in the Kreinitz experiment (K). Here, the focus was on the *tree species level* and the *fungus species level* and employed Shannon diversity, as well as host and non-host proportions of the local tree neighbourhood as predictors. The Kreinitz experiment also allowed to weight tree neighbourhood effects by the basal area as a proxy for neighbour tree size. Finally, response variable were tested for inter-annual variation as repeated sampling was conducted in three subsequent growing seasons.

The aim of Chapter 4 was to study functional tree diversity effects on foliar fungal pathogens of 16 tree species at the BIOTREE experimental site at Bechstedt (BB). At *community level*, functional diversity effects were tested separately as effects of functional richness, functional evenness and functional divergence. In addition, tree species identity effects were investigated. At *tree species level*, effects of tree species' leaf traits, as well as phylogenetic patterns of leaf traits and foliar fungal pathogen richness and infestation were analysed.

The objective of Chapter 5 was to determine tree clone diversity effects of eight birch clones on foliar fungal pathogens in the Satakunta experiment (S). The role of tree clone richness was tested at *community level*, but the effects of tree clone richness and the Shannon diversity of the tree clones, as well as of tree clone density of the local tree neighbourhood were analysed at *individual level*, *tree clone level* and *fungus species level*.

Glossary

Biodiversity: Refers to genetic, species and ecological diversity over all temporal and spatial scales (Harper & Hawksworth 1994).

Clone diversity / genetic diversity / genotype diversity / intra-specific diversity: Intra-specific genetic diversity of individuals of a particular plant species within a particular plant community.

Clone density / genotype density / host density / host proportion / species abundance / species density / species proportion: Availability of a particular plant host species/clone within a particular plant community based on number or proportion of individuals of that species/clone.

Clone identity / species identity: Refers to the degree of susceptibility of a particular plant species/clone and its resulting impact on overall plant communities' pathogen richness or infection.

Clone richness / genotype richness / species richness: The number of different plant species/clones in a plant community.

Disease-resistant clone / disease-resistant genotype / disease-resistant host / disease-resistant species: The inherent ability of a plant host to overcome or retard, completely or to some degree, the infection of a pathogen (Shurtleff & Averre III 1997).

Disease-prone clone / disease-prone genotype / disease-prone species / susceptible clone / susceptible species: The inability of a plant host to resist the effect of a pathogen (Shurtleff & Averre III 1997).

Disease risk / disease incidence / infection risk: The ability of a host plant species, plant clone or a plant community to contract disease (Baker 1978).

Disease severity / fungal infestation / pathogen damage / pathogen infestation / pathogen load: The measure of damage done by a plant pathogen (Shurtleff & Averre III 1997).

Ecosystem functioning: Refers to ecosystem properties, ecosystem goods and ecosystem services (Christensen et al. 1996).

Functional diversity: Inter-specific trait diversity of plant species in a particular plant community (Petchey & Gaston 2006).

Fungal species richness / number of fungus species / pathogen species richness: The number of pathogen species on a host individual or species or community.

Generalist pathogen / generalized pathogen: A pathogen species that is able to use a broad range of host species as microhabitat.

Host-specific pathogen / specialist pathogen / specialized pathogen: Pathogen species that is able to use only a single or few closely related host species as a host (Shurtleff & Averre III 1997).

Shannon diversity: Diversity and abundance of individuals of different plant species/clones in the local neighbourhood of a particular target plant individual.

Species diversity: Inter-specific diversity of individuals of different plant species in a particular plant community.

This thesis tested the following main hypotheses in particular experiments (see also Figure 1-1, Table 1-1):

H1 Pathogen species richness increases and pathogen load decreases with increasing tree species richness/Shannon diversity (BK, K) and tree clone richness/Shannon diversity of tree clones (S).

Tree communities with high tree diversity are expected to provide more favourable microhabitats for different specialist foliar fungal pathogen species, but pathogen transmission of a particular fungus species is less effective at high tree host diversity, thus pathogen infestation is lower as compared to communities with low tree diversity.

H2 Inter-annual variation of both pathogen species richness and load decreases with increasing Shannon diversity (K).

Tree species-rich communities are expected much more to buffer inter-annual variation of the response variable than tree monocultures.

H3 Pathogen species richness and pathogen load decrease with increasing functional tree diversity (BB).

Low functional diversity is brought about by tree species of similar trait values, which often results from close relatedness. Phylogenetically closely related tree species suffer from a similar risk of infection by closely related foliar fungal pathogen species, thus they suffer from an increase risk of fungi of different, but closely related species.

H4 Pathogen species richness decreases and pathogen load increases with increasing host tree species proportions in the local neighbourhood (K) and host tree clone density (S).

High tree host availability is expected to facilitate pathogen transmission of compatible foliar fungal pathogen species, thus rising pathogen infestation, whereas pathogen richness decreased because of fewer differences in available microhabitats.

H5.1 At the *community level*, both pathogen species richness and pathogen load increase with the presence of disease-prone tree species (BK, BB) and tree clones (S). The contrary effect is expected with the presence of disease-resistant tree species (BK, BB) and tree clones (S).

Identity effects of tree species/tree clones are expected due to their particular susceptibility to foliar fungal pathogens.

H5.2 With regard to the local tree neighbourhood, pathogen species richness and load are expected to depend on the identity of particular non-host tree species (BK), on the proportion of a particular non-host tree species (K) and on the density of a particular less compatible tree clone (S).

Non-host tree species proportion and tree clone density in the local neighbourhood are expected to facilitate or hinder foliar fungal pathogen transmission by providing a pathogen reservoir or a physical barrier, respectively, and to affect pathogen development by altering microclimatic conditions.

2. SPECIES RICHNESS AND SPECIES IDENTITY EFFECTS ON OCCURRENCE OF FOLIAR FUNGAL PATHOGENS IN A TREE DIVERSITY EXPERIMENT

Lydia Hantsch, Uwe Braun, Michael Scherer-Lorenzen, Helge Bruelheide

Ecosphere 4:81

2.1 Abstract

Current theory on transmission rates of plant pathogens predicts a strong influence of host richness on the degree of infection. In addition, identity effects, caused by the presence of particular species in a community, may also drive biodiversity and ecosystem functioning relationships, with *selection* or *sampling effects* being particularly important. We tested the effect of tree species richness and tree species identity effects on foliar fungal pathogens on four forest tree species of the temperate zone making use of the BIOTREE tree diversity experiment in Germany. We hypothesized that fungal species richness is positively and fungal pathogen load negatively related to tree species richness. In addition, we tested whether species number of foliar biotrophic fungi and pathogen load depend on tree community composition and on the presence or absence of particular disease-prone tree species. All foliar fungi were identified macro- and microscopically and subjected to statistical analyses at three hierarchical levels, at the *plot level*, the level of single *tree species* and the level of individual *fungus species*. There was a negative effect of tree richness on the pathogen load of common powdery mildew species. Moreover, we found strong tree species identity effects at the *plot level* as the presence of *Quercus* resulted in a high pathogen load. Thus, for the first time we experimentally showed that disease risk and pathogen transmission of foliar fungal pathogens in temperate forest tree ecosystems may depend on tree richness and on the presence of particular disease-prone species.

Keywords

biodiversity and ecosystem functioning, BIOTREE experiment, disease dilution effect, ecosystem processes, Erysiphales, *Fagus sylvatica*, local neighbourhood, *Quercus*, shannon diversity, tree species identity effects, tree species richness effects

2.2 Introduction

It has been hypothesized that biodiversity is able to reduce passive pathogen transmission by different mechanisms, such as host distance and abundance, pathogen behaviour and the environmental conditions shaped by hosts and non-hosts (Keesing et al. 2006, 2010). Pathogen transmission rates are one of a multitude of ecosystem processes that might be affected by biodiversity (Loreau et al. 2001; Hooper et al. 2005; Balvanera et al. 2006). Furthermore, biodiversity might affect the average susceptibility of individuals in the community, by influencing the ability to resist pathogen attacks (Shurtleff & Averre 1997 p. 321). Less susceptible individuals will result in lower community dynamics, thus causing more stable communities (Pautasso et al. 2005). The disease-diversity hypothesis states that high species or high genetic diversity in a community confers disease resistance (Heybroek 1982; Burdon 2001).

At the community scale, the *plot level*, there might be different mechanisms that result in such diversity effects. For example, a drier microclimate in mixtures than in monocultures has been found to be the cause for the decrease in fungal disease levels in experimental rice fields with genetically diverse mixtures of rice cultivars (Zhu et al. 2005). From the host species' perspective, lower levels of pathogen load would be considered positive, since an intact leaf area would provide a higher photosynthetic gain. As a fungal infection finally results in a higher mortality of single leaves or whole plants, and thus, presents a type of disturbance, a decreased pathogen load would finally be translated into a higher net primary production of the whole plant community (Mitchell 2003; Berger et al. 2007). Thus, biodiversity ecosystem functioning would be negatively affected by leaf pathogens, because flux rates of matter and energy in the system would be reduced (Jiang et al. 2008). In contrast, host species richness is expected to have positive effects on pathogen species richness, as a wider niche space is provided for the different pathogen species (Bond & Chase 2002). Positive or neutral biodiversity and ecosystem functioning effects on pathogen richness might also occur if additional plant species are important for completing the pathogens' life cycles (Cheatham et al. 2009; Johnson et al. 2009; Mundt et al. 2011). This is the case for many rust fungi (*Puccinia* spp.), which have a hetero-oecous life style and use different plant species as alternate hosts for sexual reproduction (Cheatham et al. 2009). Positive effects of host species richness are representing a parallel to the effect of *associational susceptibility* with respect to herbivory. A meta-analysis of herbivore abundance and damage Vehvilainen et al. (2007) showed that mixed stands received more damage than monocultures, mainly caused by

generalist herbivores. Similarly, moose browsing tended to increase with the number of tree species in the mixture (Vehvilainen & Koricheva 2006).

In addition to host diversity effects, biodiversity and ecosystem functioning relationships at the community scale can be driven by identity effects, could be caused by the presence of particular species in a community, with *selection* or *sampling effects* being particularly important (Loreau & Hector 2001). Positive or negative selection effects occur when, on average, species that perform accordingly higher or lower than average in the monoculture increase their relative abundance in diverse communities. In addition, sampling effects result from the increasing probability for a species with a particularly high or low contribution to ecosystem functioning to be present in species-rich communities (Loreau & Hector 2001).

Both diversity and identity effects do also occur at the scale of the local neighbourhood the *tree species level* and the *fungal species level*. Thus, the disease-diversity hypothesis can be extended to individual hosts, allowing comparing different host species in their degree of susceptibility as well as different fungal species in their degree of infectiousness. With increasing host species richness the transmission risk for a specialist fungal species will decrease because of a decreased host density in the local neighbourhood (Figure 2-1a). Then, particular tree species in the neighbourhood might either increase or decrease the pathogen load of a target tree, thus having positive or negative effects on the hosts pathogen load (Figure 2-1b). Both types of identity effects can be considered different cases of neighbour-mediated facilitation. The first case would be a neighbour-species mediated facilitation of the pathogen species, the latter a neighbour-species mediated facilitation of the host species. Such identity mechanisms have been demonstrated by sowing non-host plant species in grassland diversity experiments, which resulted in a decreased pathogen load (Mitchell et al. 2002). Similarly, Roscher et al. (2007) showed a reduction of disease intensity of rust fungi on the host species *Lolium perenne* with increasing species richness.

The question whether positive or negative selection effects prevail in a host-pathogen system depends on whether the system is dominated by susceptible or more resistant host species. It has recently been suggested that ecosystem functions involving more than one trophic level, such as host-pathogen systems, might be predominantly characterized by negative selection effects (Jiang et al. 2008), because less susceptible host species might be dominant more often than susceptible ones. However, empirical evidence for this proposition is scarce and contradictory.

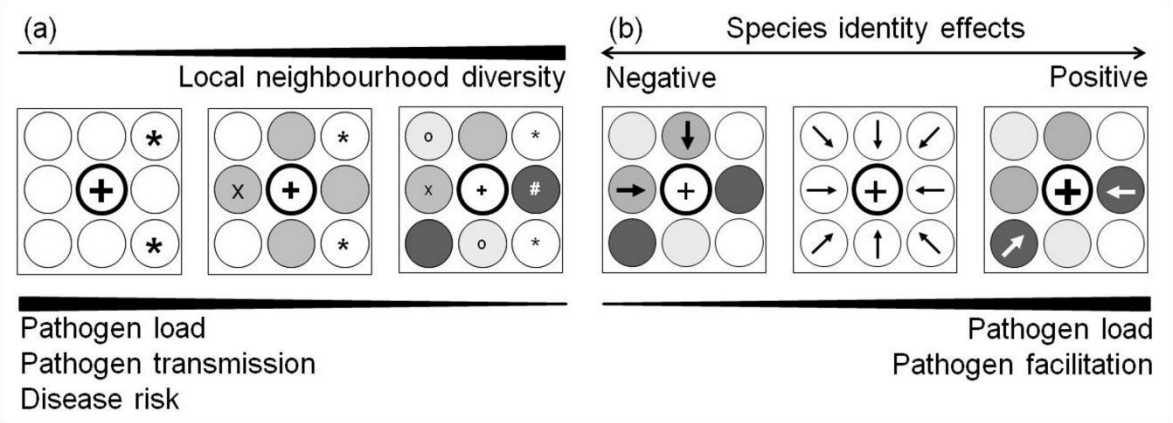

Figure 2-1 Graphical illustration of how biodiversity can affect fungal pathogen richness and fungal pathogen load at the local neighbourhood scale in experimental plant communities of constant plant species density. Plant individuals are represented by circles, with different colours being different species. Target individuals are marked by a thick black outline. The symbols in the circles represent specialized biotrophic fungal pathogens, with different symbols indicating different species and different size of symbols showing a different degree of pathogen load. Circles with arrows indicate that the plant species changes the local environment of a target species. (a) Host species richness effects resulting from a reduced pathogen transmission risk with increasing plant species richness. (b) Species identity effects brought about by particular plant species that might either increase or decrease the pathogen load of the target individual, depending on whether the species in the neighbourhood changes the environmental conditions in a favourable or unfavourable manner for the specialized biotrophic pathogen.

Pathogens do not only respond to host richness but do also have effects on host species performance and composition. They can, like other consumers (Duffy 2003), change the outcome of competition between host species (Mordecai 2011). Pathogens can promote coexistence among host species by regulating the relative abundance of hosts. According to the Janzen-Connell hypothesis, pathogens can cause local negative density dependence of host species, and thus, affect the spatial host abundance patterns (Janzen 1970; Connell 1971; Bagchi et al. 2010). However, the situation in most ecosystems might be even more complex, as beside hosts and pathogens further components are involved in food-webs (Duffy 2002). In general, biotic networks and the epidemiology of directly transmitted infectious diseases are fundamentally linked (Keeling & Eames 2005). This entanglement of factors and the confounding of causes and effects make it extremely difficult to analyze the relationship between host and pathogen species richness in observational studies. The obvious solution to this problem is an experiment in which host density is kept constant and host species richness is manipulated. Such tree diversity experiments have become available only recently (Verheyen 2012).

In this study we make use of the German tree diversity experiment BIOTREE (Scherer-Lorenzen et al. 2007). On the community scale, we tested the hypotheses that 1) the species number of foliar biotrophic fungi is positively and the pathogen load is negatively related to

the host tree species richness. We also expected strong tree species identity effects and hypothesized that 2) species number of foliar biotrophic fungi and pathogen load depend on community composition and on the presence or absence of particular disease-prone tree species. With focus on the local neighbourhood scale, we tested 3) whether pathogen load is negatively related to the tree species richness (Figure 2-1a) and 4) to the presence or absence of particular tree species (Figure 2-1b).

To our knowledge this is the first study that addresses biodiversity effects in tree hosts-foliar fungal pathogen systems in a full tree-experimental setting.

2.3 Material and Methods

Study site

The BIOTREE project adopts a *synthetic community approach* to study tree diversity effects on ecosystem functioning. Study sites were established by experimentally creating a gradient of tree species richness under relatively homogeneous site conditions. The project has been described in detail elsewhere (Scherer-Lorenzen et al. 2007), so that we report the most important design feature only. The study site at Kaltenborn was selected from the three BIOTREE sites and is located in South-West Thuringia (N 50°47', E 010°13'), Germany. Elevation is about 320-350 m a.s.l., mean annual temperature is 7.8°C and mean annual precipitation is about 650 mm (Scherer-Lorenzen et al. 2007). The climate is subatlantic, the soils are acidic and sandy. In 2003/2004, 16 plots were established that contained either 1 (n = 4), 2 (n = 6), 3 (n = 4) or 4 (n = 2) tree species (*Quercus*, *Fagus sylvatica*, *Picea abies*, *Pseudotsuga menziesii*, Scherer-Lorenzen et al. 2007). Individuals of the genus *Quercus* mainly belonged to the species *Quercus petraea*, but there were also a few individuals of *Quercus robur* and potentially some hybrids of both *Quercus* species planted. The occurrence of this within genus mixture is caused by a less strict differentiation between those *Quercus* species in the tree nurseries as well as by the high hybridization rate between both species. Since these two species are known to be closely related and do not differ much ecologically, we refer to them as *Quercus*. The replicates of the four-species mixture always contain the same species composition, while at all other diversity levels, species composition differs between replicates. The plots have a size of 120 x 48 m and are divided into three subplots which will receive a different management in the future (unmanaged, managed, managed with additional tree species), but are equivalent for the purpose of our study. Each subplot consists of 30 mono-specific patches of 8 x 8 m. Mimicking local forestry practices, patches with

Quercus and *F. sylvatica* were planted at higher densities, comprising 28 tree individuals, whereas those with *P. abies* and *P. menziesii* have 16 tree individuals. Detailed information about the plot design and tree establishment success at the Kaltenborn site is given in Scherer-Lorenzen et al. (2007) and Don et al. (2007).

Tree individuals were randomly selected for foliar fungal pathogen analyses in every plot, mostly using the unmanaged subplots and excluding all patches at the outer border of a plot to avoid edge effects when calculating local neighbourhood composition. The random selection of trees resulted in the selection of both trees growing in the centre and at the edge of each tree species patch. The mean tree size of sampled individuals at the time of sampling in September 2010 varied between 2.4 + 0.4 m for the smallest species (*Quercus*) and 3.5 + 0.7 m for the tallest species (*Pseudotsuga*), respectively. In total, we sampled six individuals in each of the monocultures, three individuals per species in each of the two-species mixture plots and two individuals per species in each of the three and four species mixtures. In total, 100 trees were sampled.

Leaf sampling

On deciduous trees, two branches, growing in opposite directions were selected in the upper as well as in the lower part of the crown and 20 leaves were collected from every branch. On conifers, eight shoots were sampled with needles of both the recent and all previous years. Leaves and needles were dried immediately after sampling at 60°C for three days and then stored in the dark at approximately 20°C.

Macro- and microscopic analyses

For macro- and microscopic analyses we used a random subset of 10 leaves or 100 needles per tree individual of each of the 100 sampled individuals. We included only visible pathogenic foliar fungi in this study, but excluded visible saprophytic and epiphytic fungi, since they have different ecological functions. In addition, one has to be aware that there were also endophytic fungi, which might also have an impact on the host, but were not investigated in this study. Thus, the restriction of fungal species inventory to visible parasitic fungi leads to an exclusion of all other fungi living on the leaf surface as well as within the tissue, resulting in a smaller amount of fungal species diversity.

Light microscopy was used to identify pathogenic fungal taxa to the species level (after Brandenburger 1985; Ellis & Ellis 1997; Braun & Cook 2012). Depending on the fungal developmental stage identification was not always possible at the species level. In one case we could only assign a taxon without fructification to the phylum level of Ascomycota.

Binocular analyses of the collected and dried leaves allowed the quantification of pathogen load of fungal taxa on the whole surface of leaves and needles. Pathogen load was estimated as percent leaf area damage on a percentage scale with categories of 0 %, 1-5 %, 6-10 %, 11-25 %, 26-50 %, 51-75 % and 76-100 %.

Data analyses

Both fungal species richness and pathogen load were related to tree species richness/Shannon diversity of the local tree neighbourhood and to tree species identity. Local tree neighbourhood was calculated from all eight patches around the central patch with the target individual, including also the trees in the central target, and thus, comprising 9 x 16 (n = 144, conifers) to 9 x 28 (n = 252, deciduous trees) trees. Effects were assessed at three different hierarchical levels. At the community scale, the *plot level*, all tree und fungal species and their pathogen load were jointly analyzed (n = 100 tree individuals), providing insights in overall pathogen diversity and pathogen load with respect to community diversity. Using sequential linear models, in a first model, the effect of tree species richness as main predictor was accounted for. However, that model included a certain part of remaining variance, which might be explainable by further biodiversity effects. Thus, in a second step, the residuals of that model were used for testing species identity effects, using presence/absence coding for the identity of all four tree species in the experiment and following the procedure outlined in Bell et al. (2009). At the local neighbourhood scale, fungal species richness and pathogen load data were analyzed separately by tree species at the *tree species level*, taking each of the four tree species as target species (n = 25 tree individuals per tree species). This analysis by tree species separation was done due to differences of host species' susceptibility and species specific host-pathogen interactions. In addition, at the *fungus species level*, pathogen load was evaluated separately for every fungal taxon on a particular tree species (n = 25 tree individuals per tree species), since infectiousness and biology of each fungal taxa were different. We used linear mixed effects models (*lme*, package nlme, Bates 2011) at the *tree species level* and *fungus species level*, including plot as random factor (R Development Core Team 2008), testing for specific effects of Shannon diversity of the local neighbourhood and neighbour identity. As in the ordinary linear models, residuals of the model with Shannon diversity as main predictor were tested for species identity effects of all four tree species in a subsequent mixed model. F tests and p values were obtained from the mixed models with the *cftest* command (package multcomp). The variance components analysis (varcomp procedure) was used to assess the proportion of total random variation to be attributed to plot. The amount of

variation explained by the model was obtained from regressing predicted against observed responses. All figures were produced with R.

2.4 Results

Statistical analysis showed that *Quercus* seemed to be the tree species in the Kaltenborn-Experiment that was most affected, in terms of pathogen load and with four different foliar pathogen species also with respect to number of pathogen species. Two of the pathogens, with the highest frequency of occurrence and quantity of infection, were powdery mildew species (*Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam., *Erysiphe hypophylla* (Nevod.) U. Braun & Cunningt.). The other two fungi species occurred only in low frequency and coverage on the leaves, *Microstroma album* (Desm.) Sacc. and an unidentified species of Ascomycota (Berk.) Caval.-Sm.). A third powdery mildew species (*Phyllactinia orbicularis* (Ehrenb.) U. Braun) was detected on *Fagus sylvatica*, but this fungus species showed a lower pathogen load than the powdery mildew species on *Quercus*. *Fagus sylvatica* was host of a second foliar pathogen species (*Apiognomonia errabunda* (Roberge ex Desm.) Höhn.). In contrast, foliar pathogens were neither encountered on *Pseudotsuga menziesii* nor on *Picea abies*.

At the community scale, linear model analysis at the *plot level* showed no significant tree species richness effects, neither on fungal species richness nor on pathogen load (Figure 2-A1, Table 2-1). However, the presence of *Quercus* within the community positively affected fungal species richness and pathogen load, indicating a higher fungal species richness and pathogen load when *Quercus* was present in a plot. In contrast, *F. sylvatica*, *P. menziesii* and *P. abies* exhibited mostly negative tree species identity effects on both response variables (Table 2-2).

Table 2-1 Linear model results at the *plot level*. Effect of tree species richness on number of fungus species and pathogen load (%) across all plots (n = 16).

Response variable	Df	SS	F	p
Number of fungus species	1	14.504	2.784	0.117
Residuals	14	72.933		
Pathogen load (%)	1	57.29	0.499	0.491
Residuals	14	1604.60		

Table 2-2 Linear model results at the *plot level*. Effect of tree species identity on number of fungus species and pathogen load (%) across all plots (n = 16). Significant results are indicated in bold fonts.

	Number of fungus species		**Pathogen load (%)**	
Identity effect	Estimate	p	Estimate	p
Quercus	3.375	<0.001	15.094	<0.001
F. sylvatica	0.125	0.563	-5.365	0.053
P. menziesii	-1.875	<0.001	-3.813	0.154
P. abies	-1.625	<0.001	-5.917	0.036

With a focus on the local neighbourhood scale, at the *tree species level*, Shannon diversity of the local tree neighbourhood had no significant effects on fungal species richness (Table 2-3). There was a marginally significant positive effect on pathogen load of *Quercus* while there were no effects on pathogen load of the other tree species (Figure 2-A2, Table 2-3). In contrast to the *plot level*, no species identity effects were encountered at the *tree species level* (Table 2-A1).

Similarly, at the local neighbourhood scale at the *fungus species level*, we found that pathogen loads of the two powdery mildew species *Erysiphe alphitoides* and *Erysiphe hypophylla* on *Quercus* were negatively related to Shannon diversity of the local tree neighbourhood (Figure 2-2A, Table 2-4). However, there was no effect of tree species neighbourhood richness on *Microstroma album* and the unidentified leaf-spotting ascomycet species on *Quercus* (Table 2-4). Figure 2-2B shows for the host *F. sylvatica* that Shannon diversity of local tree neighbourhood affected the powdery mildew *Phyllactinia orbicularis*, too, whereas there was no effect on *Apiognomonia errabunda* (Table 2-4). As for the *tree species level*, no tree species identity effects were detected at the *fungus species level* (Table 2-A2).

Table 2-3 Linear mixed model results at the *tree species level.* Effect of Shannon diversity of the local tree neighbourhood on number of fungus species and pathogen load (%) for all target trees (n = 100, plot variance = 22.2 % and 31.8 % of total random variation, respectively) and for the tree species *Quercus* (plot variance = 8.5 %) and *Fagus sylvatica* (plot variance = 47.1 %) across all tree individuals of each tree species (n = 25). Df of the numerator = 1, df of the denominator = 83 for all target trees and df = 15 for *Quercus* and *F. sylvatica*. Note that no fungal pathogens were detected on *Picea* and *Pseudotsuga*.

		All target trees		***Quercus***		***F. sylvatica***	
Response variable		F	p	F	p	F	p
Number of fungus species	Intercept	23.242	< 0.001	-	-	-	-
	Shannon Diversity	0.047	0.828	-	-	-	-
Pathogen load (%)	Intercept	9.07	0.003	58.487	< 0.001	7.347	0.016
	Shannon Diversity	0.619	0.434	3.209	0.094	2.448	0.139

Table 2-4 Linear mixed model results at the *fungus species level*. Effect of Shannon diversity of the local tree neighbourhood on pathogen load (%) of specialized fungus species for the tree species *Quercus* and *Fagus sylvatica* across all tree individuals of each tree species (n = 25). Df of the numerator = 1, df of the denominator = 15. Significant results are indicated in bold fonts. Plot variance of total random variation: *E. alphitoides* = 8.5 %, *E. hypophylla* < 0.1 %, *M. album* = 3.9 %, *Sp. of Ascomycota* = 28.5 %, *P. orbiculata* = 44.0 %, *A. errabunda* = 21.5 %.

Pathogen load (%) of fungus species on *Quercus*			**Pathogen load (%) of fungus species on *F. sylvatica***		
	F	p		F	p
Intercept	40.478	< 0.001	Intercept	6.677	0.02
Erysiphe alphitoides	4.388	0.054	*Phyllactinia orbicularis*	4.317	0.055
Intercept	53.76	< 0.001	Intercept	6.151	0.026
Erysiphe hypophylla	4.689	0.047	*Apiognomonia errabunda*	0.044	0.836
Intercept	3.439	0.084			
Microstroma album	0.04	0.845			
Intercept	6.721	0.02			
Species of Ascomycota	0.262	0.616			

2.5 Discussion

Loss of biodiversity might have extensive, positive or negative, impacts on disease transmission in natural ecosystems (Borer et al. 2009; Haas et al. 2011). Our study showed that tree diversity plays a role in biotrophic fungal pathogen infections of common European forestry species. We have to reject the first hypothesis that species number of foliar biotrophic fungi at the *plot level* is positively and pathogen load is negatively related to the number of host tree species. There are several explanations for this outcome at the community scale. First, the number of host tree species might not have been able to affect overall species number and pathogen load of foliar biotrophic fungi, because the two conifer species have not been infected by any fungal pathogen. Thus, at the community scale, there was only a gradient of two competent tree species instead of the four tree species in the whole experiment. The immunity of conifers to foliar biotrophic fungi is a feature observed before and might be brought about by both constitutive and inducible defences (Erbilgin & Colgan 2012). Second, none of the biotrophic fungi in our study were generalist species or depended on multiple hosts. Our results support the idea that host species richness mainly favours generalist pathogens rather than specialized pathogens, which was shown for generalist vs. specialist herbivores (Koricheva et al. 2006). Third, the young age of the tree individuals in the BIOTREE experiment may have retarded potential host richness effects (Scherer-Lorenzen et al. 2007).

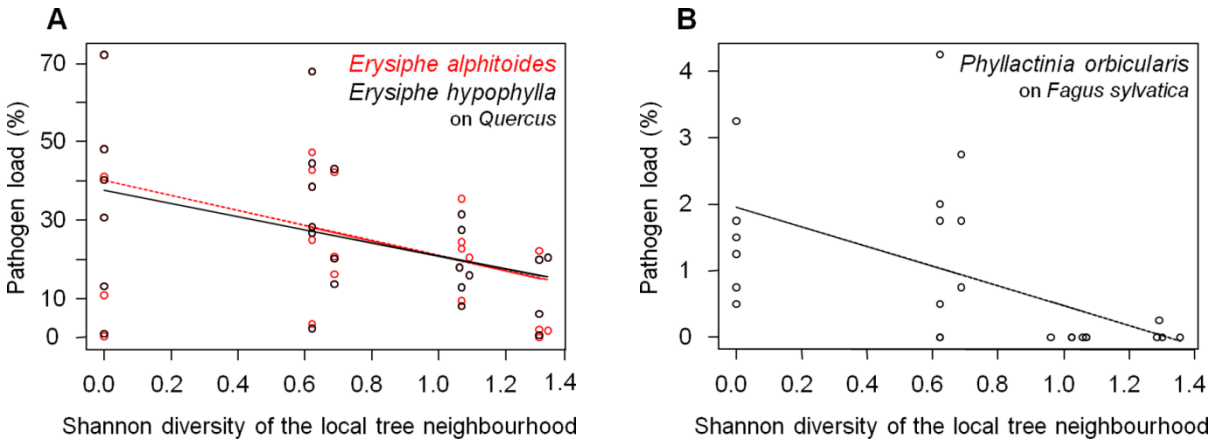

Figure 2-2 Relationship between Shannon diversity of the local tree neighbourhood and pathogen load of A) *Erysiphe alphitoides* (red; $R^2 = 0.288$, $p = 0.054$, $n = 25$) and *E. hypophylla* (black; $R^2 = 0.169$, $p = 0.047$, $n = 25$) on *Quercus* and B) *Phyllactinia orbicularis* on *Fagus sylvatica* ($R^2 = 0.66$, $p = 0.055$, $n = 25$).

As expressed in the second hypothesis, strong tree species identity effects on fungal species attack at the *plot level* were caused by the presence of particular disease-prone tree species. The high host-specificity of foliar biotrophic pathogens is the ecological consequence of the highly specific molecular mechanisms of host-pathogen interactions (Keen 1990; Chisholm et al. 2006). The strong identity effects mainly depended on a positive effect of *Quercus* and a negative one of *Fagus sylvatica*, *Pseudotsuga menziesii* and *Picea abies*. In addition, the susceptibility of *Quercus* to fungal leaf diseases is not easily explained by the species' leaf traits. The leaves of *Q. petraea* as those of *Q. robur* are neither short-lived, nor nutrient rich or poorly defended, as proposed by the host physiological phenotype hypothesis (Cronin et al. 2010). On the contrary, *Q. petraea* leaves have exceptionally high contents of tannin and non-tannin phenolics (Estiarte et al. 2007; Eichenberg et al., under review). Alternatively, the load of host-specific pathogens might be less dependent on the host's traits but on the host species' range sizes and local abundance, as demonstrated by Schuldt et al. (2012) for host-specific herbivory.

In contrast, at the *tree species level* we encountered a negative effect of the tree richness in the local neighbourhood on pathogen load of foliar biotrophic fungi, thus supporting the third hypothesis with focus on the local neighbourhood scale. This effect was even clearer at the *fungus species level*, where tree richness reduced the pathogen load of several powdery mildew species. Such negative biodiversity effects have been described previously for grasslands (Mitchell 2002; Roscher et al. 2007) and also for crops (Zhu et al. 2005), but not yet for forest communities. The negative tree richness effects on pathogen load of foliar biotrophic fungi probably resulted from a dilution effect with increasing tree species richness,

because two trees species carried no pathogens at all, thus including more zeros in the numerator while increasing the denominator.

In contrast to the fourth hypothesis, tree species identity effects were not encountered at the *tree species level* and *fungus species level*. In contrast to the community scale, where species identity effects result from host-specific contributions to the plot's pathogen load and are solely brought about by the presence or absence of disease-prone host tree species, species identity effects at the local neighbourhood scale would only operate through a change in environmental conditions, such as microclimate, or through changes in competitive hierarchies. No such effects were encountered in our study. This implies that the dilution effect brought about by tree richness at the local scale does not depend on the characteristics of the trees in the local neighbourhood. Thus, solely the density of the target tree seems to be the key factor for fungal pathogen load, while the characteristics of the diluting matrix are unimportant. This clearly points to reduced transmission rates of pathogens as the main cause of the observed dilution effect at the local neighbourhood scale (Keesing et al. 2010; Haas et al. 2011).

In conclusion, our study provides clear evidence for biodiversity effects in disease risk and pathogen transmission of specialized biotrophic fungi in temperate forest systems. To our knowledge this is the first time that biodiversity effects were experimentally demonstrated for tree host-foliar fungal pathogen systems on four different tree species. Furthermore, our findings clearly point out the paramount importance of species identity effects at the community scale, while tree species richness effects were only apparent at the local neighbourhood scale. This indicates that these two drivers of ecosystem functioning might operate at different spatial scales, which has not been tested so far.

2.6 Acknowledgements

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2.7 Appendices

Table 2-A1 Linear mixed model results at the *tree species level*. Effect of tree species identity of the local neighbourhood on number of pathogen species and pathogen load (%) for all target trees (n = 100, plot variance of total random variation 22.2 % and 31.8 %, respectively) and for the tree species *Quercus* (plot variance = 8.5 %) and *Fagus sylvatica* (plot variance = 47.1 %) across all tree individuals of each tree species (n = 25).

		Number of pathogen species		**Pathogen load (%)**	
Target species	**Identity effect**	Estimate	p	Estimate	p
All tree species	*Quercus*	0.484	0.060	3.984	0.108
	F. sylvatica	0.023	0.922	-1.411	0 .551
	P. menziesii	-0.3	0.224	-1.006	0.669
	P. abies	-0.207	0.392	-1.552	0.512
Quercus	*F. sylvatica*			-3.425	0.612
	P. menziesii			5.998	0.385
	P. abies			-3.503	0.604
F. sylvatica	*Quercus*			0.033	0.927
	P. menziesii			-0.331	0.371
	P. abies			0.273	0.454

Table 2-A2 Linear mixed model results at the *fungus species level*. Effect of tree species identity of the local neighbourhood on pathogen load (%) of specific fungus species for the tree species Quercus and Fagus sylvatica across all tree individuals of each tree species (n = 25). Plot variance of total random variation: E. alphitoides = 8.5 %, E. hypophylla < 0.1 %, M. album = 3.9 %, Sp. of Ascomycota = 28.5 %, P. orbiculata = 44.0 %, A. errabunda = 21.5 %.

	Pathogen load (%) of fungus species on *Quercus*							
	Erysiphe alphitoides		***Erysiphe hypophylla***		***Microstroma album***		***Species of Ascomycota***	
Identity effect	Estimate	p	Estimate	p	Estimate	p	Estimate	p
F. sylvatica	-2.579	0.714	-2.666	0.708	-0.433	0.624	-0.509	0.289
P. menziesii	5.195	0.468	6.732	0.36	-0.432	0.625	0.174	0.705
P. abies	-3.606	0.61	-4.768	0.509	0.798	0.378	0.29	0.532
	Pathogen load (%) of fungus species on *F. sylvatica*							
	Phyllactinia orbicularis		***Apiognomonia errabunda***					
Identity effect	Estimate	p	Estimate	p				
Quercus	0.052	0.859	-0.031	0.834				
P. menziesii	-0.262	0.382	-0.176	0.261				
P. abies	0.195	0.51	0.19	0.229				

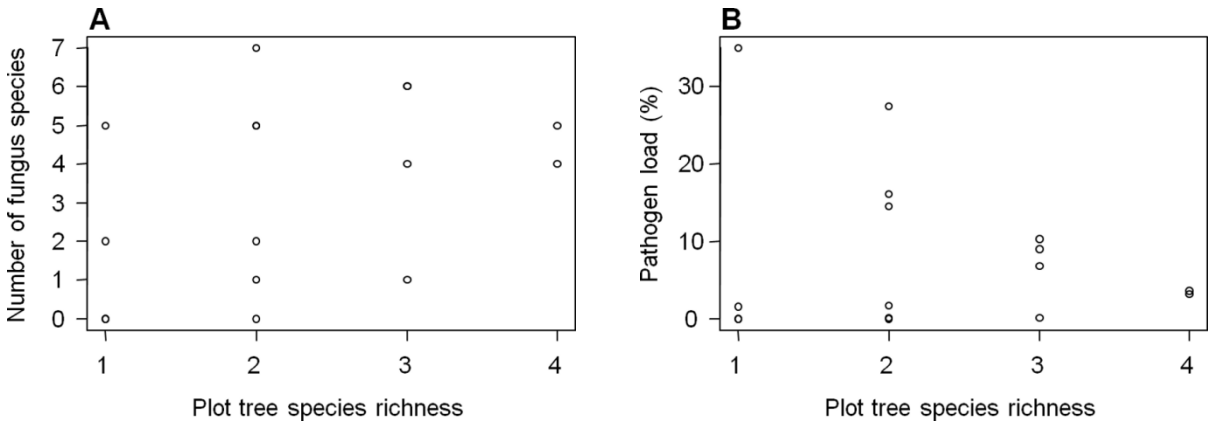

Figure 2-A1 Relationship between plot tree species richness and A) the number of fungus species encountered in the whole plot (R^2 = 0.315, p = 0.117, n = 16) and B) average pathogen load per plot (R^2 = 0.224, p = 0.491, n = 16).

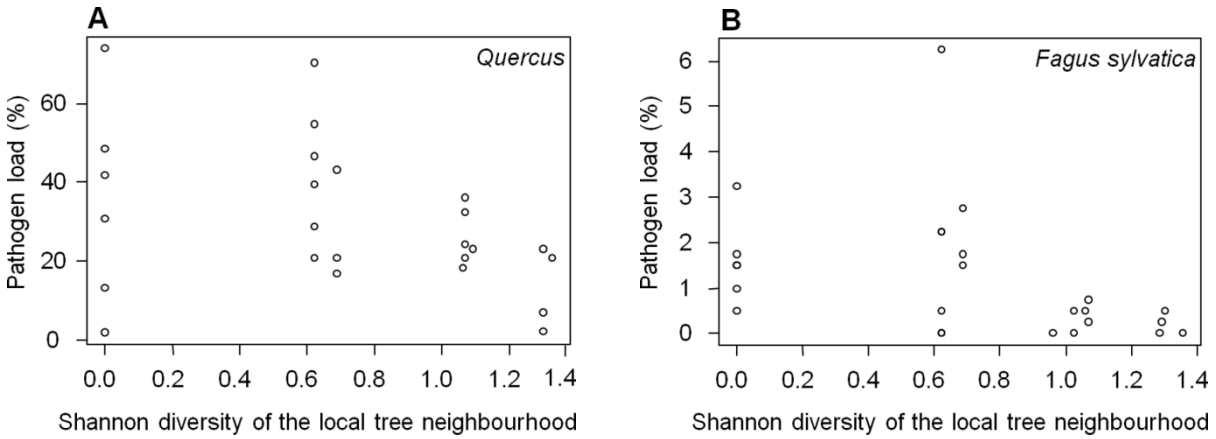

Figure 2-A2 Relationship between Shannon diversity of the local tree neighbourhood and pathogen load on A) *Quercus* (R^2 = 0.252, p = 0.094, n = 25) and B) *Fagus sylvatica* (R^2 = 0.644, p = 0.136, n = 25).

3. EFFECTS OF TREE DIVERSITY, HOST AND NON-HOST SPECIES PROPORTION ON FOLIAR FUNGAL PATHOGENS

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Journal of Ecology (under review)

3.1 Abstract

The degree of plant pathogen infestation of a host plant is expected to be strongly influenced by plant species diversity in the local neighbourhood. Species diversity might operate through local proportion of the host species as suggested by the resource hypothesis as well as through presence or proportion of particular non-host plant species in the local neighbourhood. Since pathogen infestation may affect host performance negatively, it can mediate the effects of local biodiversity on ecosystem functioning and stability. We tested the effect of tree diversity, proportion of host species and of non-host species in the local tree neighbourhood on foliar fungal pathogens of *Tilia cordata* and *Quercus petraea* by using the Kreinitz tree diversity experiment in Germany. We hypothesized that mean pathogen richness increased and mean fungal infestation decreased with increasing tree diversity and decreasing host proportion, and in addition, depend on presence and proportion of particular non-host species. We collected leaves of the target species in three subsequent years and macro- and microscopically identified all foliar fungal pathogens that were visible on the leaf surface. Diversity effects of the local neighbourhood were assessed for fungal species richness and total fungal infestation on host trees as well as for infestation by individual fungal species. High tree diversity reduced the total fungal species richness and infestation of *T. cordata* and fungal infestation of *Q. petraea* as well as the infestation of three host-specialized fungal pathogen species. These effects on pathogen infestation were brought about by local tree diversity and were independent of host species proportion. In general, host species proportion had almost no effects on fungal species richness and infestation. However, we found strong proportion effects of particular non-host neighbour tree species on fungal species richness and infestation on the two host tree species as well as on the infestation by particular fungal species. For the first time, we experimentally showed for two common forestry tree species that foliar fungal pathogen richness and infestation depend on local neighbourhood biodiversity. Thus, local tree diversity can increase forest ecosystem functioning through decreased infestation by fungal pathogens.

Keywords
biodiversity and ecosystem functioning, disease dilution effect, fungal pathogen richness and infestation, Kreinitz experiment, local neighbourhood, neighbour species identity effects, *Quercus petraea*, Shannon diversity effects, *Tilia cordata*

3.2 Introduction

Anthropogenic global change is the most important driver of declining biodiversity, causing dramatic and irreversible alterations in the Earth's natural ecosystems (Knops et al. 1999; Chapin III et al. 2000; Keesing et al. 2010). The loss of species has been shown to be important for fundamental ecosystem processes and overall system performance (Naeem et al. 1994; Chapin III et al. 2000). Moreover, ecosystem stability is crucially depending on biodiversity (for the diversity-stability relationship see McCann 2000; Proulx et al. 2010) because a larger diversity should buffer ecosystems against environmental variation (Cadotte et al. 2012). In addition, a reduction in plant species richness increases ecosystem vulnerability to invasions, alters herbivore communities and enhances the spread of fungal plant diseases (Knops et al. 1999; Pautasso et al. 2005).

Many biodiversity-ecosystem functioning (BEF) experiments have shown increasing productivity with increasing biodiversity and similarly positive effects for other ecosystem functions studied (e.g. Balvanera et al. 2006; Bezemer & van der Putten 2007; Vilà et al. 2007; Potvin & Dutilleul 2009; Reich et al. 2012). However, only few BEF experiments addressed host-pathogen interactions (e.g. Roscher et al. 2007; Latz et al. 2012), although relationships across different trophic levels are considered crucial to our understanding of biodiversity functioning (Duffy et al. 2007; Mordecai 2013). Under the assumption, that a more diverse host community provides a wider range of resources for higher trophic levels, a simultaneous increase of plant and consumer richness would be expected (Koricheva et al. 2006; Scherber et al. 2010; Castagneyrol & Jactel 2012), but have not yet been demonstrated for fungal species richness. For instance, in contrast to monocultures, higher herbivore richness was demonstrated with increasing plant species richness in grassland communities (e.g. Mulder et al. 1999; De Deyn et al. 2004) as well as in forests (Jactel & Brokerhoff 2007; Vehvilainen et al. 2007; Schuldt et al. 2010). In contrast, the degree of pathogen infestation may depend on host availability, i.e. on host frequency and on local host proportion. As pathogens reduce the hosts' growth and fitness by decreasing transpiration and photosynthesis rates (Hajji et al. 2009), pathogens ensue further negative effects for the host, as not only host

performance is reduced but also plant competiveness. As an outcome, plant species coexistence can be enhanced if stronger competitors are more affected by pathogens than competitively inferior species (Janzen-Connell effects; Mordecai 2011, 2013). Such pathogen mediated negative feedbacks have been demonstrated by many recent studies that showed increased productivity of plant species mixtures as compared to monocultures (Maron et al. 2011; Schnitzer et al. 2011; Hendriks et al. 2013). Particularly reductions in pathogen infection (disease risk) and infestation (disease severity) have been described from high diversity communities in grassland-experiments (Mitchell et al. 2002; Mitchell et al. 2003; Zhu et al. 2005; Roscher et al. 2007), but have also been reviewed for forests differing in tree diversity (Pautasso et al. 2005).

Whether diversity of the producers results in an increase or decrease in species richness and infestation of higher trophic levels also depends on the degree of host specialization. With increasing host species diversity, species richness and infestation by generalized consumers are expected to increase while occurrence of specialized consumers should decrease (Koricheva et al. 2006; Jactel & Brokerhoff 2007; Sobek et al. 2009; Schuldt et al. 2011). Specialized herbivores are expected to cause less disease severity with increasing host species diversity because host plant densities decrease, which translates into reduced resource concentration (Root 1973; Schuldt et al. 2010; Figure 3-1A). Such patterns have been shown for several interactions of plants and specialized herbivores (e.g. Otway et al. 2005; Unsicker et al. 2006). The same pattern would be expected for plant-fungal pathogen relationships, as most fungal pathogens are highly specialized on one host species (Prell 1996), and thus, exclusively depend on the availability of this specific resource. Reduced resource availability, i.e. a decrease in host proportion, might be the key mechanism of how biodiversity reduces fungal infestation rates. The higher the diversity of plants that co-occur with the host plant, the lower the probability that pathogens encounter host plants (Burdon & Chilvers 1982; Bell et al. 2006; Garcia-Guzman & Dirzo 2006; Sobek et al. 2009). For instance, Mundt et al. (2011) showed a suppressed epidemic spread of disease by a decrease in host frequency independent from plant species diversity. In addition, in Californian coastal forests, pathogen transmission was reduced and tanoak killing was hampered by lower densities of tanoaks in mixtures with non-host neighbouring species (Cobb et al. 2012). Furthermore, sudden oak death decreased with lower host stem density (Meentemeyer et al. 2008). In contrast, enhanced abundance of particular host species increased disease transmission in grasslands (Mitchell et al. 2002). In consequence, disease severity increases with resource concentration,

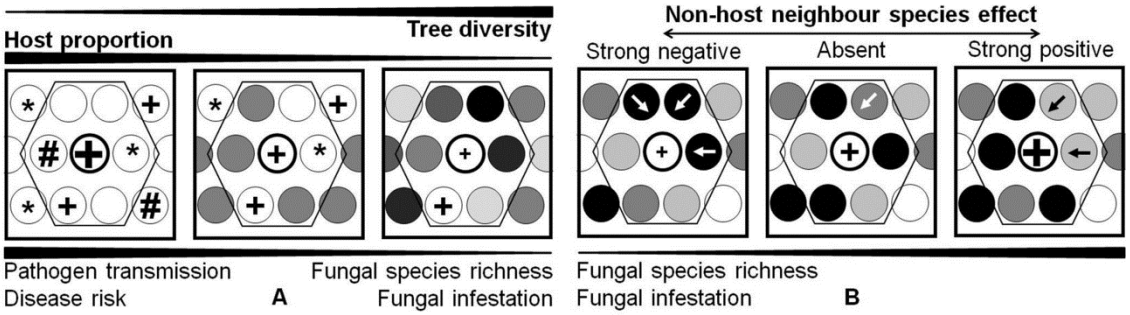

Figure 3-1 Graphical illustration of how tree diversity of the local neighbourhood can affect fungal pathogen richness and fungal pathogen infestation at a local neighbourhood scale in experimental tree communities of constant individual density. Individuals are represented by circles, with different shades of grey being different species. Target individuals are located in the centre marked by a thick black outline and the local neighbour individuals are those within the hexagon. The symbols in the circles represent pathogens, with different symbols indicating different fungal pathogen species and different size of symbols showing a different degree of fungal pathogen infestation. Circles with arrows indicate that the neighbour species changes the local environment of a target species. A) Fungal species richness of specialized pathogens and their average fungal infestation of a target individual are expected to decrease with increasing tree species diversity. One reason for these effects is the reduction of pathogen transmission among neighbour individuals in communities of high tree diversity because of larger distances between potential host individuals. Dilution of host individuals is attended by a reduction of the host proportion with increasing tree species diversity and leads to a reduction of the overall disease risk of a tree species. (B) Local non-host neighbour species proportions effects, at a given neighbour species richness, might either increase or decrease fungal species richness and fungal infestation of a target individual. These effects could either depend on the presence and proportion of a particular neighbour species and whether the tree species changes the environmental conditions in a favourable or unfavourable manner for the pathogen. The first case would be a neighbour-species mediated facilitation of the pathogen species, indicating an increase in fungal infestation. The latter a neighbour-species mediated facilitation of the host species, resulting in a decrease of fungal infestation by the particular non-host neighbour species.

being at maximum in mono-specific stands with highest host proportion (Figure 3-1A; Root 1973; Otway et al. 2005; Johnson et al. 2012).

In addition, many studies have encountered strong species identity effects, which were often more pronounced than species diversity or host proportion effects (De Deyn et al. 2004; Sobek et al. 2009; Nadrowski et al. 2010). Such identity effects can occur with regard to pathogen infestation, since particular non-host species in the neighbourhood of a target host plant might increase or decrease the pathogen infestation (Figure 3-1B). Neighbour-mediated facilitation has been demonstrated, for instance, by sowing non-host plant species in grassland diversity experiments, which resulted in a decreased pathogen infestation (Mitchell et al. 2002). Such neighbourhood effects might work through modification of sun exposure, local microclimate or soil conditions (Bahnweg et al. 2008). Such indirect effects might be subsumed under the associational resistance hypothesis (Tahvanainen & Root 1972), which proposes that a diverse host community might reduce pathogen infestation due to a higher

structural heterogeneity. Thus, more heterogeneous microclimates within such communities force individuals to adjust to different temperature, light and moisture regimes (Tahvanainen & Root 1972). In addition, microclimatic properties are fundamental for fungal pathogen development since growth of mycelia, sexual and asexual reproduction and spread of spores depend on critical temperatures and air humidity (Tainter & Baker 1996). Thus, disease risk of a plant community will also depend on microclimatic complexity. In the case of trees, microclimatic complexity is enhanced by varying canopy sizes and crown structures of neighbour plant species in a particular neighbourhood composition (Jactel et al. 2009; Calonnec et al. 2013). As neighbour size is one of the most influential trait (Potvin & Dutilleul 2009; Castagneyrol et al. 2013), for instance by influencing microclimate (Bourke 1970), it is important to compare neighbourhoods that are as evenly structured as possible, e.g. by comparing trees of equal age.

Moreover, adding abiotic environmental effects to the key relationship between pathogen and host results in a fundamental triangle where all bivariate relationships are modified by the third variable (Bourke 1970; Warren & Mordecai 2010). On the one hand, favourable conditions, such as temporary high humidity (Bourke 1970; Laneri et al. 2010) and warm temperature (Tainter & Baker 1996; Gutknecht et al. 2012), drive pathogen development, reproduction and persistence (Warren & Mordecai 2010). On the other hand, climatic extremes (e.g. winter frost, hail, summer drought, storms) might damage plant individuals by wounding, and thus, facilitating pathogen infection directly (i.e. increased disease spread) or indirectly (Bourke 1970; Thomas et al. 2002; Bock et al. 2010). In consequence, pathogen species richness and infestation vary with time, depending on the climatic conditions within season and between seasons (Root 1973; Jarosz & Burdon 1992; Lappalainen et al. 1999; Laneri et al. 2010). However, the degree of variation might also depend on host diversity, as there are some hints that temporal ecosystem stability increases with plant diversity (Tilman 1996; Tilman et al. 2006; Eisenhauer et al. 2011). However, so far a lower variation of pathogen infestation with increasing host diversity has never been shown in BEF experiments.

We used one of the European BEF tree diversity experiments, the Kreinitz experiment in Germany, to analyze the influence of tree diversity, proportion and species identity of non-host species, as well as inter-annual variation in weather conditions on foliar fungal species richness and fungal infestation. We focused on two tree species (*Quercus petraea* and *Tilia cordata*), which are both known for their susceptibility to foliar fungal pathogens (Heuser & Zimmer 2002; Gonthier et al. 2006; Roslin et al. 2007; Cobb et al. 2010; Deszprez-Loustau et al. 2010; Tack et al. 2012). Using a macroscopic and microscopic analysis, all foliar fungal

pathogens on the upper and lower leaf surface were identified over three subsequent years (2010-2012). Here, we tested the following hypotheses separately for each of the two tree species: 1) Increasing tree diversity increases fungal species richness and decreases fungal infestation and 2) increasing host proportion increases fungal infestation. 3) Fungal species richness and fungal infestation depend on the presence and proportion of particular non-host species. 4) Inter-annual variation in fungal species richness and fungal infestation decreases with increasing tree diversity.

3.3 Material and Methods

Study site

The tree diversity experiment in Kreinitz was established in 2005 with the aim to analyse above-below-ground links in the biodiversity-ecosystem functioning relationship. The experimental site is located in Saxony (51°23'10" N, 13°15'43" E) and was established on a former arable field. Elevation is about 110-120 m a.s.l., the soils are weakly acidic (pH 4.6-6.3) with sandy soil texture. Out of a species pool of six tree species (*Fagus sylvatica*, *Fraxinus excelsior*, *Picea abies*, *Pinus sylvestris*, *Quercus petraea*, *Tilia cordata*), 49 different communities were created, comprising bare ground (n = 1 plot), monocultures of all species (n = 6), all possible 2-species mixtures (n = 15), all possible 3-species mixtures (n = 20), all possible 5-species mixtures (n = 6) and the 6-species mixture (n = 1) (Figure 3-2A). Exactly the same communities were replicated once, resulting in a total of 98 plots, each with 49 different species compositions in either of the two blocks. Plot size was 25 m² (5 m * 5 m) containing in total 30 tree individuals of the respective tree species mixture. Tree species were planted randomly in five rows with a between-row distance of 1 m and a within-row distance of 0.8 m (Figure 3-2B). Dead trees were replaced during the first two years. Site management included mowing between the plots as well as weeding by using the herbicide Roundup® during the first 4 years after establishment of the experiment.

Study species and leaf sampling

For foliar fungal analysis, *Q. petraea* and *T. cordata* tree individuals were selected randomly in all plots that contained at least one of the two study species, choosing locations in the core area of a plot (see dotted line within the plot, Figure 3-2B). The outermost row of trees in a plot was not sampled to avoid edge effects, and in addition, to guarantee the presence of all six potential tree individuals in the local neighbourhood of the target trees. However, in some

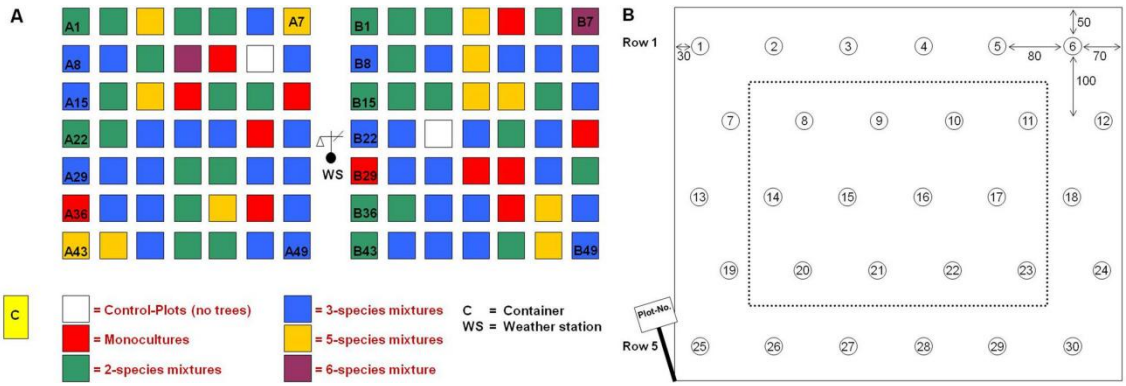

Figure 3-2 The experimental design of the tree diversity experiment in Kreinitz. (A) Different tree species mixtures (different colours) were arranged randomly within 49 plots and exact mixtures repeated by the two blocks (Block A, B). (B) General plot design includes 30 tree individuals arranged within five rows, though equal number of individuals per tree species depends on tree species mixtures, but tree individuals were randomly distributed. Note the core area (dotted line) within the plot.

rare cases we had to sample edge individuals when potential core individuals had died. We sampled six individuals in each of the monocultures, three individuals per species in each of the 2-species mixture plots, two individuals per species in each of the 3-species mixture plots and one individual in each of the 5- and 6-species mixture plots. This sampling was repeated over three years (2010-2012), resulting in 282 trees (94 tree individuals * 3 years) for each of the study species. On each of the total of 564 individuals two branches exposed to opposite directions in the upper as well as in the lower part of the crown were selected and five leaves per branch were collected. The leaves were dried immediately after sampling at 60°C for three days and then stored in the dark at room temperature.

Macro- and microscopic analyses

For macro- and microscopic analyses we used a random subset of 10 leaves per tree individual. We identified taxa of fungal pathogens on the leaf surface to the species level using light microscopy (after Brandenburger 1985; Ellis & Ellis 1997; Braun & Cook 2012). Endophytic fungi were not included in this micro- and macroscopic approach, neither were all visible epiphytic and saprophytic fungi. Depending on the fungal developmental stage, identification was not always possible at the species level. Because of missing fructification, we assigned a few fungi only to the genus level (*Zygosporium* sp. and *Ramichloridium* sp.) or phylum level (Ascomycota). Furthermore, we quantified pathogen infestation of fungal taxa on the upper and lower surface of leaves by using stereomicroscope analyses. The total percentage of leaf area infested by each fungal pathogen species was visually estimated and rated on a scale with seven damage classes: 0 %, 1-5 %, 6-10 %, 11-25 %, 26-50 %, 51-75 % and 76-100 % (Schuldt et al. 2012; Hantsch et al. 2013). Fungal infestation per leaf was

calculated from summing up the infestations of all fungal species per leaf. Fungal infestation per individual was calculated by averaging fungal infestation of all analyzed leaves of this individual.

Weather conditions

The tree diversity experiment in Kreinitz included a residential meteorological station (see Figure 3-2A), which measured temperature and precipitation in 1 hour intervals. The data showed that 2010 was the coldest and most humid year (mean temperature = 9.85°C, precipitation = 881.71 mm), whereas 2011 and 2012 featured warmer and drier local weather conditions (mean temperature = 11.65°C/11.41°C, precipitation = 657.11 mm/559.47 mm). We then used those variables as predictors for fungal species richness and fungal infestation in the different years.

Data analysis

Both fungal species richness and fungal infestation were related to tree diversity (Shannon diversity), proportion of the host species (of the six neighbour individuals) and to proportion of non-host species in the local tree neighbourhood (of the six neighbour individuals). Tree diversity, host proportion and non-host species proportion were calculated from all six trees in the local neighbourhood around the target tree, but excluding the target tree and were based on basal areas of neighbour individuals as a proxy for neighbour tree size (Figure 3-1B). Thus, Shannon tree diversity varied between 0 and 1.561, host species proportion between 0.167 and 1 and non-host species proportion between 0 and 1. Since individuals within plots were planted randomly, the exact composition of the local neighbourhood of each focal tree individual might differ even in the same tree species mixtures. This also means that the two blocks were not exact replicates of specific neighbourhood compositions. Effects were assessed at two different hierarchical levels. At the *tree species level,* fungal species richness and fungal infestation were analyzed as an effect of tree diversity separately by tree species. In a parallel approach, effects of host species proportions and non-host species proportions were used instead of tree diversity to predict fungal species richness and fungal infestation. A significant effect of the proportion of the host species would indicate that potential tree diversity effects operate through dilution of the host species, whereas an effect of a particular non-host tree species would indicate an operation through environmental changes by the presence of a particular neighbour tree species. At the *fungus species level*, fungal infestation was evaluated separately for every fungal taxon, using the same models as above but separately by fungus species. All calculations were done with linear mixed effects models

(*lme*; package nlme; Pinheiro 2013), including block and plot nested in block as random factors and using a Gaussian error distribution (R Core Team 2013). The *varcomp* procedure (package ape) was used to assess the proportion of total random variation explained by plot. The amount of variation explained by the total model was obtained from regression predicted against observed responses. All figures were produced with R.

3.4 Results

In total, we encountered four and five foliar fungal pathogen species on *Tilia cordata* and *Quercus petraea*, respectively. *Tilia cordata* was host of the most abundant fungal pathogen *Passalora microsora* (Sacc.) U. Braun and the less abundant pathogen *Asteromella tiliae* (F. Rudolphi) Butin & Kehr, which covered both often more than a tenth of the leaf surface. *Tilia cordata* was also host of a frequent but small unknown ascomycete (identification not possible due to lacking fructification) and the rare fungus *Apiognomonia errabunda* (Roberge) Höhn. *Quercus petraea* was host of the most frequent fungal pathogens *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam. and *Erysiphe hypophylla* (Nevod.) U. Braun & Cunningt., which are both powdery mildew species and covered often more than one-tenth of the leaf surface. Two other, but less frequent, fungal pathogens belonged to the genera of *Zygosporium* Mont. and *Ramichloridium* Stahel ex de Hoog, respectively. In addition, we identified also an unknown small ascomycete on leaves of *Q. petraea* (fructification insufficient for a final determination).

At the *tree species level*, for *T. cordata*, fungal species richness and overall fungal infestation significantly declined with increasing tree diversity of the local neighbourhood (Figure 3-3A, B, Table 3-1). Fungal species richness of *T. cordata* was similar over the years. A significant interaction of tree diversity and year indicated a stronger decrease in fungal species richness with increasing tree diversity in 2012 than in 2011 or 2010 (Figure 3-3A, Table 3-1). In contrast, fungal infestation was generally higher in 2010 than in 2011 or 2012. There were no main effects of host species proportion of the local neighbourhood on fungal species richness and on fungal infestation (Table 3-2). However, fungal species richness showed a significant interaction between host proportion and year, since fungal species richness increased with increasing host proportion in 2012, but decreased in 2010 and 2011 (Table 3-2). Among all analyses of proportion effects of hetero-specific local non-host species, there were two significant proportion effects for fungal infestation (Table 3-2). Thus, overall fungal infestation on *T. cordata* increased with increasing proportion of *F. sylvatica*, but decreased

with increasing proportion of *P. sylvestris* in the local neighbourhood (Figure 3-4A, B, Table 3-2). Furthermore, there were some interactions between non-host species proportion and years, indicating a reduction in fungal infestation with increasing proportion of *P. abies*, but only in the years 2011 and 2012. In addition, fungal species richness decreased with increasing proportion of *Pinus sylvestris* in the years 2011 and 2012, but increased in 2010 (Table 3-2).

Tree diversity of the local neighbourhood had no effect on fungal species richness, but reduced also fungal infestation of the second study species *Q. petraea* (Figure 3-3C, D, Table 3-1). In addition, there were significant differences in fungal species richness and fungal infestation between the years, with generally lower values in 2010 than in the other years (Figure 3-3C, D, Table 3-1). Again, there was no host species proportion effect of the local neighbourhood on fungal pathogens (Table 3-2). There were two main effects of the proportion of non-host neighbour species on fungal infestation (Table 3-2). Thus, high proportion of *F. excelsior* enhanced fungal infestation (Figure 3-4C, Table 3-2), while high

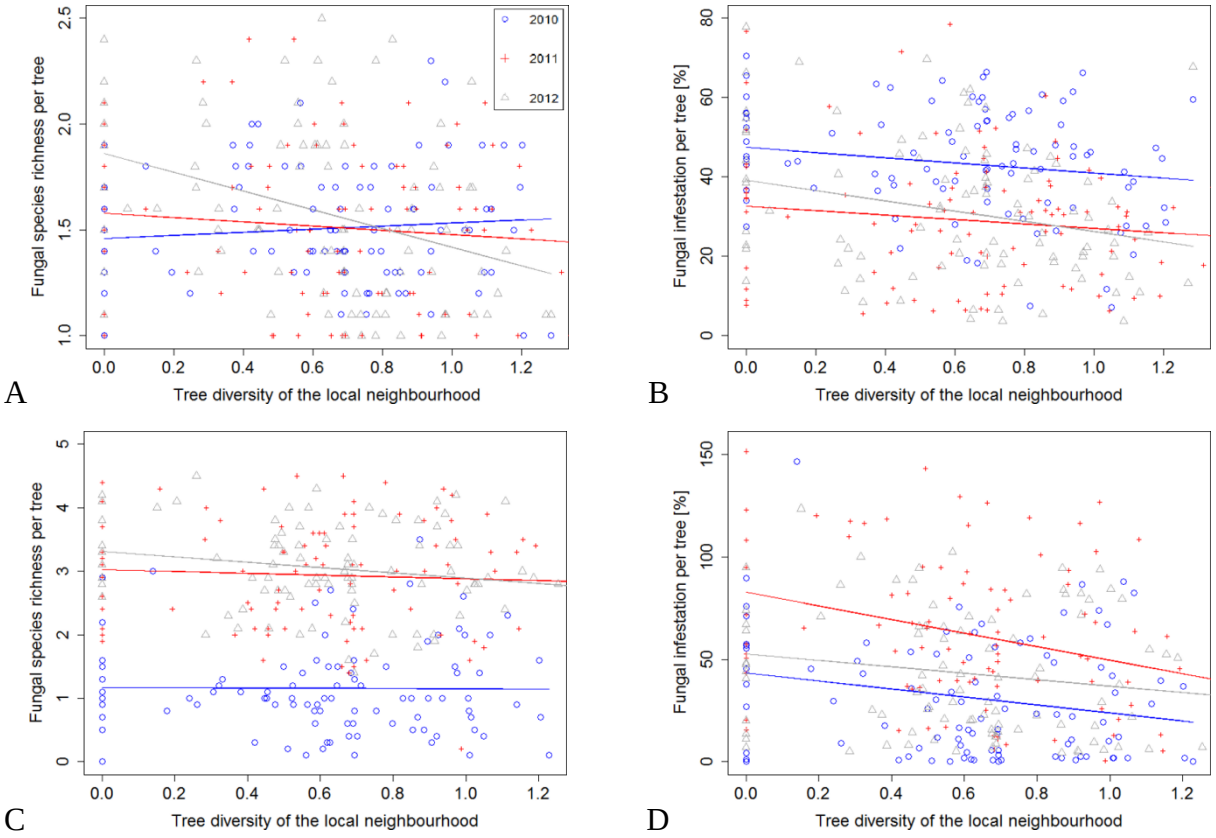

Figure 3-3 The relationship between tree diversity of the local tree neighbourhood and fungal species richness (A, C) and the overall fungal infestation (B, D) of *Tilia cordata* (A: $R^2 = 0.277$, $p = 0.045$; B: $R^2 = 0.475$, $p = 0.046$) and *Quercus petraea* (C: $R^2 = 0.705$, $p = 0.102$; D: $R^2 = 0.417$, $p = 0.004$) individuals, respectively, in the years 2010-2012. Tree diversity is quantified as Shannon diversity, with zero indicating a monoculture. n = 282. Fungal species richness and fungal infestation were averaged over all leaves per tree individual.

Table 3-1 Linear mixed effect model results at the *tree species level.* Effect of tree diversity of the local tree neighbourhood on fungal species richness and fungal infestation for the tree species *Tilia cordata* and *Quercus petraea* across all tree individuals of each tree species (n = 282) within the years 2010-2012. DF = 233. Significant results are indicated in bold fonts. Variance of the random factors block and plot, respectively: Fungal species richness *T. cordata* = 2.9 % and 12.1%, *Q. petraea* = 2.5 % and 25.6 %; Fungal infestation *T. cordata* = <0.1 % and 27.4 %, *Q. petraea* = <0.1 % and 22.5 %.

		Tilia cordata		***Quercus petraea***	
Response variable	**Explanatory variable**	F	p	F	p
Fungal species richness	Intercept	922.426	<0.001	417.423	<0.001
	Tree Diversity (TD)	4.055	0.045	2.69	0.102
	Year	1.761	0.174	233.196	<0.001
	TD x Year	7.562	0.001	0.954	0.387
Fungal infestation	Intercept	507.3	<0.001	220.135	<0.001
	Tree Diversity (TD)	4.03	0.046	8.389	0.004
	Year	31.429	<0.001	28.296	<0.001
	TD x Year	1.01	0.366	1.075	0.343

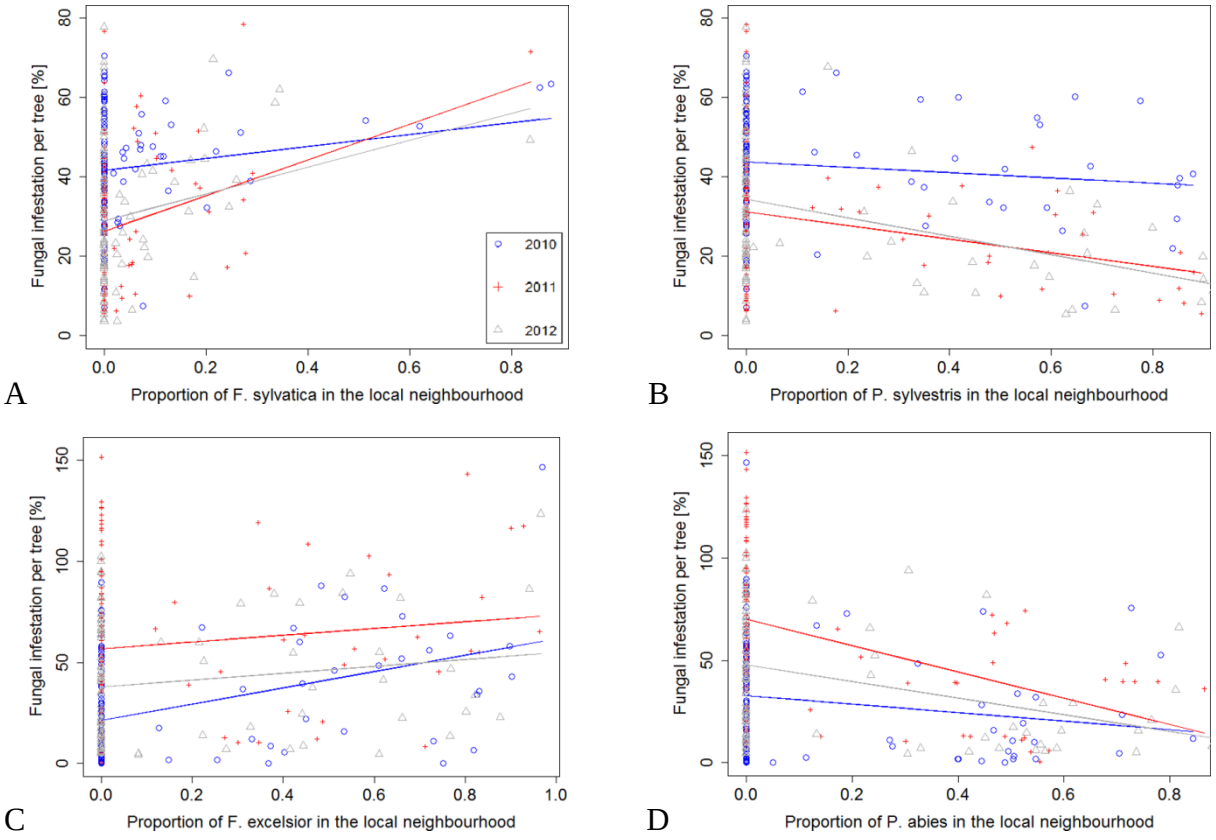

Figure 3-4 The relationship between non-host neighbour proportion (A: *Fagus sylvatica*, B: *Pinus sylvestris*, C: *Fraxinus excelsior*, D: *Picea abies*) of the local tree neighbourhood and the overall fungal infestation of *Tilia cordata* (A: $R^2 = 0.482$, p = 0.001; B: $R^2 = 0.487$, p = 0.001) and *Quercus petraea* (C: $R^2 = 0.421$, p = 0.011; D: $R^2 = 0.391$, p < 0.001) individuals, respectively, in the years 2010-2012. Non-host proportion was calculated as proportion of the six neighbour individuals, with zero indicating the absence of *F. sylvatica* in the neighbourhood. n = 282. Fungal infestation was averaged over all leaves per tree individual.

Table 3-2 Linear mixed effects model results at the *tree species level.* Effect of tree species proportions of the local tree neighbourhood on fungal species richness and fungal infestation for the tree species *Tilia cordata* and *Quercus petraea* across all tree individuals of each tree species (n = 282) within the years 2010-2012. DF = 233. Significant results are indicated in bold fonts. Host species proportions are local neighbourhoods conspecific to the target tree and marked in grey. Proportion = P., *Fagus sylvatica* = Fs., *Fraxinus excelsior* = Fe., *Picea abies* = Pa., *Pinus sylvestris* = Ps., *Quercus petraea* = Qp., *Tilia cordata* = Tc.

Proportion of neighbour tree species	***Tilia cordata*** **Fungal species richness**		**Fungal infestation**		***Quercus petraea*** **Fungal species richness**		**Fungal infestation**	
	F	P	F	p	F	p	F	p
Intercept	797.88	<0.001	532.47	<0.001	53.834	<0.001	203.494	<0.001
P.Fs.	0.636	0.426	12.395	0.001	1.101	0.295	1.133	0.288
Year	1.883	0.154	29.791	<0.001	143.74	<0.001	27.796	<0.001
P.Fs. x Year	0.323	0.725	2.049	0.131	0.098	0.907	0.205	0.815
Intercept	834.44	<0.001	434.443	<0.001	350.911	<0.001	218.018	<0.001
P.Fe.	0.054	0.817	0.779	0.378	0.739	0.391	6.647	0.011
Year	1.867	0.157	31.483	<0.001	156.14	<0.001	29.096	<0.001
P.Fe. x Year	0.849	0.429	2.169	0.117	10.085	<0.001	1.775	0.172
Intercept	780.496	<0.001	452.118	<0.001	370.838	<0.001	308.425	<0.001
P.Pa.	0.456	0.5	1.184	0.277	2.148	0.144	18.071	<0.001
Year	1.808	0.166	32.153	<0.001	145.986	<0.001	28.313	<0.001
P.Pa. x Year	0.027	0.973	3.235	0.041	2.219	0.111	3.055	0.049
Intercept	781.01	<0.001	545.266	<0.001	356.56	<0.001	204.174	<0.001
P.Ps.	0.826	0.365	11.849	0.001	0.484	0.487	0.748	0.388
Year	1.945	0.145	31.615	<0.001	147.143	<0.001	29.219	<0.001
P.Ps. x Year	6.242	0.002	2.757	0.066	2.923	0.056	4.474	0.012
Intercept	793.674	<0.001	439.613	<0.001	357.114	<0.001	200.31	<0.001
P.Qp.	0.087	0.769	0.018	0.893	0.193	0.661	0.382	0.537
Year	1.824	0.164	31.609	<0.001	151.181	<0.001	28.227	<0.001
P.Qp. x Year	1.039	0.356	2.105	0.124	6.157	0.003	1.914	0.15
Intercept	814.632	<0.001	523.984	<0.001	356.29	<0.001	205.925	<0.001
P.Tc.	0.332	0.565	2.423	0.121	0.261	0.61	1.391	0.24
Year	1.867	0.157	31.746	<0.001	144.247	<0.001	28.243	<0.001
P.Tc. x Year	5.864	0.003	0.962	0.384	0.234	0.792	1.065	0.346

proportion of *P. abies* reduced fungal infestation (Figure 3-4D, Table 3-2) on *Q. petraea* in all years. In contrast to the patterns observed on *T. cordata*, there were also positive non-host proportion effects on *Q. petraea*. Moreover, a significant interaction indicated that increasing proportion of *F. excelsior* enhanced fungal infestation in 2011 and 2012, but reduced fungal infestation in 2010 (Table 3-2).

At the *fungus species level*, we analysed tree diversity, host and non-host proportion effects separately by fungal and tree species. Among the fungal species on *T. cordata P. microsora* and the unknown ascomycete showed a (marginal) response to tree diversity of the local neighbourhood (Table 3-3). Fungal infestation of *P. microsora* decreased with increasing tree

diversity in all study years. Fungal infestation was significantly higher in 2010 than in 2011 and 2012. Similarly, fungal infestation of the unknown ascomycete decreased with increasing tree diversity, but only in 2011 and 2012, indicated by a significant interaction of tree diversity and year (Table 3-3). There was also an interaction between tree diversity and year for the infestation by *A. tiliae*, resulting in a decrease of fungal infestation with increasing tree diversity in 2012, but an increase in 2010 and 2011 (Table 3-3). Similar to the *tree species level*, there was no evidence that the infestation of any of the fungal pathogen species depended on host species proportion of the local neighbourhood (Table 3-4). However, infestation of the unknown ascomycete showed a significant interaction, indicating an increase with increasing host proportion in 2011 and 2012, but a reduction in 2010 (Table 3-4). Furthermore, there were several non-host species effects on particular fungi on *T. cordata*. Thus, fungal infestation of *P. microsora* increased with increasing proportion of *F. sylvatica*, but decreased with proportion of *P. sylvestris* (Table 3-4). A significant interaction between proportion of *P. abies* and year revealed that infestation only decreased with increasing proportion of *P. abies* in 2011 and 2012, but not in 2010 (Table 3-4). In addition, the fungal infestation of *A. errabunda* increased with the proportion of *F. excelsior* in the local neighbourhood (Table 3-4). Furthermore, there were significant interactions between the proportion of *P. sylvestris* and year, indicating an increase of infestation of *A. tiliae* in 2010 and 2011 and of the unknown ascomycete in 2010, but a reduction of infestation in 2012, and 2011 and 2012, respectively, with increasing proportion of *P. sylvestris* (Table 3-4).

Among all fungal species that occurred on *Q. petraea* the infestation by *E. hypophylla* was significantly affected by tree diversity of the local neighbourhood (Table 3-3). However, fungal infestation of all species differed significantly between the years with general lower infestation in 2010 than in 2011 or 2012 (Table 3-3). Similar to the effects observed on *T. cordata*, host species proportion of the local neighbourhood had no main effect on the infestation of any fungal pathogen species. However, a significant interaction between host proportion and year showed an increase in fungal infestation of the unknown ascomycete with increasing host proportion in 2012, while there was a reduction in 2010 and 2011 (Table 3-4). Again, there were several non-host species effects on particular fungi on *Q. petraea*. Thus, infestation of *E. alphitoides* decreased with increasing proportion of both *P. abies* and *T. cordata* in the local neighbourhood, but increased with proportion of *F. excelsior* (Table 3-4). In addition, a significant interaction showed that infestation of this fungus also increased with increasing proportion of *P. sylvestris* in the years 2011 and 2012, but decreased in 2010 (Table 3-4). Similar to *E. alphitoides*, fungal infestation of *E. hypophylla* decreased with

increasing proportion of *P. abies* in the local neighbourhood (Table 3-4). Furthermore, fungal infestation of the unknown ascomycete on *Q. petraea* showed a significant interaction between proportion of *F. excelsior* and year, indicating an increase in fungal infestation with increasing proportion of *F. excelsior* in the local neighbourhood in 2010 and 2011, but a reduction in 2012 (Table 3-4). In addition, the fungal infestation of *Ramichloridium* sp. increased with increasing proportion of *F. sylvatica* or *F. excelsior*, but decreased with increasing proportion either of *P. sylvestris* or *T. cordata* (Table 3-4).

3.5 Discussion

We showed for two common tree species that fungal pathogen species richness and fungal pathogen infestation depended on biodiversity of the local tree neighbourhood and on local weather conditions. Increasing local tree diversity resulted in decreasing fungal species richness and infestation on *Tilia cordata* in general and in decreasing infestation of the main fungal species *Passalora microsora* and an unknown ascomycete in particular. Although there were no local tree diversity effects on fungal species richness of *Quercus petraea*, there were effects on overall fungal infestation and in particular on infestation of one of the main fungal pathogens on oak, *Erysiphe hypophylla*. An important result was that these effects mainly relied on tree diversity or on the proportion of non-host species in the local neighbourhood and not predominantly on the proportion of the host, and thus, in most cases were not simple dilution effects by decreasing host proportion.

Tree diversity effects

While we found no evidence for the first hypothesis that fungal species richness increases with increasing tree diversity, we could confirm the second half of this hypothesis. Fungal infestation on both *T. cordata* and *Q. petraea* decreased with increasing tree diversity. In addition, we also expected to find a reduction in fungal infestation of single fungal species since the majority of biotrophic fungi exhibits a narrow host spectrum (e.g. Takamatsu et al. 2006), and thus, could not benefit from higher tree species mixtures. We could confirm this expectation as we encountered a reduction of fungal infestation of three different fungi with increasing tree diversity. However, one of the fungi in our study, *Apiognomonia errabunda*, may have benefitted from the presence of *T. cordata*, *Q. petraea* and *Fagus sylvatica* because all three tree species shared this pathogen (e.g. Sogonov et al. 2007). The results of tree diversity on the degree of fungal infestation of *Q. petraea* and *T. cordata* confirmed the

Table 3-3 Linear mixed effect model results at the *fungus species level*. Effect of tree diversity of the local tree neighbourhood on fungal infestation of host-specific fungal species for the tree species *Tilia cordata* and *Quercus petraea* across all tree individuals of each tree species (n = 282) within the years 2010-2012. Df = 233. Significant results are indicated in bold fonts. Variance of the random factors block and plot, respectively: *T. cordata*: *Passalora microsora* = <0.1 % and 28.9 %, *Apiognomonia errabunda* = 0.18 % and <0.1 %, *Asteromella tiliae* = 1.8 % and 15.7 %, Species of Ascomycota = <0.1 % and 8.0%; *Q. petraea*: *Erysiphe alphitoides* = 3.0 % and 11.9 %; *Erysiphe hypophylla* = <0.1 % and 21.9 %, Species of Ascomycota = <0.1 % and 19.4 %, *Zygosporium* sp. = <0.1 % and 13.5 %, *Ramichloridium* sp. = 9.5 % and 16.2 %.

	Tilia cordata									
Explanatory	***Passalora microsora***		***Apiognomonia errabunda***		***Asteromella tiliae***		**Species of Ascomycota**			
variable	F	p	F	p	F	p	F	p		
Intercept	444.11	<0.001	2.407	0.122	10.695	0.001	230.307	<0.001		
Tree Diversity (TD)	3.619	0.058	0.181	0.671	0.095	0.759	5.087	0.025		
Year	33.444	<0.001	0.772	0.463	2.231	0.11	0.001	0.999		
TD x Year	0.498	0.608	1.351	0.261	4.369	0.014	6.253	0.002		
	Quercus petraea									
	Erysiphe alphitoides		***Erysiphe hypophylla***		***Species of Ascomycota***		***Zygosporium sp.***		***Ramichloridium sp.***	
	F	p	F	p	F	p	F	p	F	p
Intercept	18.787	<0.001	188.949	<0.001	223.521	<0.001	138.457	<0.001	51.206	<0.001
Tree Diversity (TD)	3.241	0.073	7.874	0.005	0.031	0.86	0.344	0.558	0.234	0.629
Year	20.807	<0.001	15.655	<0.001	71.802	<0.001	62.608	<0.001	73.187	<0.001
TD x Year	0.845	0.431	0.828	0.438	0.501	0.607	1.138	0.322	0.363	0.696

Table 3-4 Linear mixed effect model results at the *fungus species level*. Effect of tree species proportions of the local tree neighbourhood on the fungal infestation of host-specific fungal species for the tree species *Tilia cordata* and *Quercus petraea* across all tree individuals of each tree species (n = 282) within the years 2010-2012. Df = 233. Significant results are indicated in bold fonts. Host species proportions are local neighbourhoods con-specific to the target tree and marked in grey. Proportion = P., *Fagus sylvatica* = Fs., *Fraxinus excelsior* = Fe., *Picea abies* = Pa., *Pinus sylvestris* = Ps., *Quercus petraea* = Qp., *Tilia cordata* = Tc.

	Tilia cordata								***Quercus petraea***									
Proportion of neighbour tree species	***Passalora microsora***		***Apiognomonia errabunda***		***Asteromella tiliae***		**Species of Ascomycota**		***Erysiphe alphitoides***		***Erysiphe hypophylla***		**Species of Ascomycota**		***Zygosporium sp.***		***Ramichloridium sp.***	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Intercept	471.062	<0.001	2.131	0.146	11.447	0.001	199.975	<0.001	19.419	<0.001	174.464	<0.001	223.801	<0.001	138.373	<0.001	46.52	<0.001
P.Fs.	12.287	0.001	0.451	0.503	0.529	0.468	1.269	0.261	1.004	0.318	1.156	0.283	0.435	0.51	0.655	0.419	10.79	0.001
Year	31.98	<0.001	0.728	0.484	2.055	0.131	0.006	0.994	20.171	<0.001	15.556	<0.001	71.489	<0.001	61.392	<0.001	72.762	<0.001
P.Fs. x Year	2.496	0.085	0.155	0.857	0.392	0.676	0.607	0.546	0.015	0.985	0.302	0.74	0.14	0.869	0.025	0.978	0.651	0.522
Intercept	387.624	<0.001	1.974	0.161	10.029	0.002	195.585	<0.001	17.911	<0.001	175.636	<0.001	222.045	<0.001	140.661	<0.001	50.255	<0.001
P.Fe.	0.919	0.339	6.317	0.013	2.261	0.134	0.085	0.771	17.472	<0.001	1.514	0.22	1.029	0.312	0.216	0.642	5.671	0.018
Year	33.537	<0.001	0.901	0.408	1.952	0.144	0.004	0.996	21.394	<0.001	16.13	<0.001	81.703	<0.001	63.139	<0.001	78.376	<0.001
P.Fe. x Year	2.041	0.132	0.432	0.65	0.216	0.806	2.41	0.092	2.486	0.085	2.496	0.085	14.427	<0.001	2.306	0.102	4.26	0.015
Intercept	402.833	<0.001	2.406	0.122	11.509	0.001	197.56	<0.001	20.79	<0.001	260.258	<0.001	226.427	<0.001	143.216	<0.001	51.483	<0.001
P.Pa	1.242	0.266	1.236	0.267	0.603	0.438	0.409	0.523	7.306	0.007	17.622	<0.001	0.508	0.477	2.706	0.101	0.114	0.737
Year	34.292	<0.001	0.743	0.477	2.169	0.117	0.003	0.997	20.84	<0.001	15.745	<0.001	72.803	<0.001	62.123	<0.001	74.742	<0.001
P.Pa. x Year	3.115	0.046	0.302	0.74	1.182	0.309	0.78	0.46	3.185	0.043	1.836	0.162	2.377	0.095	1.048	0.353	2.932	0.055
Intercept	486.47	<0.001	2.355	0.126	10.538	0.001	202.001	<0.001	22.84	<0.001	172.181	<0.001	226.242	<0.001	139.689	<0.001	43.811	<0.001
P.Ps.	11.233	0.001	0.91	0.341	0.067	0.796	1.184	0.278	1.854	0.175	0.64	0.424	0.675	0.412	0.44	0.508	9.404	0.002
Year	33.402	<0.001	0.76	0.469	2.169	0.117	0.004	0.996	21.429	<0.001	16.093	<0.001	72.282	<0.001	61.504	<0.001	72.595	<0.001
P.Ps. x Year	1.858	0.158	0.205	0.815	3.379	0.036	5.041	0.007	5.544	0.004	2.351	0.098	1.689	0.187	0.117	0.89	0.075	0.928
Intercept	391.747	<0.001	2.224	0.137	10.203	0.002	197.244	<0.001	17.88	<0.001	180.216	<0.001	224.271	<0.001	139.346	<0.001	51.747	<0.001
P.Qp.	0.023	0.879	1.24	0.267	0.741	0.39	0.253	0.615	1.341	0.248	1.774	0.184	0.237	0.627	0.572	0.45	1.028	0.312
Year	33.815	<0.001	0.739	0.479	2.079	0.127	0.004	0.996	20.77	<0.001	15.628	<0.001	74.122	<0.001	63.134	<0.001	73.451	<0.001
P.Qp. x Year	2.411	0.092	2.325	0.1	0.051	0.95	0.596	0.552	0.312	0.732	2.778	0.064	3.417	0.035	1.963	0.143	1.003	0.369
Intercept	462.652	<0.001	2.129	0.146	10.999	0.001	208.65	<0.001	23.541	<0.001	172.538	<0.001	226.251	<0.001	141.253	<0.001	61.14	<0.001
P.Tc.	2.027	0.156	0.513	0.475	0.001	0.98	1.291	0.257	4.243	0.041	0.222	0.638	0.24	0.625	0.48	0.489	5.351	0.022
Year	33.662	<0.001	0.775	0.462	2.175	0.116	0.003	0.997	20.745	<0.001	15.686	<0.001	71.578	<0.001	61.79	<0.001	74.625	<0.001
P.Tc. x Year	0.488	0.615	0.194	0.824	2.785	0.064	7.86	0.001	1.307	0.273	0.601	0.549	0.198	0.821	0.507	0.603	0.011	0.989

results of different powdery mildew species on *Quercus* and *F. sylvatica* from another experiment, i.e. the BIOTREE experiment in Kaltenborn (Hantsch et al. 2013). Both studies calculated Shannon diversity of the local neighbourhood, comprising six tree individuals of the local neighbourhood in the Kreinitz experiment, but 252 neighbour tree individuals in the Kaltenborn experiment. However, we have also to consider that both the local neighbourhood scale and the plot size (Kreinitz experiment: 25 m²; Kaltenborn: 5760 m²) might have been too small for local tree diversity effects on fungal species richness of *Q. petraea* and to particular fungal pathogen species on host tree species. It is possible that different fungal species are affected by tree diversity at different scale, depending on their dispersal ability or their dependence on the microclimate provided by the local tree neighbourhood, which depends on canopy structure and architecture as well as on stand management (Jactel et al. 2009; Calonnec et al. 2013). This also points out the limitations of young tree plantation as compared to mature forests, for which several studies showed lower susceptibility of fungal pathogens with increasing tree species diversity (see review by Pautasso et al. 2005). It is well known that ontogeny exerts a strong influence on microbial phyllosphere community (Peñuelas et al. 2012). Moreover, it has been shown that resistance to fungal attacks also relies on constitutive and induced defence. For example, infection success of *Apiognomonia errabunda* was shown to depend on endogenous levels of constitutive phenolic compounds (Bahnweg et al. 2008). As the constitutive and induced production of secondary metabolites strongly differs between young and adult trees (Erbilgin & Colgan 2012), ontogeny seemed to an important determining factor for defence response. In consequence, the absence of tree diversity effects on fungal pathogen richness on *Q. petraea* requires follow-up investigations and underscores the request of long-term experiments. However, the results of fungal infestation on the two tree species in our study confirm those from grassland experiments (Mitchell et al. 2003; Roscher et al. 2007) and point to associational resistance caused by modifying the microclimate (Tahvanainen & Root 1972). Thus, for the first time we demonstrated associational resistance for fungal pathogens on tree species, which shows close parallels to specialized herbivores, as described for *Quercus robur* (Jactel & Brokerhoff 2007; Castagneyrol et al. 2013).

Host proportion effects

We have not found much support for our second hypothesis that increasing host proportion of *T. cordata* and *Q. petraea* enhances the degree of fungal infestation. However, significant interactions of host proportion with year revealed that fungal infestation by unknown ascomycetes increased with host proportion in 2012, under driest weather conditions, but

decreased 2010 and 2011 (exception: ascomycete of *T. cordata* increased also 2011). The absence of host dilution effects was unexpected since several studies showed decreased pathogen infestation or transmission with decreasing host availability (e.g. Bell et al. 2006; Mundt et al. 2011; Cobb et al. 2012). The absence of main host proportion effects in combination with the presence of tree diversity effects of the local neighbourhood on fungal pathogens of *T. cordata* and *Q. petraea* shows that the diversity mechanism does not operate through dilution of the host species, as hypothesized in Figure 3-1A. Thus, diversity does not operate through decreasing the concentration of resources but by modifying the pathogen's local environment. Our results contradict the 'resource concentration hypothesis', which proposes that specialized organisms are concentrated in habitats where their resource is most abundant (Root 1973; Burdon & Chilvers 1982). Furthermore, results do also not confirm other findings on density-dependency of fungal pathogens. For instance, for a rust fungus on wheat, Mundt et al. (2011) found an increase of epidemic spread with increasing host frequency. In addition, for a neo-tropical tree species, Bell et al. (2006) showed a density-dependent seedling mortality caused by plant pathogens, with higher mortality in dense stands. However, opposing relationships have also been described, at least for host-herbivore relationships, where a negative association between host density and herbivore richness (Johnson et al. 2012) or abundance (Bañuelos & Kollmann 2011) was shown.

Non-host neighbour proportion effects

In contrast to host species proportion, proportion of particular non-host neighbour tree species affected fungal species richness and fungal infestation. Thus, we confirmed our third hypothesis on the existence of density-dependent non-host neighbour identity effects. Thus, at the *tree species level* and the *fungus species level*, increasing proportion of both conifer tree species as well as of *T. cordata* reduced fungal infestation. The final mechanism is difficult to conceive, with microclimate modified by canopy structure and architecture being the most possible explanation (Calonnec et al. 2013). However, the role of microclimate in associational resistance has not been tested experimentally yet. Alternatively, different neighbourhoods might affect foliar fungi through changed chemical composition of leaves or simply by shielding target trees from spores. At least, we could exclude facilitation by the understory plants that might serve as alternate hosts or shelter (Jactel et al. 2009), since understory vegetation was more or less absent in the experimental plots because of a dense canopy cover. However, as different neighbourhoods were either positive for the pathogen or positive for the host in particular years, they represent a case of unspecific and inconsistent facilitation. Thus, a few non-host tree species effects (interactions) seem to be partial

idiosyncratic, being operative in some cases only in particular years and in particular non-host neighbour combinations. These findings confirm those observed in grassland diversity experiments where the presence of non-host plants species facilitated the host species (Mitchell et al. 2002). In addition, such host species facilitation might be also shaped by crown architecture of the surrounding non-host neighbours and here in particular by higher vertical structuring and canopy density which is due a dispersal barrier (Calonnec et al. 2013) and provided by both conifer species as well as for *T. cordata*. However, there were also positive non-host neighbourhood effects, and thus, increasing proportion of *F. sylvatica* and *F. excelsior* enhanced fungal infestation at the *tree species level* and the *fungus species level*. Such positive identity effects indicate tree species-specific fungal pathogen facilitation. *Fagus sylvatica* might exert such effects because of lowest growth rates, whereas *F. excelsior* featured higher growth rates than those of the other tree species in the experiment, resulting in a lower and higher tree height, respectively (Castagneyrol et al. 2013). However, the latter tree species was highly infected by ash dieback symptoms and thus, exhibited early defoliation and thus increasing crown thinning (Kowalski 2006). These tree species traits possibly affecting the microclimatic conditions (Bourke 1970) which might have resulted in increased temperatures, which in turn has increased fungal infestation (Calonnec et al. 2013).

Inter-annual variation

In general, we encountered a pronounced variation in fungal species richness and fungal infestation between years, which clearly demonstrates the species-specific dependence of fungal development and timing of life cycle on local weather conditions (Tainter & Baker 1996; Jactel et al. 2009). The effect was different for the two target tree species with higher fungal infestation in the colder and more humid year 2010 on *T. cordata* and in the warmer and drier years 2011 and 2012 on *Q. petraea*. In addition to our results for *Q. petraea*, fungal species richness and fungal community composition of *Quercus ilex* increased under dry summer conditions (Peñuelas et al. 2012). Similarly, Lappalainen et al. (1999) found differences in endophyte composition and infestation on *Betula* species between two subsequent years. Furthermore, the high fungal infestation observed in some fungal species in the experiment is not extraordinary, but has been described also in the literature. For instance, Bernadovičová and Ivanová (2008) noticed also massive and continuous occurrence of *Passalora microsora* in urban tree plantings in Slovakia. However, we could not confirm our fourth hypothesis that the inter-annual variation in fungal species richness and fungal infestation decreased with increasing tree diversity. The general pattern is a strong, but species-specific dependence of fungal infestation on climatic conditions and tree diversity

effects that are only played out under certain climatic conditions. Thus, diversity did not confer higher temporal stability in these tree plantations, which forms a contrast to the results from grassland ecosystems, for which a reduced inter-annual variation with increasing plant species diversity has been reported (Tilman et al. 2006; Eisenhauer et al. 2011).

Conclusions

Our experimental study provides evidence for tree diversity effects on fungal species richness and fungal infestation on two common trees species at the local scale, with lower fungal infestation in more diverse tree species communities. Our central finding was that this effect was not caused by simple host dilution effects, i.e. by decreasing host proportion, but by tree diversity of the local neighbourhood. Moreover, we could show that particular local non-host neighbour species impede or facilitate fungal pathogen infestation. Probably, the transfer of the results from this small scale experiment to the large scale of planted or natural forests can only be made with strong reservations. In any case, out results apply to young tree plantations, and thus, might be valuable for the design of new forest plantations. Mixing tree species at the scale of the local neighbourhood can reduce pathogen transmission and infection. Subsequent studies now will have to show whether these differences in fungal infestation translate into negative feedbacks on tree growth and survival.

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4. NO PLANT FUNCTIONAL DIVERSITY EFFECTS ON FOLIAR FUNGAL PATHOGENS IN EXPERIMENTAL TREE COMMUNITIES

Lydia Hantsch, Uwe Braun, Josephine Haase, Oliver Purschke, Michael Scherer-Lorenzen, Helge Bruelheide

Fungal diversity (submitted)

4.1 Abstract

Foliar fungal pathogens affect forest ecosystem processes through highly species-specific effects on growth and survival of trees. As many ecosystem processes in forests strongly depend on the functional diversity of tree species, a tight relationship would also be expected between functional tree diversity and foliar fungal pathogen infestation. Testing for such a relationship in the German tree diversity experiment BIOTREE in Bechstedt, we hypothesized that pathogen richness and pathogen load decrease with increasing functional diversity of tree communities. Using macro- and microscopic analyses, we assessed pathogen richness and pathogen load on 16 tree species in plots that differed in functional diversity but had the same tree species richness. We found no effects of functional diversity, neither on pathogen richness nor pathogen load. However, we encountered strong species identity effects as susceptible tree species contributed positively to the community's pathogen richness and load. Furthermore, testing for effects of particular leaf traits and the geographical distribution range size of the host species revealed a significant effect of total leaf phenolics, which was contrary to expectation as pathogen richness increased with increasing content in polyphenolics. Our study showed that host species identity was more important for richness and load of foliar fungal pathogens than functional diversity of host trees. The positive relationship between pathogen richness and phenolics in leaves, in combination with the finding that pathogen richness is highly conserved in tree species, point to an evolutionary arms race of hosts and pathogens by boosting both defence and infectivity.

Keywords

biodiversity–ecosystem functioning, BIOTREE experiment, host defence traits, phylogenetic pattern, red queen hypothesis, tree species identity effects

4.2 Introduction

Functional diversity is considered crucial for maintaining ecosystem functions (Petchey 2004; Petchey & Gaston 2006). In the past decades there has been an increasing concern that the increased loss of biodiversity might decrease the functional diversity of plant communities and finally compromise ecosystem functioning (Petchey & Gaston 2002a, b; Thuiller et al. 2006). Grassland studies have shown that community stability depends on species diversity across different trophic levels (Loreau et al. 2001; Lafferty et al. 2008; Proulx et al. 2010; Roscher et al. 2011). In particular, parasites and pathogens play an important role for the stability of communities because they impair the host's carbon uptake and biomass production (Hajji et al. 2009; Allen et al. 2010; Eisenhauer et al. 2011). If pathogens affect dominant hosts more than subordinate ones, they cause a negative density-dependence, with the ultimate effect of increased plant species diversity (Janzen 1970; Connell 1971; Bagchi et al. 2010). Conversely, tree diversity has the potential to reduce passive pathogen transmission by increased host distance and decreased host abundance (Keesing et al. 2006, 2010). Recently, we demonstrated these effects for experimental forest communities, where tree species diversity reduced the degree of foliar fungal pathogen infestation in several species (Hantsch et al. 2013; Hantsch et al. unpublished data). However, not attempt has been made yet to relate foliar fungal pathogen infestation to functional attributes of host species and to functional tree diversity.

Functional diversity is based on one or several functional traits that affect the functioning of communities. The general idea is that the variance in trait values of these relevant traits rather than mean values have an effect on the function considered. Mathematically, the variance in trait values can be expressed in various ways, resulting in different functional diversity (FD) measures (Walker et al. 1999; Petchey & Gaston 2002b; Botta-Dukát 2005; Böhnke et al. 2013). In addition to the variance in trait values, the overall infestation by fungal pathogens at the community level strongly depends on the resistance to infection and infestation of a particular host species. Furthermore, most fungal pathogens show very high host specificity (Prell 1996). Thus, the presence of susceptible or resistant host species increases or decreases the community's pathogen load. In addition, non-host neighbour trees of an infected tree can alleviate or intensify a foliar fungal disease within a community (Hantsch et al. 2013; Hantsch et al. unpublished data). In biodiversity-ecosystem functioning (BEF) research, these species-specific consequences have been termed species identity effects (Specht et al. 2008; Hantsch et al. 2013). If every species is functionally unique (*singular hypothesis*: Naeem et al. 2002), the loss of every species translates into a loss of ecosystem functioning. In grassland

communities, for instance, this uniqueness has been shown for soil microbial organisms (Eisenhauer et al. 2010) and herbivores (Specht et al. 2008). In forest communities, host uniqueness has a strong effect on composition of endophytic fungi, thus differences in fungal community composition were higher between host species than between tissue types (Sun et al. 2012). Alternatively, different species might have similar functions in an ecosystem (*redundancy hypothesis*: Walker 1992; Naeem & Li 1997). In consequence, ecosystem functioning would not be compromised with a loss of some species in the community (Cardinale et al. 2012). In grassland communities, for instance, this redundancy has been shown for soil decomposer organisms and activity of heterotrophic soil organisms (Spehn et al. 2000; Scherer-Lorenzen 2008). However, it is unclear how many species can be lost without losing ecosystem functioning.

The high host-specificity of fungal pathogens is the ecological consequence of highly specific molecular mechanisms of host-pathogen interactions (Keen 1990, Chisholm et al. 2006). Plant defence is often induced by gene expression of particular conserved gene families (Duplessis et al. 2009; Hacquard et al. 2011). Defence mechanisms can occur at different developmental stages, and affect for instance the resistance to penetration or to haustorial development after penetration (Azaiez et al. 2009; Fernández-Aparicio et al. 2009). The pathogens therefore have to overcome hosts' defence mechanisms to develop within the host and to feed on living cells (Kloppholz et al. 2011). For instance, a rust fungus may exude disease effectors that modulate the cell physiology of host cells and can suppress host immunity. According to the Red Queen hypothesis (van Valen 1973) pathogens have co-evolved with their hosts in an "arms race" (McDonald & Linde 2002; Parker & Gilbert 2004; Thines & Kamoun 2010). Although the Red Queen hypothesis primarily had a micro-evolutionary scope and aimed at explaining the origin of genetic variation within the host and the pathogen species (Clay & Kover 1996a) it can be extended to macro-evolutionary patterns to explain inter-specific variation. Thus, it would be expected that closely related host species share certain functional traits that are phylogenetically conserved and confer resistance. Furthermore, closely related species are also expected to suffer from pathogen infestation of closely related pathogen species to a similar degree. Among all traits that affect pathogen resistance, secondary metabolites are thought to play a key role. Plants produce numerous secondary metabolites, which all in principle might have a defence function, such as terpenoids, alkaloids, sterols and polyphenolics (Blodgett et al. 2005; Iason et al. 2005). Among all these compounds, polyphenolics play a central role as they occur in high amounts in plants (Shanmugam et al. 2010). For instance, the resistance of potatoes to fungal pathogens of the genus *Phytophthora*

depends on phenolic compounds (Kröner et al. 2012). Furthermore, an increase in leaf phenolic content of *Rosa hybrida* contributed to a decrease of disease severity by the rose powdery mildew (Shetty et al. 2011). However, plant susceptibility does also depend on morphological traits and nutrient content. For instance, McElrone et al. (2005) showed a reduced leaf spot disease severity of *Acer rubrum* L. with decreasing leaf nitrogen contents and increasing carbon to nitrogen ratio and tannin contents. It might well be that damage caused by foliar fungal pathogens shows parallels to that caused by herbivores. , Mraja et al. (2011) observed higher herbivore damage of *Plantago lanceolata* L. with increasing specific leaf area and increasing leaf nitrogen content. As in particular the content of polyphenolics is highly phylogenetically conserved (Eichenberg et al. unpublished data), host differences in susceptibility to foliar fungal pathogen attack should be reflected in phylogenetic distances between host species (Gilbert & Webb 2007).

In addition to morphological or chemical defence traits of the host species, pathogen richness and pathogen load might also depend on host's geographical distribution range size (Mitchell et al. 2010). The larger the host's distribution range, the more opportunities there have been for pathogens to come into contact with this host species, and the more different pathogen species would be expected to occur on that host species (Humphries et al. 1986). Such patterns have been demonstrated for ectomycorrhizal fungi (Tedersoo et al. 2013), insects and the degree of insect herbivory on tree species but not yet for foliar fungal pathogens. For instance, a positive relationship has been shown for insect richness and tree species' geographical distribution range size (Lavandero et al. 2009; but see also the review by Lewinsohn et al. 2005). Similarly, the degree of herbivory damage on subtropical broad-leaved tree species has been found to be positively related the host tree species distribution range (Schuldt et al. 2012). Thus, we would also expect that a host species is the more affected by pathogens, the larger its geographical distribution range size.

Of all the BEF experiments with trees in the global network TreeDivNet (www.treedivnet.ugent.be), the BIOTREE experiment in Bechstedt (Thuringia, Germany) is the only one that has been established to analyse effects of functional tree diversity. Using a total pool of 16 tree species, communities have been created that differ in functional diversity of tree species but have the same species richness of four tree species (Scherer-Lorenzen et al. 2007). We used the Bechstedt study site to explore the relationship between foliar fungal pathogens and functional tree diversity and tested the following hypotheses: 1) High functional tree diversity decreases pathogen richness and pathogen load. 2) The presence of particular disease-prone or disease-resistant tree species either increases or decreases

pathogen richness and pathogen load, respectively. 3) Pathogen richness and pathogen load depend on particular leaf traits and on geographical distribution range size of the host species, with higher pathogen richness and pathogen load for tree species with larger geographical distribution range size. 4) Defence traits (i.e. phenolic and tannin content) and the degree of fungal infestation are phylogenetically conserved.

4.3 Material and Methods

Study site and experimental setup

The design and the establishment of the BIOTREE experiment have been described in detail by Scherer-Lorenzen et al. (2007) and are only shortly summarized here. The Bechstedt study site is located in Central Thuringia (N 50°54', E 11°05'), Germany. Elevation is about 400-415 m a.s.l. and the bedrock is limestone (Scherer-Lorenzen et al. 2007). The climate is subatlantic with mean annual temperature of 7.9°C and mean annual precipitation of 553 mm. A total of 25 plots were established in 2003/2004 with four tree species per plot, but different tree species compositions (Scherer-Lorenzen et al. 2007). Tree species composition was created in accordance to functional trait similarity of 16 different tree species (listed in Table 1). The functional similarity gradient was based on nine different traits which are indicative for productivity, resource use and nutrient cycling: leaf habit (evergreen or deciduous), a ranked expert guess on light requirements as adults, height growth vigour, mean annual increment growth and rooting vigour, crown architecture (monopodial, sympodial-narrow or sympodial-broad), root architecture (shallow-rooted, heart-rooted, tap-rooted) as well as species mean values of leaf nitrogen concentration and carbon to nitrogen ratio of litter (Scherer-Lorenzen et al. 2007). Functional trait similarity was calculated by Gower's distance, taking into account the mixed scale of categorical, ordinal and cardinal variables. Functional diversity (FD) was then calculated from total branch length of a trait dendrogram (Petchey & Gaston 2002b). In the following, we refer to this measure as "FD of the original design", as it was used to create the 25 different tree species communities in the different plots. However, as the traits used might not be relevant for foliar fungal infestation, we have created further FD measures (see below).

The plots are hexagon-shaped and have a size of 1700 m² (maximum width = 43.5 m, maximum length = 56 m). Each plot is divided into 44 circular mono-specific patches with a diameter of 7 m, each containing 20 tree individuals. An inventory conducted in winter 2011 showed that the tree species greatly differed in height, with *Fagus sylvatica* and *Larix decidua*

being the smallest (1.04 m) and tallest species (6.86 m), respectively (Table 1). Further information about the Bechstedt study site and tree establishment success is given in Scherer-Lorenzen et al. (2007) and Don et al. (2007).

For foliar fungal pathogen analyses, we selected tree individuals randomly in all plots, but excluded patches on the outer border of a plot to avoid edge effects. In September 2010, we sampled a total of eight individuals per plot, with two individuals per tree species, which were also randomly chosen within patches. In total, 200 tree individuals were sampled. As the tree species were not planted in the same frequency across all communities, in the whole experiment the number of sampled trees differed among tree species (Table 1).

Leaf sampling

For deciduous trees, two branches were selected growing in opposite direction in the upper as well as in the lower part of the crown and 20 leaves were collected per branch. For the evergreen conifer in the experiment (*Pinus sylvestris*), needles of the recent and all previous years, and for the deciduous conifer (*Larix decidua*) only needles of the recent year were collected for a total of eight shoots. Directly after sampling, leaves and needles were dried at 60°C for three days and then stored in the dark at approximately 20°C.

Macro- and microscopic analyses

Macro- and microscopic analyses were done on a random subset of 10 leaves or 100 needles per tree individual on each of the 200 sampled individuals. Focusing on the ecological role of parasitism, fungal species inventory was restricted to visible parasitic fungi (biotrophy), excluding all other fungi living on the leaf surface as well as endophytic fungi. Qualitative microscopic analyses were used for determining pathogen richness, allowing the identification of biotrophic foliar fungal taxa to the species level by light microscopy (after Brandenburger 1985; Ellis & Ellis 1997; Braun & Cook 2012). However, as species identification based on morphology depends on the fungal developmental stage, in a few cases of absent fructification, particular taxa could not be identified at the species level but were assigned to the phylum Ascomycota (see Table 1). Quantitative macroscopic analyses were done on the whole surface of leaves and needles, estimating pathogen load of each foliar fungal taxon by stereomicroscopy. Pathogen load was visually recorded as leaf area damage using a percentage class system with seven damage classes: 0 %, 1-5 %, 6-10 %, 11-25 %, 26-50 %, 51-75 % and 76-100 % (Schuldt et al. 2012; Hantsch et al. 2013).

Leaf traits

We measured the following traits on the trees of the Bechstedt experiment: specific leaf area (SLA), leaf thickness and leaf toughness as physical defence traits, and carbon content (C), nitrogen content (N) carbon to nitrogen ratio (C/N), total phenolic content and tannin content as chemical traits. Leaf trait analyses were done on healthy leaf material of five individuals per tree species, which were selected randomly (total n = 80) in August 2010 (Appendix for information on the measurement procedures). All leaf traits were analysed for inter-relationships. Significant correlations with Person's $r > 0.75$ were encountered between polyphenolics and tannins ($r = 0.936$, $p < 0.001$), leaf thickness and leaf toughness ($r = 0.924$, $p < 0.001$) as well as N and C/N ratio ($r = -0.945$, $p < 0.001$). A principal component analysis conducted with all leaf traits clearly revealed the leaf economics spectrum (Wright et al. 2002) on the first axis (see Figure 1 in Appendix).

Data analysis

In each of the 25 plots, functional diversity (FD) of the community was assessed based on i) the set of nine traits used in the original design, that describe productivity, resource use and nutrient cycling of the trees, and on ii) the eight leaf traits measured in the current study. For the latter, we used several FD indices to test for different aspects of FD (Mason et al. 2003; Villéger et al. 2008; Schleuter et al. 2010). Villéger et al. (2008) proposed three important FD indices: the functional niche space that is occupied by the species in the community (functional richness, FR_D), the regularity of species' abundances within this functional niche space (functional evenness, FE_m) and the degree of divergence in trait values (functional divergence, FD_Q). We used Rao's quadratic entropy (Rao 1982) for calculating FD_Q as a predictor for pathogen richness and pathogen load, using both the nine original design traits and the eight leaf traits. Functional richness and functional evenness was calculated according to Villéger et al. (2008), using the eight leaf taits, and also tested for effects on pathogen richness and load. We surveyed FD indices for effects on pathogen richness and pathogen load using linear model regressions.

Alternatively, we tested for species identity effects within communities, using the presence/absence information coded as binary variable for each of the 16 tree species in the experiment as predictor for pathogen richness and load. We tested to which degree pathogen richness and pathogen load at the community level (n = 25) can be predicted from species identity information, using the species' mean values over all sampled tree individuals per tree

species and the particular tree species composition of every plot. Subsequently, we compared predicted with realized pathogen richness and pathogen load for each community

In addition, we used multiple regressions to test for the joint explanatory power of several predictors. For both pathogen richness and load, we run a forward selection of predictors and chose the model with the lowest AIC. In addition to leaf traits, we also used geographical distribution range size as predictor for pathogen richness and pathogen load in a linear model. To assess the most relevant traits for predicting pathogen richness and load, we used a redundancy analysis (RDA, Legendre & Legendre 2012), based on pathogen richness and load as constraining variables and all others as predictors. Thus, the RDA to explore visually which set of predictors best explains pathogen richness and pathogen load.

To assess whether closely related species shared similar trait values, range sizes or levels of pathogen infestation, we assessed the phylogenetic signal in the species' ecological characteristics, using Blombergs's *K* (Blomberg et al. 2003). *K* values > 1 indicate more phylogenetically signal in species' characteristics than expected from a Brownian motion model of trait evolution, implying that closely related species are ecologically similar. A phylogenetic tree for the 16 species in our study was constructed from a dated, ultrametric supertree for Central European vascular plant species (Daphne 1.0, Durka & Michalski 2012). The significance of the phylogenetic signal was tested by randomizing (999 times) the species names across the tips of the phylogenetic tree and comparing the observed values of *K* to those expected by chance. Leaf trait values, tree species' distributional range size and fungal pathogen proxies were log-transformed and centred (scaled to mean = 0 and sd = 1) prior to the phylogenetic analysis.

All statistics were done and all figures created with R 3.0.0 (R Core Team 2013).

4.4 Results

In total, we detected 29 fungal taxa on all 16 tree species (Table 1). The most disease-prone tree species both in terms of pathogen richness and pathogen load were *Quercus petraea*, the three *Acer* species and *Tilia cordata*. In addition, *Sorbus aucuparia* showed high pathogen richness and *Populus tremula* exhibited high a pathogen load (see Figure 2 in Appendix). In contrast, disease-resistant tree species were the two conifer species, *Larix decidua* and *Pinus sylvestris*, which did not carry any foliar fungal pathogens.

Table 1 List of all 16 tree species at the Bechstedt study site, including their abbreviation as used in the Figures, mean height [m] of the tree species based on the inventory in winter 2011 and averaged across all individuals of all plots, total number of sampled individuals per tree species and their mean pathogen richness and mean pathogen load [%] in autumn 2010 (n = 16). In addition, all foliar fungal pathogen species that were observed on a tree species are listed.

Tree species / Abbreviation	**Mean tree height [m]**	**Number of sampled individuals**	**Mean pathogen richness**	**Mean pathogen load [%]**	**Fungal taxa**
Acer campestre Acca	2.651	10	2.000	8.075	*Phloeospora aceris* (Lib.) Sacc. *Rhytisma acerinum* (Pers.) Fr. *Sawadaea bicornis* (Wallr.) Miyabe
Acer platanoides Acpl	3.249	18	2.722	54.903	*Phloeospora aceris* (Lib.) Sacc. *Rhytisma acerinum* (Pers.) Fr. *Sawadaea tulasnei* (Fuckel) Homma Sterile non-identified ascomycete
Acer pseudoplatanus Acps	2.277	10	1.8	37.325	*Rhytisma acerinum* (Pers.) Fr. *Sawadaea bicornis* (Wallr.) Miyabe Sterile non-identified ascomycete
Betula pendula Bepe	5.234	16	1.063	9.844	*Marssonina betulae* (Lib.) Magnus *Venturia ditricha* (Fr.) P. Karst.
Carpinus betulus Cabe	2.068	16	1.188	2.125	*Monostichella robergei* (Desm.) Höhn. *Mycosphaerella punctiformis* (Pers.) Starbäck Sterile non-identified ascomycete
Fagus sylvatica Fasy	1.039	6	1.0	1.958	*Apiognomonia errabunda* (Roberge) Höhn. *Phyllactinia orbicularis* (Ehrenb.) U. Braun
Fraxinus excelsior Frex	2.618	14	1.0	4.054	*Venturia fraxini* Aderh.
Larix decidua Lade	6.855	8	-	-	-
Pinus sylvestris Pisy	2.178	10	-	-	-
Populus tremula Potr	5.073	10	1.2	50.225	*Melampsora populinum* (Jacq.) Lév. Sterile non-identified ascomycete
Prunus avium Prav	2.913	10	1.2	3.7	*Thyrostroma carpophilum* (Lév.) B. Sutton Sterile non-identified ascomycete
Quercus petraea Qupe	1.455	14	3.571	47.089	*Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam. *Erysiphe hypophylla* (Nevod.) U. Braun & Cunningt. *Microstroma album* (Desm.) Sacc. *Trabutia quercina* (F. Rudolphi ex Fr.) Sacc. & Roum. Sterile non-identified ascomycete
Sorbus aucuparia Soau	2.471	16	1.75	4.0156	*Gymnosporangium cornutum* Arthur ex F. Kern *Phyllosticta aucupariae* Thüm. *Venturia inaequalis* (Cooke) G. Winter
Sorbus torminalis Soto	2.339	14	1.0	13.714	*Venturia inaequalis* (Cooke) G. Winter
Tilia cordata Tico	2.140	12	1.833	31.479	*Asteromella tiliae* (F. Rudolphi) Butin & Kehr *Passalora microsora* (Sacc.) U. Braun Sterile non-identified ascomycete
Ulmus glabra Ulgl	2.968	16	0.938	4.344	*Phloeospora ulmi* (Fr.) Wallr.

The functional diversity (FD) based on the original design traits that described productivity, resource use and nutrient cycling of the trees and rely on branch lengths of a trait dendrogram were closely related to FD_Q based on Rao's quadratic entropy of the same traits (Figure 1A). Similarly, FD_Q calculated from the eight leaf traits assessed in the current study was significantly related to FD_Q calculated from the original design traits (Figure 1B).

The FD indices showed no significant relationship to pathogen richness or pathogen load (Table 2). The general trend in slopes was negative, indicating a decreasing pathogen richness and pathogen load with increasing FD_Q and FR_D, as well as with decreasing FE_m. In contrast, there were species identity effects as mean community pathogen richness was significantly increased by the presence of *Acer platanoides* (Table 2). Overall pathogen load in communities was significantly increased with the presence of the disease-prone tree species *A. platanoides*, *P. tremula* and *T. cordata* and decreased with the presence of disease-resistant species *Fraxinus excelsior* and *Ulmus glabra* (Table 2). Thus, the presence or absence of a species is a suitable predictor for pathogen richness and pathogen load per community, which could be also seen when plotting observed against predicted values calculated from overall species means (Figure 3 in Appendix). The residuals of this relationship were neither related to any FD index, nor to the presence or absence of any of the tree species (data not shown).

None of the species traits could satisfyingly explain pathogen richness or load of a tree species. This can be concluded from the redundancy analysis (Figure 2), where all traits could only explain 13.86 % variance of both responding variables. A subsequent permutation test showed that the constrained variables pathogen richness and load did not significantly explain

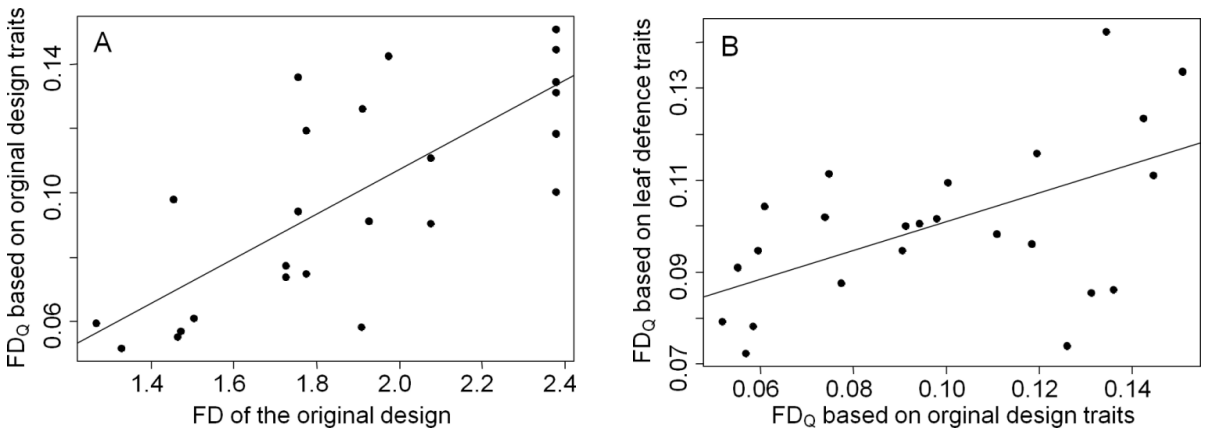

Figure 1 Relationship between A) functional diversity based on Rao's quadratic entropy (FD_Q) of the original traits, related to productivity, resource use and nutrient cycling, and functional diversity of the original plot design, derived from branch lengths of a dendrogram based on the same traits ($p < 0.001$) and B) functional diversity based on Rao's quadratic entropy (FD_Q) of leaf traits and functional diversity based on Rao's quadratic entropy (FD_Q) of the original traits ($p = 0.003$) ($n = 25$).

Table 2 Linear regression model results for the effect of functional diversity (FD) indices and tree species identity on mean pathogen richness and mean pathogen load [%] per plot. FD indices were calculated per plot (n = 25) using Rao's quadratic entropy (FD_Q) based on the original traits of the Bechstedt design that describe productivity, resource use and nutrient cycling as well based on leaf traits that describe physical and chemical defence. FR_D and FE_m are functional richness and functional evenness, respectively, according to Villéger et al. (2008). Mean pathogen richness and mean pathogen load were centred before tested for tree species identity effects. Significant results are indicated in bold fonts.

Predictor	**Pathogen richness**		**Pathogen load [%]**	
	Estimate	p	Estimate	p
Original traits				
FD_Q	-14.915	0.218	-110.972	0.088
Leaf traits				
FD_Q	-9.338	0.674	-35.25	0.772
FR_D	-69.219	0.804	-904.2	0.552
FE_m	10.767	0.166	7.331	0.866
Species identity				
A. campestre	-1.37	0.201	-1.381	0.683
A. platanoides	2.022	0.037	13.555	0.001
A. pseudoplatanus	-1.31	0.247	3.444	0.35
B. pendula	-0.847	0.342	-1.543	0.593
C. betulus	0.058	0.942	-1.987	0.452
F. sylvatica	-0.157	0.898	-3.096	0.453
F. excelsior	-1.066	0.388	-8.771	0.05
L. decidua	-0.004	0.997	-0.12	0.97
P. sylvestris	0.344	0.755	-2.364	0.519
P. tremula	0.265	0.767	9.082	0.011
P. avium	-0.99	0.236	-4.387	0.122
Q. petraea	1.129	0.16	5.075	0.067
S. aucuparia	-0.097	0.913	-5.46	0.088
S. torminalis	-0.459	0.589	-0.06	0.983
T. cordata	1.625	0.074	6.249	0.043
U. glabra	-0.287	0.767	-9.186	0.016

the whole set of traits used in the RDA ($p = 0.426$, $n = 1000$ permutations). Testing the importance of trait by trait in a multiple regression analysis with forward selection revealed that total phenolics be the only significant predictor for pathogen richness, with increasing pathogen richness with increasing content of polyphenolics (Figure 3, full data not shown). None of the leaf traits was related to the species pathogen load. Similar to the majority of leaf traits, pathogen richness and load were independent of the tree species' geographical distribution range size (data not shown).

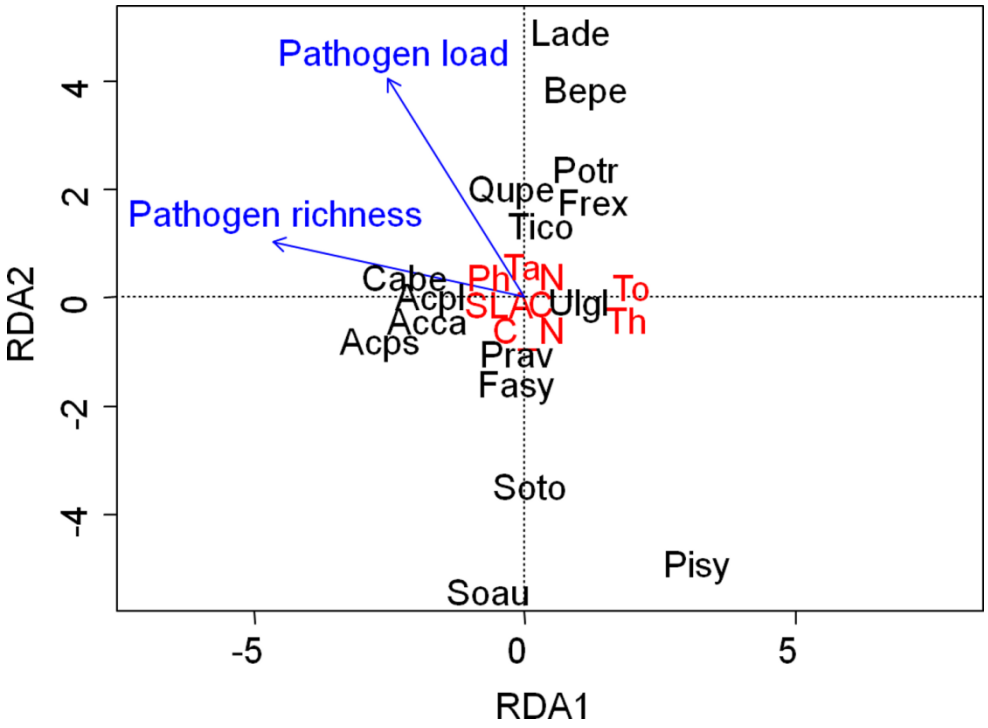

Figure 2 Redundancy analysis for mean pathogen richness and mean pathogen load [%] per tree species including mean leaf traits (red) as explaining variables (n = 16; proportions of variance explained by constrained and unconstrained axes are 13.86 % and 86.14 %, respectively). Leaf traits: C = Carbon, N = Nitrogen, Ph = Phenolics, SLA = Specific leaf area, Ta = Tannins, Th = Thickness, To = Toughness. Species abbreviations are given in Table 1.

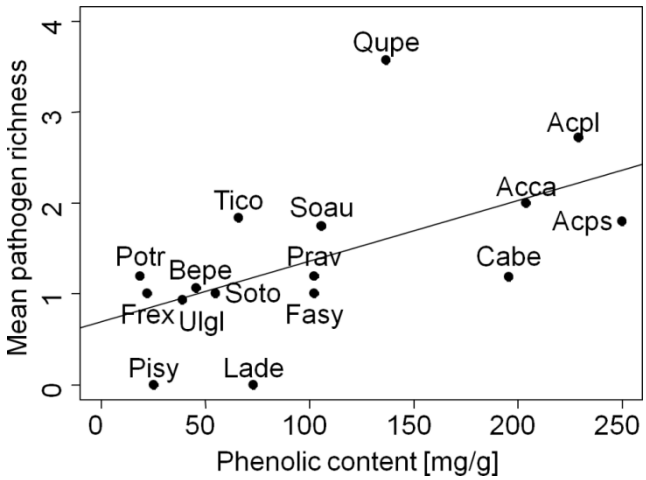

Figure 3 Relationship between mean pathogen richness and mean total phenolic content [mgg^{-1}] per tree species (n = 16; p = 0.021).

Significant phylogenetic signals were found for pathogen richness and load as well as for phenolic and tannin content (Figure 4, Table 3). The highly significant conservatism of pathogen richness and load appeared to be mainly caused by the disease-resistant conifer species which did not show any pathogen infections (Figure 4). Although phenolic and tannin content were slightly less phylogenetically conserved than expected from a Brownian motion model of trait evolution, *K* values were larger than those expected by chance, indicating low but significant phylogenetic signal, with high trait values for the species of the Aceraceae, but only partly for species of the Betulaceae (i.e. *Carpinus betulus*). None of the other traits showed a significant phylogenetic signal (Table 3).

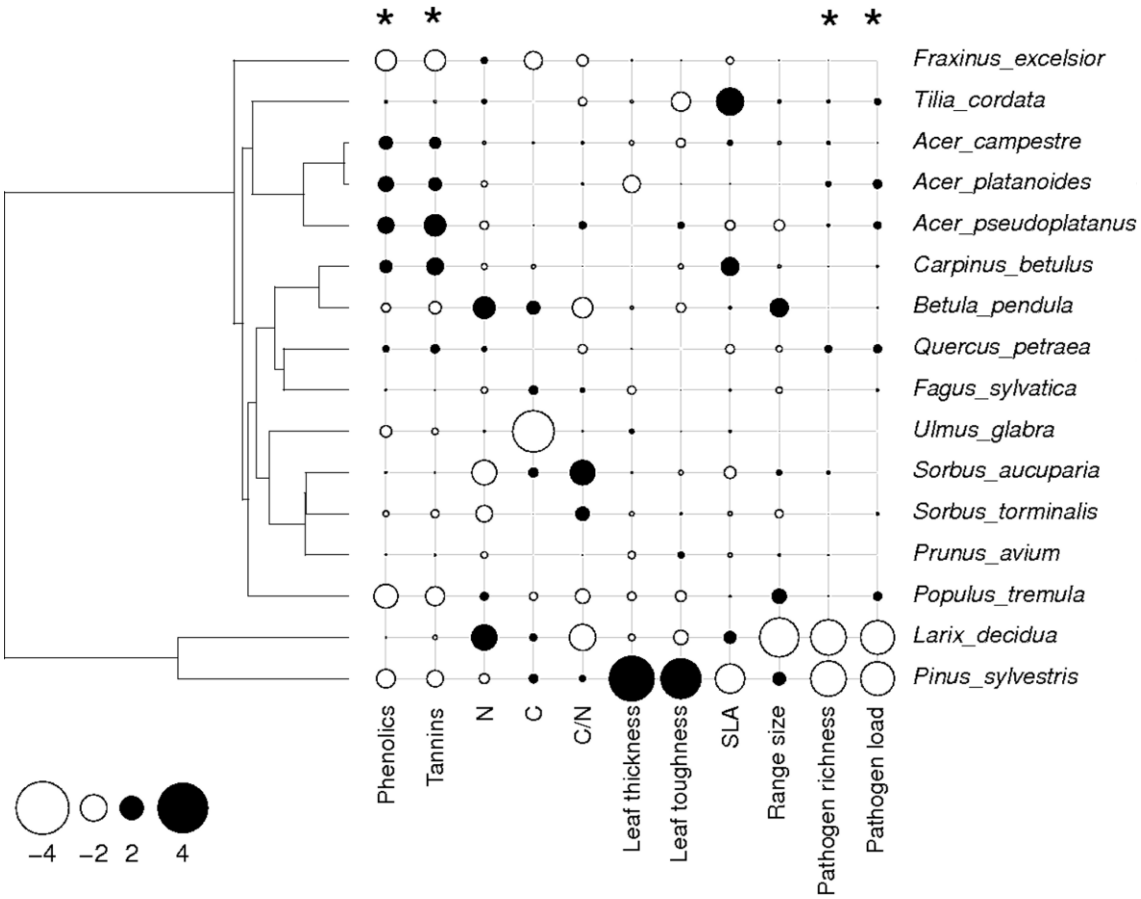

Figure 4 Phylogenetic tree of all 16 tree species and species mean values of leaf traits, pathogen richness and pathogen load [%] and geographical distribution range size (n = 16). Trait values were log-transformed and standardized prior to the analysis. Large white and black circles show large negative and positive trait values, respectively. Asterisks show the significance of the phylogenetic signal according to Blomberg's *K* (see Table 3).

Table 3 Results of phylogenetic tree analysis (see Figure 4) on species mean leaf traits, pathogen richness and pathogen load [%] and geographical distribution range size (n = 16). Trait conservatism is seen in large Blomberg's K while values close to zero indicate a less phylogenetically conserved pattern than expected from a Brownian motion model. Significant results are indicated in bold fonts.

Leaf traits	***K***	**p**
Phenolics	0.553	0.014
Tannins	0.449	0.045
N	0.424	0.091
C	0.383	0.173
C/N	0.472	0.059
Leaf thickness	0.517	0.138
Leaf toughness	0.494	0.096
SLA	0.37	0.185
Range size	0.411	0.13
Pathogen richness	6.556	0.002
Pathogen load	1.76	0.005

4.5 Discussion

In contrast to expectations, we did not encounter effects of functional diversity (FD) on pathogen richness or pathogen load but found strong species identity effects. None of the traits included in our study was able to predict pathogen load. On the contrary, the content of phenolics as a typical leaf defence trait was positively related to pathogen richness. None of these relationships has yet been shown in any observational study or other biodiversity–ecosystem functioning (BEF) experiments.

Functional diversity

At the scale of the BIOTREE experiment in Bechstedt with 16 tree species that strongly differed in trait values, the choice of FD measure did not matter. FD based on branch lengths of a trait dendrogram (Petchey & Gaston 2006) and on Rao's quadratic entropy (Villéger et al. 2008) was significantly related to each other. This is mainly explained by the perfect evenness of species abundances in the experiment, where each of the four tree species per plot was planted with the same number of individuals. However, we did not expect that FD was also related between the different traits used. FD based on the original design traits that described productivity, resource use and nutrient cycling and FD based on the leaf traits that describe chemical and physical defence were tightly related. This shows that both types of traits are related to each other and that leaf defence traits as part of the leaf economics spectrum also reflect productivity (Wright et al. 2002). However, our study generally provided no evidence

that FD had effects on pathogen richness or pathogen load. This contrasts to grassland experiments where plant functional group diversity affected higher trophic levels, such as the presence and density of insects (Sabais et al. 2011) and the resistance and recovery from herbivory (Scherber et al. 2010). However, an absence of FD effects has been also encountered in grassland experiments, where functional group diversity had no effect, for instance, on herbivore resistance (Specht al. 2006; Sonnemann et al. 2012). The absence of FD effects pointed on the functional redundancy of groups or single species as has been shown for soil organisms (Spehn et al. 2000; Scherer-Lorenzen 2008). The assumption of functional redundancy in the relationship of host tree species and foliar fungal pathogens in the BIOTREE experiment in Bechstedt is supported by the findings of Reich et al. (2012) who compared long- and short-term results from BEF experiments and found that community composition was more redundant in early years and species became more and more functionally unique with time. As the Bechstedt experiment is still a plantation in the sapling stage, with an age of tree individuals of approximately 10 years when we conducted our study, our results might serve as a baseline for future studies. However, the absence of FD effects might also have been caused by missing important traits that were not included in the study (Petchey & Gaston 2006; Swenson et al. 2011). This interpretation is supported by the fact that none of the physical and chemical defence traits was strongly related to pathogen richness or load (see below). This point out the necessity to consider further so far disregarded traits beyond the "soft traits" that are easily measurable (Weiher et al. 1999; Díaz et al. 2004).

Tree species identity

FD effects might have been masked by strong identity effects of particular tree species in the community (Nadrowski et al. 2010; Mouillot et al. 2011). Thus, the presence of particular disease-prone or disease-resistant tree species can overrule any FD effects, which might also have been the case in our study. We clearly showed that the communities' pathogen richness and pathogen load increased with the presence of disease-prone species. Such strong identity effects were also encountered by us at another site of the BIOTREE experiment at Kaltenborn, where the presence of the disease-prone tree genus *Quercus* led to a significant increase in a community's pathogen load (Hantsch et al. 2013). Similarly, Rajala et al. (2013) pointed out host tree identity as key factor for endophyte community composition and abundance. In grasslands, Moore and Borer (2012) identified grass host identity as main predictor for yellow dwarf virus abundance in open meadows. All these species identity effects give support for the idea of functional uniqueness of host species (Naeem et al. 2002).

Predicted vs. observed pathogen richness and pathogen load

The proportion of the different species in a community and overall species means in pathogen richness and pathogen load were sufficient predictors for the observed pathogen richness and pathogen load. Neither the particular species composition in a community nor functional diversity did explain any remaining residual variation, indicating that interactions between tree species in a community did not affect pathogen richness and load.

Leaf traits and geographical distribution range size

Neither any of the leaf traits nor the geographical distribution range size was a predictor for a species' pathogen richness or load. This contrasts findings from other studies that showed that pathogen load were related to both chemical and physical leaf traits (Valkama et al. 2005). Similarly, comparing 18 species of clover Bradley et al. (2003) found that a larger leaf size supported spore germination because larger leaves captured more water and retained it longer than small leaves. Amongst all tested leaf traits there was only one significant relationship to pathogen richness, which increased with increasing content in total phenolics. This pattern was brought about by the three *Acer* species and by *Carpinus betulus*. In particular, the high pathogen richness and high content in phenolics within the *Acer* genus in combination with the strong phylogenetic signal for these characteristics suggests an "arms race" of increasing levels of plant defence and increasing numbers of fungal pathogen species adapted to the defensive compounds (Clay & Kover 1996a, b). Although phenolics are ubiquitously produced by a general defence pathway to repel fungi and to suppress their conidial germination or appresorium formation (Shetty et al. 2011), several phenolics were found to be pathogen-specific and to be induced after attack by a particular pathogen (Shanmugam et al. 2010; Kröner et al. 2012). Our study is not the only example, where a fungal richness increased with the content in phenolics. In a study on the tropical plant species *Coccoloba cereifera* Sanchez-Azofeifa et al. (2012) showed that endophyte richness increased with leaf polyphenolic content to specific leaf weight ratio. In contrast, a study about medicinal plants provided evidence for decreasing number of endophytic fungal taxa with increasing total phenolic content (Huang et al. 2008). Furthermore, we did not confirm a reduction in fungal pathogen load with increasing defence levels such as content in phenolics, which has been shown in several other studies (Blodgett et al. 2005; Pociecha et al. 2009; Kröner et al. 2012). Furthermore, the absence of effects of foliar carbon and nitrogen contents on pathogen load was unexpected since higher foliar nitrogen content has been shown, for instance, to increase fungal development and disease severity of the Swiss needle cast disease (El-Hajj et al. 2004). In addition, reduced leaf nitrogen content and increased carbon to nitrogen ratio or tannin

content has been determined to decrease *Phyllosticta minima* disease severity of *Acer rubrum* (McElrone et al. 2005).

In contrast to our prediction, tree species' geographical distribution range size showed no impact on pathogen richness or pathogen load. Consistent to our results, there were also no effects of geographic range size of host trees for species richness of ectomycorrhizal fungi (Tedersoo et al. 2013). However, such a positive relationship has been shown previously for pathogen richness and range size of 124 plant species introduced to North America (Mitchell et al. 2010). In addition, the host species' geographic range size has been found to influence insect species richness (Jones & Lawton 1991; Lavandero et al. 2009).

High levels of phylogenetic conservatism in leaf defence traits and fungal infestation

We expected to find host defence leaf traits and the degree of fungal infestation to be phylogenetically conserved. The leaf content of tannins and total phenolics as well as pathogen richness and load were highly phylogenetically conserved. At least part of this high conservatism was brought about by the two disease-resistant gymnosperm species, which formed a strong contrast to all angiosperm tree species. Accordingly, phylogenetic signals are rarely described in the literature for pathogen or herbivore load. For example, Whitfield et al. (2012) did not encounter phylogenetically conserved patterns for the abundance of tropical insect herbivores in New Guinea. However, phylogenetic signals for symbiotic ectomycorrhizal fungi have recently been studied by Tedersoo et al. (2013). In their study 75 % variation of fungal species richness and 20 % variation of fungal community composition were explained by host phylogeny. In contrast to our findings, a phylogenetic signal of the host species' range size has been described for several plant pest and pathogen groups (Gilbert et al. 2012). Thus, further studies are required to provide more general conclusions of how phylogenetic relatedness affects host traits and biogeographical characteristics as well as fungal pathogens.

Conclusion

In conclusion, functional richness, functional evenness and functional divergence was not yet found to be important for fungal pathogen richness and pathogen load in these young mixed tree communities. The strong identity effects of disease-prone and disease-resistant host tree species on fungal pathogen richness and load point to a functional uniqueness of host tree species. Finally, leaf polyphenolics seem to play a more complex role as host defence traits than anticipated and might indicate an evolutionary arms race, which seem to be an important

mechanism for the evolution of biotrophic fungal pathogens on common temperate tree species.

4.6 Acknowledgements

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4.7 Appendices

Measurements of leaf traits

1. Physical traits: Morphological leaf traits can act as a physical defence against pathogens. In our analyses we included physical traits that were widely used in similar studies, i.e. specific leaf area (SLA [cm^2g^{-1}]), leaf thickness [mm], and leaf toughness [N]. The traits were measured for each of the 16 tree species (Table 1) on leaves or needles of a total of five plant individuals (from five different plots), according to the protocol provided by Cornelissen et al. (2003). The leaves or needles were collected from two opposite directed branches of the upper part of the crown in August 2010. For deciduous tree species, 2-4 outer canopy leaves (depending on tree size) per branch were sampled, including petiole and for *Fraxinus excelsior* the whole compound leaf. For coniferous tree species, needles of the current year's

section were taken from two terminal outer canopy shoots per branch. Ten needle pairs or 15 needles per shoot were sampled for *Pinus sylvestris* and *Larix decidua*, respectively. Only mature leaves and needles unaffected by herbivory or pathogens were collected. Leaf area was measured by leaf area meter (AM300, ADC BioScientific Ltd., Herts, England) or by digital scanning of the (full compound) leaves (including petiole and rachis) and needles, depending on tree species. Using the latter, leaf area was estimated by the Lafore program (free software, University of Oldenburg). SLA (cm^2g^{-1}) was calculated after oven-drying (60°C) of the fresh leaves as leaf area divided by leaf dry mass. Leaf thickness and leaf toughness were measured 4-5 times (depending on leaf size) on a subset of two leaves per branch, excluding rachis and leaf veins, or on two needles per shoot and branch, measure each needle once in the centre. Leaf thickness (mm) was measured by a digital micrometer (0-25 mm, TSB Kommunikationstechnik, Edling, Germany), whereas leaf toughness was analysed by dial tension gauge models with peak hold (546-135 DTG-50NP, range 0.06 N-0.5 N for thinner leaves, and 546-136 DTG-100NP, range 0.1 N-1 N for tougher leaves, Mitutoyo Messgeräte Leonberg GmbH, Leonberg, Germany).

2. Chemical traits: Phytochemicals determine both the nutritional quality and the chemical defence mechanisms of plant species. We determined leaf C and N content [%] and the C/N ratio [g/g], and the total phenolic and tannin content [mg g^{-1}] of the leaves and needles of the 16 tree species (Table 1). A second set of leaves and needles was sampled again on five individuals of each species in August 2010 using the same procedure as described above. Subsequent to the harvest fresh leaf material was air-dried and stored in the dark and under room temperature until further proceeding. Using a pooled sample per species, leaves and needles were dried over night at 40°C before ground for further analyses (Scheibenschwingmühle-TS, Siebtechnik, Germany). C and N content [%] as well as C/N ratio were determined with an elemental analyzer (vario El cube, Elementar, Hanau, Germany). In addition, total phenolic content was assessed using the Modified Prussian Blue

Method (Price & Butler 1977; Graham 1992) and total tannin content was determined by the Radial Diffusion Assay (Hagerman 2002) for increased sensitivity, but with minor modification as described by Eichenberg et al. (unpublished data).

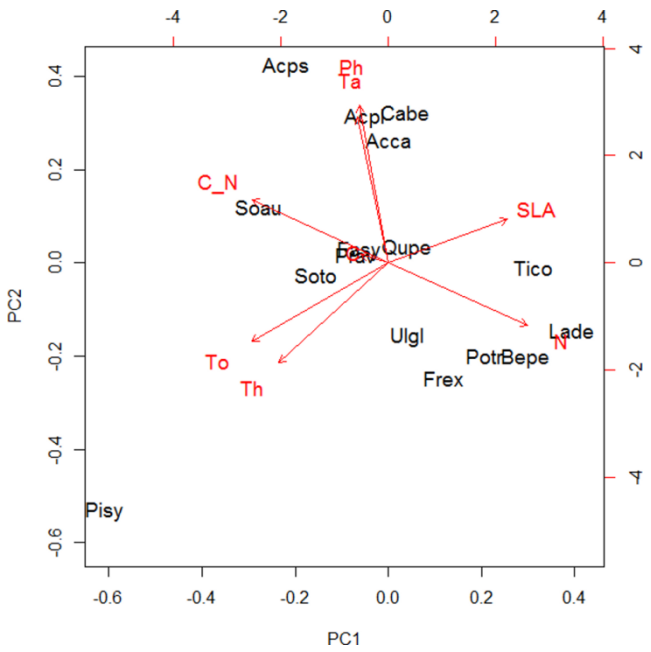

Figure 1 Principal components analysis showing the leaf economics spectrum on the first PCA axis. The analysis was based on species-mean leaf traits of all 16 tree species (n = 16). Leaf traits: C = Carbon, N = Nitrogen, Ph = Phenolics, SLA = Specific leaf area, Ta = Tannins, Th = Thickness, To = Toughness. Species abbreviations are given in Table 1.

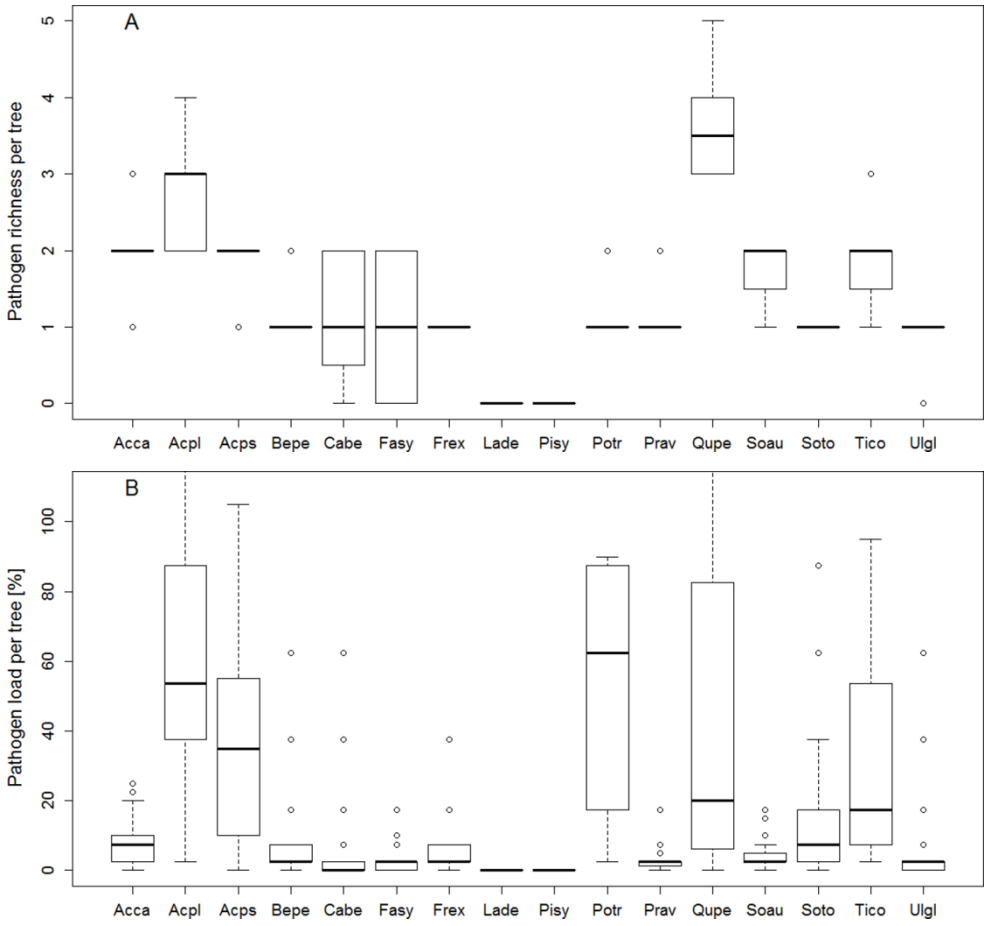

Figure 2 A) Mean pathogen richness and B) mean pathogen load [%] per tree individual (± SD), shown separately by tree species. Data were pooled over all sampled individuals per tree species (n = 8 to 18 per tree species; for exact n see Table 1).

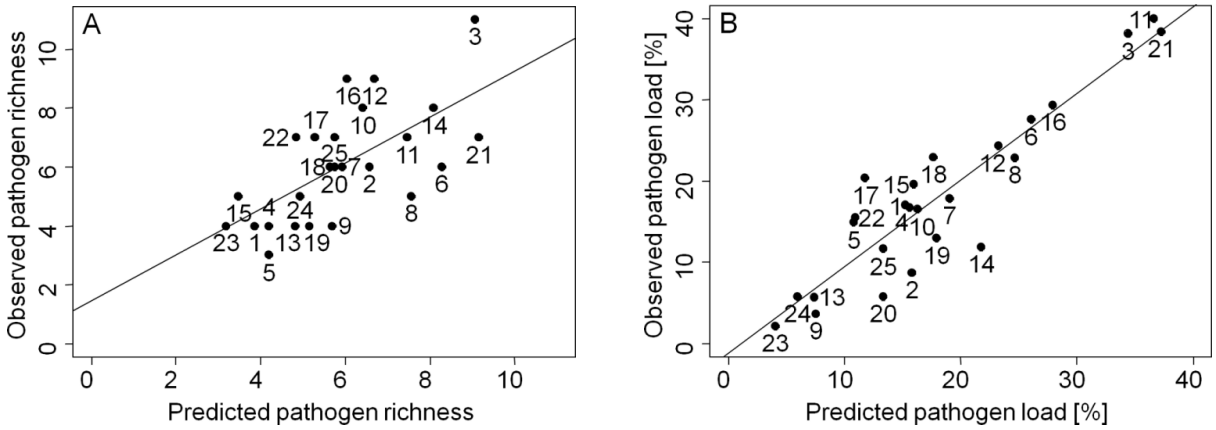

Figure 3 Mean observed vs. mean predicted A) pathogen richness ($p < 0.001$) and B) pathogen load [%] ($p < 0.001$) per plot (n = 25). For species combinations per plot see Scherer-Lorenzen et al. (2007).

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5. TREE CLONE DIVERSITY EFFECTS ON FOLIAR FUNGAL PATHOGENS IN A BIRCH-CLONE EXPERIMENT

Lydia Hantsch, Uwe Braun, Walter Durka, Julia Koricheva, Helge Bruelheide

(In preparation)

5.1 Abstract

Loss of species and genotype diversity affects ecosystem functioning in many ways, and hence, might also influence pathogen infestation. Genetic host diversity may play a central role for fungal diseases because pathogen transmission is less effective when susceptible and non-susceptible host genotypes are mixed in the community. We used a Finnish forest diversity experiment where communities were composed from different clones of *Betula pendula* to study the role of tree clone diversity on foliar fungal pathogen richness and infestation at different spatial scales. We hypothesised that at the *community level* pathogen species richness increases and pathogen load decreases with tree clone richness and clone diversity. In addition, we tested the hypothesis that pathogen load of a target birch clone increases with density of the same birch clone in the local neighbourhood. Finally, identity and density of susceptible or resistant birch clones were expected to increase or decrease pathogen richness and load, respectively, at all spatial scales. Leaves of randomly selected target trees were collected in monocultures, 2-, 4- and 8- clone mixtures and all visible foliar fungal pathogens on the leaf surface were identified micro- and macroscopically. Pathogen load was estimated separately per fungus species on each leaf. Statistical analyses revealed a positive tree clone richness effect on overall pathogen species richness at the *community level*, but negative effects on overall pathogen load of two birch clones. In contrast, increasing density of the same birch clone in the local neighbourhood of a target birch clone did not facilitate its overall pathogen load. Nevertheless, particular birch clones contributed disproportionally to pathogen load. While the density of susceptible birch clones increased pathogen richness and load at all spatial scales, less susceptible birch clones exhibited inverse effects. In conclusion, the encountered effects of tree clone richness on foliar fungal pathogen richness and load as well as identity and density effects of particular birch clones indicate the importance of genetic diversity for foliar fungal diseases and might provide fundamental knowledge for forest management practices in mono-specific stands.

Keywords
Atopospora betulina, biodiversity–ecosystem functioning, *Betula pendula*, clone density, clone diversity, clone identity, *Discula betulina*, pathogen load, pathogen richness, *Venturia ditricha*

5.2 Introduction

Observational and experimental studies have demonstrated that biodiversity contributes to the maintenance of ecosystem functioning (McCann 2000; Hooper et al. 2005; Cleland 2012). In agriculture and forestry, the key function of the ecosystem is productivity, which in turn is often linked to pathogen infestation as pathogens may cause enormous losses in biomass and grain yield (Akanda & Mundt 1997; Zhu et al. 2000; Berger et al. 2007; Fernández-Aparicio et al. 2009; Balmelli et al. 2013). Foliar fungal pathogens particularly have been shown to influence the productivity in grasslands through damages in photosynthetic capacity or root production (Mitchell 2003). In contrast, either forest and grassland diversity can contribute to community productivity through conferring an increased resistance to foliar fungal pathogen infection and infestation (Mitchell et al. 2003; Roscher et al. 2007; Hantsch et al. 2013).

When searching for biodiversity–trophic structure relationships to foliar fungal pathogens plant biodiversity has mainly been quantified as plant species diversity. However, there is an increasing concern that other levels of biodiversity such as the genetic diversity within species have been neglected (Pautasso et al. 2005). It is well-known that genetic variation is of fundamental importance for the fitness and adaptability of the plant population considered (Walker 1997; Cadotte et al. 2012), but it is much less known about the link to population functioning. For example, it has been demonstrated that genetically diverse populations are less vulnerable to diseases (Burdon 1987; Schmid 1994). Thus, we ask in this paper to which degree genetic diversity, expressed as the number and composition of intra-specific genotypes, has consequences for infestation by foliar fungal pathogens (Hooper et al. 2005; Hughes et al. 2008).

Human-managed agricultural and forest ecosystems with only one species or even one strain or clone often suffer from large yield losses. The use of single genotypes in a monoculture strongly selects pathogens to overcome these particular resistance genes, and thus, the high density of a particular common host genotype is a main driver of pathogen emergence (de Vallavieille-Pope 2004; Burdon & Thrall 2008; Stukenbrock & McDonald 2008). Hence, increased genotype diversity might reduce the selection pressure on specialist pathogens and

might be crucial for community stability (Dinoor & Eshed 1984; Garrett & Mundt 1999; Hajjar et al. 2008). Both host resistance and pathogen virulence have been found to be genotype-specific, indicating that host genotype frequency responds to pathogen genotype frequency and vice versa (Clay & Kover 1996; Liow et al. 2011). For instance, Davelos et al. (1996) presented differences in rust fungus disease severity among plants from different patches of a clonal prairie grass. In addition, Grünwald et al. (2008) showed for several *Viburnum* genotypes remarkable differences in susceptibility to *Phytophthora ramorum* leaf infection. As most of the foliar fungal pathogens exhibit high host-specificity (Barrett et al. 2009) particular host genotypes might be preferred by particular foliar fungal genotypes. In consequence, a community rich in host-genotypes should be also rich in different foliar fungal pathogen strains (Hudson et al. 2006; Bálint et al. 2013).

Genetic diversity of plant hosts does also play a role for slowing down the transmission rates because pathogen transmission is less effective when susceptible host individuals are more sparsely distributed in the plant community and diluted by less susceptible ones (Leonard 1969; Haas et al. 2011; Keesing et al. 2010). The presence of less susceptible or resistant plant host genotypes within the plant community increases the distance among susceptible host genotypes. Hence, host genotype mixture in the plant community dilutes the proportion of susceptible hosts and in consequence reduces the amount of susceptible tissue for a specialist pathogen (Leonard 1969; Wolfe 1985; Mundt 2002; Haas et al. 2011). In addition, the presence of resistant host genotypes among susceptible ones provides a physical barrier effect, and in turn limits pathogen transmission success and disease spread (Burdon 1978; Wolfe 1985; Garrett & Mundt 1999). Thus, the lower the density of a susceptible genotype in a community, the lower is the transmission and establishment success of the specialist pathogen (Wolfe 1985). Conversely, increased pathogen transmission, which might finally develop into pathogen epidemics, depends on the density of susceptible host genotypes (Burdon 1978, 1993; Burdon & Chilvers 1982; Hudson et al. 2006). In grasslands, higher host density has been shown to increase the transmission among host individuals of specialist rust fungi (Mitchell et al. 2002). A high genetic diversity has not only been shown to decrease disease severity and limit disease spread in grasslands (e.g. Alexander et al. 1984; Wolfe 1985; Schmid 1994; Zhu et al. 2000; Valério et al. 2004), but seems also to be important for tree populations (Pautasso et al. 2005). However, as not many studies on the genetic diversity-disease resistance relationship have been carried out in forest ecosystems, it is still unclear whether and to which degree increasing genetic diversity in tree hosts decreases foliar fungal pathogen infestation as compared to grasslands or croplands. In the understory of tropical

forests, density of particular genotypes could explain the variation in foliar fungal leaf damage (García-Guzmán & Dirzo 2006). Moreover, in tropical and temporal forests, the presence of adult hosts strongly increased tree host-specific fungal pathogen infestation, thereby increasing seedling mortality (Augspurger 1983; Packer & Clay 2000; Seiwa et al. 2008). The Janzen-Connell hypothesis describes a particular case of host density-dependent effects on pathogen infestation and describes how pathogens can maintain species coexistence and promote plant species diversity (Janzen 1970; Connell 1971).

If plant host genotypes differ in their susceptibility against specialist foliar fungal pathogens, the presence and proportions of different genotypes will determine the dynamics of pathogen communities and disease severity (Zhan et al. 2002). In particular, the presence of highly susceptible host genotypes might increase foliar fungal pathogen richness and infestation levels in a plant community, whereas the presence of resistant host genotypes or less susceptible ones might decrease diseases. Hence, the ideal host genotype community composition is one in which plant hosts susceptible to the same pathogen strain do not occur as neighbours (Wolfe 1985). Such genotype identity effects might be stronger than genetic diversity effects per se (Nadrowski et al. 2010; Mouillot et al. 2011).

This study was conducted to examine the role of genetic diversity in forests on foliar fungal pathogens. We used a birch-clone genetic diversity experiment in Finland to disentangle effects of tree clone richness, tree clone diversity, density of susceptible birch clones and identity of susceptible as well as resistant birch clones on pathogen richness and infestation of foliar fungal pathogens. We tested the following hypotheses: At the *community level* we hypothesized that 1a) increasing tree clone richness increases foliar fungal pathogen species richness and decreases pathogen load. Regarding the local neighbourhood we hypothesized that 1b) either increasing tree clone richness or tree clone diversity increase pathogen species richness, but decrease pathogen load. In addition, 2) we tested the hypothesis that pathogen load of a target birch clone increases with density of the same birch clone in the local neighbourhood. Finally, 3a) the presence at the *community level* and 3b) increasing density of susceptible birch clones different from that of the target birch clone in the local neighbourhood are hypothesised to increase both pathogen richness and pathogen load on the target birch clone, whereas the reverse is expected for resistant birch clones.

5.3 Material and Methods

Study site

The birch-clone genetic diversity experiment in Satakunta was established in summer 2000 in SW Finland (61.69°N, 22.01°E) to investigate the impact of genetic diversity of different *Betula pendula* clones on ecosystem functioning. In particular, the main goal was to evaluate the role of tree clone diversity on tree growth, leaf anatomy, phenology, defensive traits, insect herbivory, natural enemies and clone survival. Hence, different birch clones have been selected that were known to display different resistance to mammalian herbivores and to pathogens (see Table 1). In the following we refer to the clone by clone ID (e.g. Blue, Red etc.) and not by the proper clone name. The full experimental design has been described by Scherer-Lorenzen et al. (2005), thus here we only summarize the features of the experiment that are important to our study. In total, 49 plots were randomly established on an approximately 2 ha clear-cut area, thereby including a tree genetic diversity gradient (Figure 1A). Using a pool of micro-propagated saplings of eight clones of silver birch, eight different single-clone communities, each five different 2- and 4-clone mixtures and one 8-clone mixture were planted. Each clone combination was replicated two or three times with exception of the monocultures of the clones Blue, Violet and Pink, which were not replicated, and the 8-clone mixture, which was replicated six times. The plot size was 400 m² (20 m * 20 m) comprising in total 100 randomly planted tree individuals (Figure 1B). Plastic vole protectors (Agrame Oy, Finland) were used to reduce early mortality caused by vole damage. Because of mortality approximately 25 % of plants were replaced in 2001–2003. In addition, plots were weeded in 2005 and 2009 to remove naturally regenerating woody plants but otherwise were kept untreated.

Leaf sampling

We used a subset of 36 plots out of the total of all 49 plots for foliar fungal pathogen analysis (see Figure 1B). We selected two replicates per clone combination with exception of the Blue-, Violet- and Pink-monoculture, where no replication was available, and the Orange-Yellow 2-clone mixture which we sampled three times. In each plot, five individuals per birch clone were randomly selected, but obviating the use of the outward-positioned tree individuals to avoid edge effects. Moreover, to allow for testing local neighbourhood effects, we ensured that all eight potential tree individuals in the local neighbourhood of a target tree individual were present. However, in some cases we had to select also target trees that were located on the edge of the plot or that were surrounded by dead individuals. In some cases we sampled

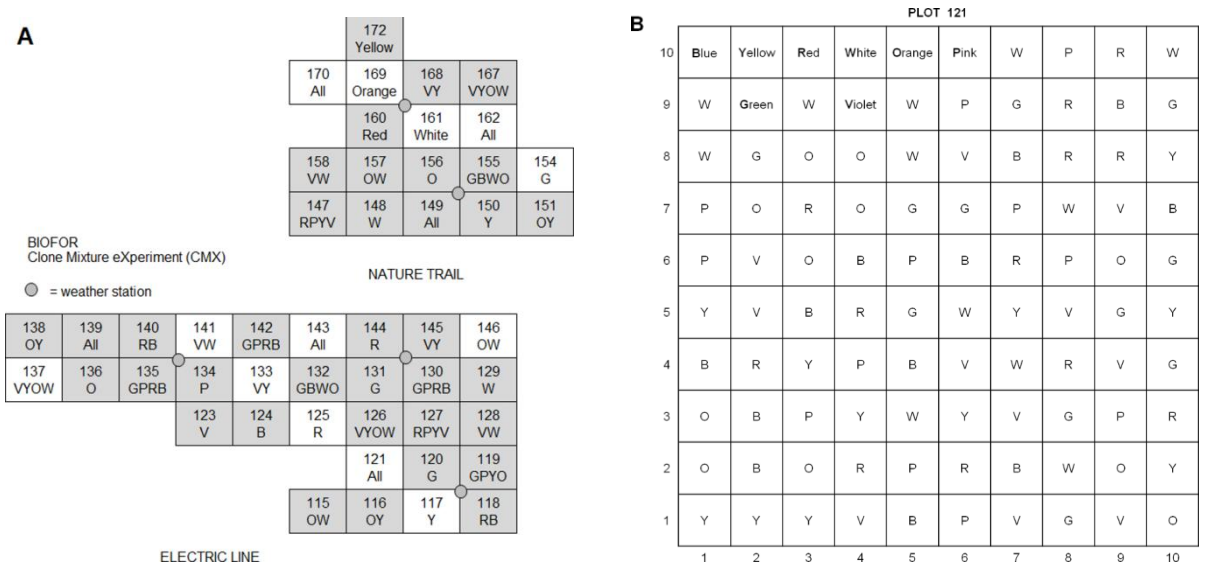

Figure 1 A) Experimental design of the Satakunta birch clone diversity experiment, comprising 49 plots of different clone compositions and a gradient in clone richness spanning from monocultures, to 2-, 4- and 8-clone mixtures. Birch clone identities are abbreviated with letters. B) Example of one plot, with a size of 10 m * 10 m and a total of 100 randomly planted individuals with similar proportions of the different birch clones.

less than five individuals per birch clone in a plot, since not more individuals of that clone had survived. To compensate for an uneven representation of clones, we selected 10 target individuals in the Blue, Violet and Pink monocultures and in one Orange-Yellow 2-clone mixture. Overall, the number of sampled individuals differed between birch clones ($n_{Blue} = 44$, $n_{Green} = 28$, $n_{Orange} = 80$, $n_{Pink} = 31$, $n_{Red} = 46$, $n_{Violet} = 59$, $n_{White} = 58$, $n_{yellow} = 79$). On each of the 425 target tree individuals, two opposite branches in the upper as well as in the lower part of the crown were chosen to randomly sample five leaves per branch. The collected leaves were dried at 60°C for three days to avoid the establishment of mould fungi and were then stored in the dark at room temperature.

Macro- and microscopic analyses

Macro- and microscopic analyses were done on a random subset of five leaves per tree individual. As this study focused on foliar fungal pathogens on the leaf surface all visible saprophytic and epiphytic fungi as well as non-visible endophytic fungi were excluded. Using light microscopy, fungal pathogen taxa were indentified to the species level (after Brandenburger 1985; Ellis & Ellis 1997; Braun & Cook 2012). Furthermore, using stereomicroscopy, fungal pathogen infestation of all fungus taxa was surveyed on the upper and lower leaf surface. We visually estimated the total damaged area caused by each fungus species by rating on a scale with seven damage classes: 0 %, 1-5 %, 6-10 %, 11-25 %, 26-50 %, 51-75 % and 76-100 % (Schuldt et al. 2012; Hantsch et al. 2013). The total fungal pathogen infestation per leaf was calculated by summing up percentage damages of all present

fungi. The total fungal infestation per individual was averaged by using a mean of fungal infestation of all analysed leaves of a particular individual.

Data analysis

Data analysis was conducted at four different hierarchical levels. At the *community level*, fungal pathogen species richness and pathogen load were related to plot tree clone richness. Hence, in a first linear model we tested effects of plot clone richness on pathogen richness and pathogen load, respectively. In a second model, the remaining variance of the residuals of the first model was tested for tree species identity effects by the presence of a particular birch clones within the plot.

Regarding the local neighbourhood, tree clone richness, tree clone diversity (Shannon diversity) and tree clone density were calculated from all eight neighbour tree individuals around a target tree, excluding the target tree. Thus, tree clone richness varied between 1 and 8, Shannon diversity of tree clones between 0 and 2.08 and tree clone density between 0 and 1. Random planting pattern within the plot led to differences in birch clone composition at the local neighbourhood, also when comparing plots with the same birch clone combinations. At the *individual level* and *tree clone level*, tree clone richness, tree clone diversity and tree clone density of the local neighbourhood were tested in parallel approaches for effects on overall foliar fungal pathogen species richness and pathogen load of all target tree individuals and separately by birch clones, respectively. In addition, we used the mean foliar fungal pathogen richness and pathogen load on the different birch clones across all monocultures and the proportion of each clone in every plot to predict the observed pathogen richness and load, respectively, for every plot. At the *fungus species level*, the same factors were tested for effects on pathogen load separately for every fungus species. All analyses at the local neighbourhood were done with linear mixed effect models (*lme*; package nlme; Pinheiro 2013), including plot as random factor and using a Gaussian error distribution (R Core Team 2013). In addition, the proportion of total variation explained by the model was obtained from regression predicted against observed responses. All Figures were produced with R 3.0.0 (R Core Team 2013).

5.4 Results

In total, three different parasitic foliar fungus species were observed. The most abundant fungus species on all silver birch clones was *Discula betulina* (J. Kickx f.) Arx with a mean and maximum pathogen load of 19.8 % and 53.5 %, respectively. Another frequent fungus

species was *Venturia ditricha* (Fr.) P. Karst. with a mean of 2.8 % and up to 20.5 % pathogen load. In contrast, *Atopospora betulina* (Fr.) Petr. was a very rare and less abundant fungus species, with a mean pathogen load below 1 %. Occurrence and load of these three fungus species was significantly different between the eight birch clones. *Discula betulina* was detected on every tree investigated and was most abundant on the clones White, Violet and Yellow (Figure 2A). *Venturia ditricha* occurred on every tree of the clone Orange and was also most abundant there, while it was almost absent on the clone Red (Figure 2B). *Atopospora betulina* was absent on the clones Blue, Green and Yellow (Figure 2C). Among all birch clones, Red and Green exhibited the lowest values of mean pathogen species richness, while Red, Pink and Blue showed the lowest mean values of pathogen load (Table 1). In contrast, the clones Orange and Violet showed the highest mean values of pathogen richness and the clones White, Violet and Yellow the highest values of mean pathogen load (Table 1).

At the *community level*, the statistical analyses revealed a positive effect of plot tree clone richness on pathogen species richness, indicating an increase in the number of fungus species with increasing birch clone availability (Table 2; Figure 3). This pattern was mainly caused by the fact that at maximum two of the tree fungus species were encountered in any of the monoculture plots. In addition, the presence of the clone Red in a mixture decreased overall pathogen richness in the plot, whereas all other birch clones did not show any identity effects neither on pathogen richness nor on pathogen load (Table 2). In contrast, tree clone richness did not affect overall pathogen load (Table 2). This is reflected in predictions which explain the importance of birch clone identity to overall plot's pathogen richness and load (Figure 4). These predictions were based on particular mean response values assessed on all target

Table 1 Silver birch clone properties and measured mean foliar fungal pathogen species richness and mean overall pathogen load (%) as well as of the three fungus species per individual for all eight birch clones. Height (cm) was measured only in monoclonal plots in 2006.

Clone ID	**Clone name**	**Height +/- SE (cm)**	**Mean number of pathogen species**	**Mean pathogen load (%)**	**Mean load (%) of *Discula betulina***	**Mean load (%) of *Venturia ditricha***	**Mean load (%) of *Atopospora betulina***
Blue	K5834	406.5±17.7	1.98	15.80	13.66	2.14	-
Green	JR ¼	134.7±16.0	1.75	22.20	21.20	1.00	-
Orange	36	393.4±10.0	2.05	22.35	15.73	6.59	0.03
Pink	K2674	317.8±13.8	1.84	14.77	13.91	0.81	0.02
Red	O154	325.4±8.56	1.15	12.76	12.69	0.07	0.01
Violet	V5818	450.4±22.1	2.02	27.88	24.29	3.55	0.04
White	K1659	397.6±10.1	1.86	29.33	27.91	1.34	0.03
Yellow	V5952	423.1±8.85	1.96	26.70	23.86	2.83	-

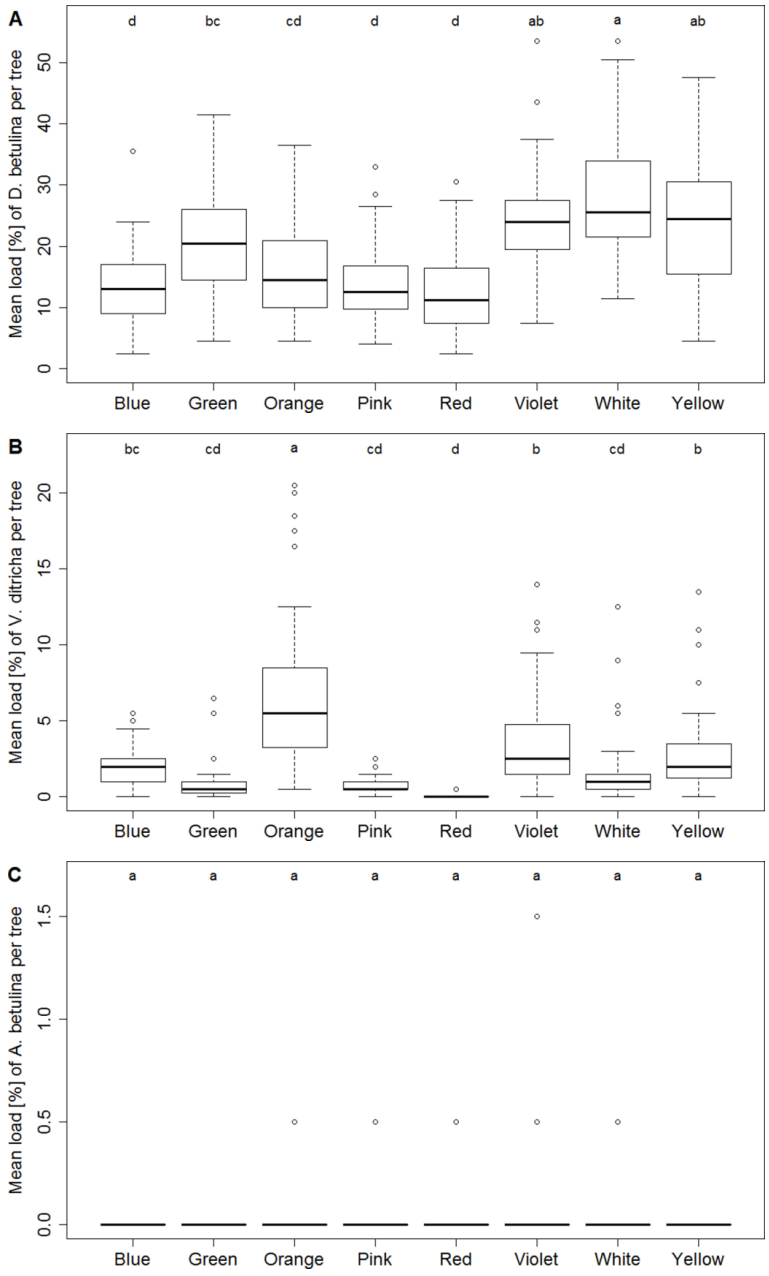

Figure 2 Mean pathogen load (%) of A) *Discula betulina*, B) *Venturia ditricha*, C) *Atopospora betulina* encountered on all target individuals for all eight clones. Small letters show significantly different differences after a Tukey post-hoc test.

individuals for a particular birch clone in its monocultures, which were averaged for all birch clones planted in a plot regarding the tree clone richness. The observed mean pathogen richness across all target individuals in a plot is well predicted from the mean pathogen richness on the different birch clones (Figure 4A). This was also the case for the observed pathogen load, which could closely be predicted from the birch clone-specific mean load values in the monocultures and the proportions of these clones in every plot (Figure 4B).

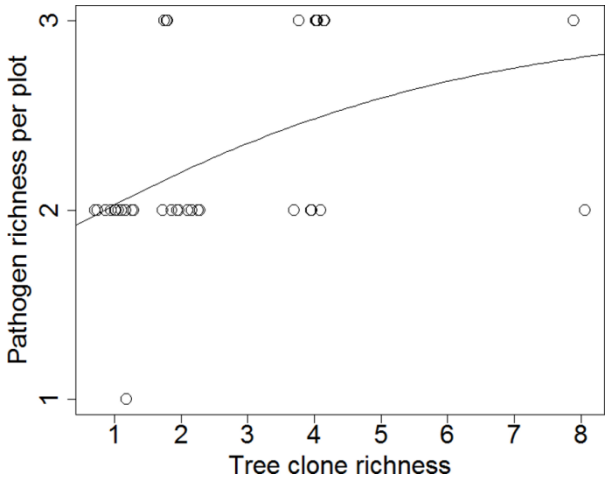

Figure 3 BEF relationship between tree clone richness and pathogen species richness of each plot at *community level* (n = 36).

At the *individual level*, both tree clone richness and tree clone diversity in the local neighbourhood did neither affect pathogen species richness nor pathogen load (Table 3). However, increasing density of the clone Yellow in the local neighbourhood increased the pathogen richness of target individuals across all clones, whereas the density of the clone Red in the local neighbourhood decreased pathogen richness (Table 3). Similar density effects were encountered for pathogen load, as an increasing density of the clones Violet or Blue in the local neighbourhood increased or decreased overall pathogen load, respectively (Table 3).

Table 2 Linear model results at the *community level* for the effect of plot tree clone richness on plot foliar fungal pathogen richness and load (%) (n = 36). Pathogen richness refers to the number of fungus species encountered in a plot. In a subsequent model, remaining variance of the first model's residuals was tested for birch clone identity effects by the presence of a particular birch clone in a particular plot. Significant results are indicated in bold fonts.

		Pathogen richness		**Pathogen load [%]**	
Model	**Predictor**	Estimate	P	Estimate	p
1	Intercept	1.937	<0.001	23.215	<0.001
	Plot clone richness	0.124	0.006	-0.21	0.757
2	Blue	0.111	0.637	-5.016	0.128
	Green	-0.039	0.86	4.003	0.197
	Orange	0.028	0.884	-4.683	0.081
	Pink	-0.012	0.961	-2.506	0.452
	Red	-0.472	0.039	-4.344	0.159
	Violet	0.24	0.264	2.708	0.359
	White	0.139	0.485	4.723	0.092
	Yellow	-0.022	0.912	4.22	0.124

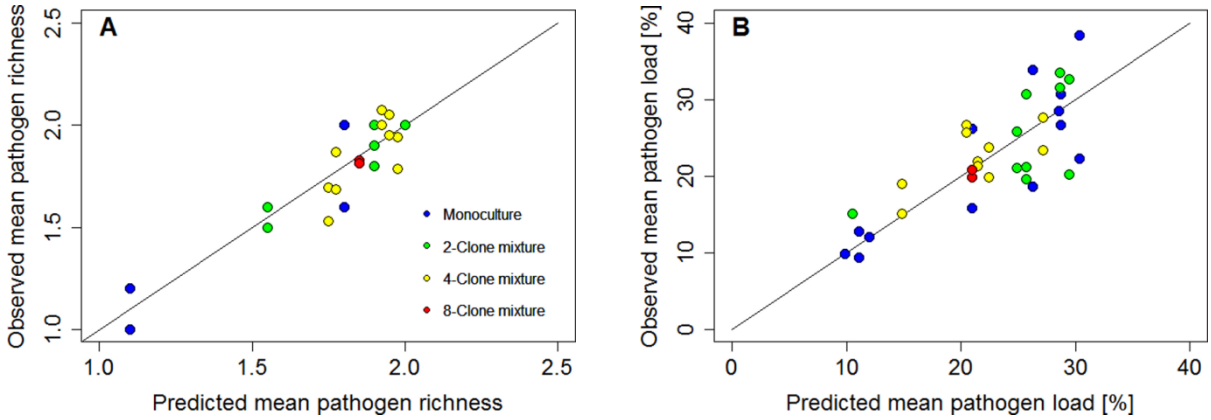

Figure 4 A) Observed vs. predicted foliar fungal pathogen richness and B) observed vs. predicted foliar fungal pathogen load of all plots at *community level* (n = 36). Note that some dots for some plots are hidden behind others. The predictions are based on birch clone-specific mean richness and load values, respectively, of the monocultures and on the proportions of these birch clones in every plot. The lines are 1:1 lines and indicate a perfect prediction.

Table 3 Linear mixed effect model results at the *individual level* for effects of tree clone richness, tree clone diversity and tree clone density in the local neighbourhood on pathogen richness and pathogen load (%) encountered on all target individuals (n = 425). Significant results are indicated in bold fonts.

	Pathogen richness		**Pathogen load [%]**	
Predictor	Estimate	p	Estimate	p
Intercept	1.825	<0.001	22.134	<0.001
Neighbourhood richness	0.013	0.559	0.221	0.724
Intercept	1.856	<0.001	22.282	<0.001
Neighbourhood diversity	0.002	0.976	0.569	0.756
Intercept	1.866	<0.001	23.608	<0.001
Blue density	-0.092	0.517	-9.722	0.01
Intercept	1.864	<0.001	22.612	<0.001
Green density	-0.071	0.607	0.474	0.9
Intercept	1.823	<0.001	22.264	<0.001
Orange density	0.204	0.068	2.311	0.473
Intercept	1.864	<0.001	23.035	<0.001
Pink density	-0.09	0.554	-4.773	0.25
Intercept	1.944	<0.001	23.514	<0.001
Red density	-0.769	<0.001	-7.513	0.034
Intercept	1.836	<0.001	21.518	<0.001
Violet density	0.173	0.196	9.208	0.012
Intercept	1.852	<0.001	22.343	<0.001
White density	0.034	0.775	2.178	0.511
Intercept	1.812	<0.001	22.18	<0.001
Yellow density	0.271	0.017	2.795	0.383

Separate analyses per birch clone at the *tree clone level*, revealed no effect of tree clone richness and tree clone diversity on pathogen species richness of any clone (Table 4). However, both tree clone richness and tree clone diversity had a positive effect on pathogen load of the Blue clone (Table 4). In contrast, tree clone richness significantly affected pathogen load of the clone Green and marginally that of the clone Violet, with decreasing pathogen load with increasing genotype richness (Table 4). There was no effect of the density of a particular birch clone in the local neighbourhood of a target tree individual on the pathogen richness and pathogen load of the same target birch clone (Table 4). However, neighbourhood analyses of particular birch clones revealed positive density effects of the clones Blue and Violet and negative effects of the clones Green and Orange on pathogen richness of particular clones (Table 4). For instance, pathogen richness of the clone Orange increased with growing densities of Blue and Violet in the local neighbourhood. In addition, pathogen richness of the clone Red increased with the density of the clone Violet. In contrast, pathogen richness of the clone Violet was diminished with increasing density of the clones Green and Orange, and pathogen richness of the clone Yellow which decreased with increasing density of the clone Green in the local neighbourhood. Similar patterns were encountered for pathogen load of particular clones, which in many cases was positively or negatively influenced by the density of particular birch clones in the local neighbourhood (Table 4). For instance, pathogen load of the clones Blue and Red increased with the density of the clone Yellow. In contrast, pathogen load of the clone Violet was reduced by increasing density of the clones Blue and Orange in the local neighbourhood, whereas the clone Yellow was negatively affected by the density of the clone Green.

At the *fungus species level*, we could not detect any effects of tree clone richness and tree clone diversity of the local neighbourhood on the three fungus species (Table 5). As for overall pathogen load, pathogen load of the most abundant fungus *D. betulina* either increased or decreased with growing density of the clones Violet or Blue in the local neighbourhood, respectively (Table 5). In addition, pathogen load of *V. ditricha* became larger with the density of the clone Orange in the local neighbourhood, but decreased with the presence of the clone Red. Pathogen load of *A. betulina* was unaffected by the density of any particular birch clone.

Table 4 Linear mixed effect model results at the *tree clone level* for effects of tree clone richness, tree clone diversity and tree clone density in the local neighbourhood on pathogen richness and pathogen load (%) encountered on all target individuals, separately for all eight birch clones. Significant results are indicated in bold fonts. D = Density, E = Estimate, Nh = Neighbourhood.

		Blue (n = 44)		**Green** (n = 28)		**Orange** (n = 80)		**Pink** (n = 31)		**Red** (n = 46)		**Violet** (n = 59)		**White** (n = 58)		**Yellow** (n = 79)	
Variable	**Predictor**	E	p	E	p	E	p	E	p	E	p	E	p	E	p	E	p
Pathogen richness	Intercept	2.007	<0.001	1.726	<0.001	2.033	<0.001	2.033	<0.001	1.109	<0.001	2.142	<0.001	1.702	<0.001	1.982	<0.001
	Nh richness	-0.01	0.519	0.005	0.937	0.006	0.739	-0.065	0.209	0.014	0.736	-0.043	0.09	0.053	0.17	-0.007	0.645
	Intercept	2.005	<0.001	1.821	<0.001	2.021	<0.001	1.992	<0.001	1.115	<0.001	2.111	<0.001	1.749	<0.001	1.997	<0.001
	Nh diversity	-0.032	0.457	-0.086	0.607	0.034	0.557	-0.18	0.177	0.038	0.737	-0.104	0.161	0.127	0.267	-0.039	0.38
	Intercept	1.953	<0.001	1.8	<0.001	2.013	<0.001	1.891	<0.001	1.154	<0.001	2.04	<0.001	1.837	<0.001	1.958	<0.001
	Blue D	0.058	0.42	-0.617	0.315	0.636	**<0.001**	-0.566	0.246	-0.023	0.936	-0.685	0.052	0.376	0.497	0.181	0.572
	Intercept	1.99	<0.001	1.582	<0.001	2.072	<0.001	1.849	<0.001	1.137	<0.001	2.029	<0.001	1.894	<0.001	1.985	<0.001
	Green D	-0.11	0.432	0.365	0.159	-0.245	0.206	-0.118	0.849	0.182	0.706	-1.491	**0.049**	-0.561	0.367	-0.559	**0.017**
	Intercept	1.969	<0.001	1.754	<0.001	2.079	<0.001	1.851	<0.001	1.147	<0.001	2.12	<0.001	1.829	<0.001	1.955	<0.001
	Orange D	0.115	0.575	-0.122	0.867	-0.07	0.489	-0.27	0.683	0.147	0.883	-1.513	**<0.001**	0.166	0.566	0.032	0.705
	Intercept	2.002	<0.001	1.815	<0.001	2.067	<0.001	1.659	<0.001	1.23	<0.001	2.021	<0.001	1.858	<0.001	1.957	<0.001
	Pink D	-0.428	0.057	-0.446	0.471	-0.32	0.281	0.388	0.066	-0.637	0.093	-0.049	0.834	0.213	0.835	0.074	0.705
	Intercept	1.992	<0.001	1.754	<0.001	2.048	<0.001	1.949	<0.001	1.217	<0.001	2.033	<0.001	1.848	<0.001	1.972	<0.001
	Red D	-0.071	0.498	-0.096	0.869	0.21	0.673	-1.379	0.056	-0.164	0.375	-0.226	0.379	0.947	0.483	-0.186	0.303
	Intercept	1.974	<0.001	1.718	<0.001	2.008	<0.001	1.884	<0.001	1.052	<0.001	2.018	<0.001	1.877	<0.001	1.954	<0.001
	Violet D	0.11	0.733	1.333	0.541	0.816	**0.002**	-0.539	0.367	1.18	**0.011**	>-0.001	0.998	-0.098	0.741	0.056	0.604
	Intercept	1.97	<0.001	1.808	<0.001	2.072	<0.001	1.839	<0.001	1.159	<0.001	1.977	<0.001	1.928	<0.001	1.97	<0.001
	White D	0.092	0.607	-1.617	0.145	-0.13	0.358	-0.015	0.987	-0.294	0.719	0.239	0.07	-0.16	0.421	-0.133	0.414
	Intercept	1.974	<0.001	1.763	<0.001	2.062	<0.001	1.872	<0.001	1.096	<0.001	1.982	<0.001	1.825	<0.001	1.942	<0.001
	Yellow D	0.131	0.741	-0.381	0.679	-0.048	0.686	-0.43	0.509	0.754	0.121	0.162	0.321	0.519	0.258	0.051	0.524

		Blue (n = 44)		**Green** (n = 28)		**Orange** (n = 80)		**Pink** (n = 31)		**Red** (n = 46)		**Violet** (n = 59)		**White** (n = 58)		**Yellow** (n = 79)	
Variable	**Predictor**	E	p	E	p	E	p	E	p	E	p	E	p	E	p	E	p
Pathogen load [%]	Intercept	11.204	<0.001	29.36	<0.001	22.603	<0.001	12.051	<0.001	13.152	<0.001	32.713	<0.001	28.993	<0.001	27.187	<0.001
	Nh richness	1.65	**0.047**	-2.357	**0.048**	-0.163	0.834	0.904	0.324	-0.123	0.88	-1.707	0.055	0.1	0.91	-0.275	0.803
	Intercept	11.522	<0.001	27.187	<0.001	21.824	<0.001	12.179	<0.001	12.507	<0.001	31.192	<0.001	28.64	<0.001	28.389	<0.001
	Nh diversity	5.109	**0.025**	-5.939	0.102	0.333	0.888	2.982	0.187	0.295	0.897	-3.855	0.117	0.729	0.782	-2.209	0.506
	Intercept	18.472	<0.001	22.169	<0.001	22.275	<0.001	15.796	<0.001	13.632	<0.001	28.831	<0.001	29.342	<0.001	26.628	<0.001
	Blue D	-5.458	0.163	-6.549	0.567	-2.547	0.766	-8.148	0.356	-4.541	0.413	-28.734	**0.05**	-0.657	0.956	-10.716	0.621
	Intercept	16.419	<0.001	18.053	<0.001	22.148	<0.001	13.687	<0.001	12.647	<0.001	27.811	<0.001	30.017	<0.001	27.625	<0.001
	Green D	1.387	0.839	8.467	0.159	-0.259	0.972	11.412	0.269	1.846	0.843	8.343	0.794	-12.236	0.337	-28.694	**0.047**
	Intercept	16.556	<0.001	23.198	<0.001	22.414	<0.001	14.783	<0.001	13.092	<0.001	29.512	<0.001	29.723	<0.001	25.259	<0.001
	Orange D	0.783	0.942	-17.438	0.206	-0.752	0.854	2.135	0.845	-18.491	0.325	-26.531	**0.008**	-2.146	0.729	5.486	0.408
	Intercept	17.956	<0.001	22.696	<0.001	21.692	<0.001	16.71	<0.001	12.595	<0.001	27.414	<0.001	29.057	<0.001	25.26	<0.001
	Pink D	-19.238	0.129	-6.738	0.565	9.76	0.397	-4.211	0.23	1.414	0.838	7.07	0.452	9.969	0.651	16.422	0.182
	Intercept	15.946	<0.001	20.494	<0.001	22.007	<0.001	13.349	<0.001	13.859	<0.001	28.321	<0.001	29.168	<0.001	26.367	<0.001
	Red D	2.912	0.615	9.049	0.384	7.6	0.7	18.241	0.113	-2.647	0.467	-6.57	0.528	8.388	0.763	-0.105	0.993
	Intercept	15.989	<0.001	22.452	<0.001	22.235	<0.001	13.994	<0.001	10.98	<0.001	25.712	<0.001	27.785	<0.001	25.401	<0.001
	Violet D	19.535	0.22	-44.651	0.253	-2.088	0.832	9.783	0.299	21.647	**0.012**	5.609	0.125	9.749	0.147	6.274	0.444
	Intercept	16.837	<0.001	22.003	<0.001	22.634	<0.001	14.89	<0.001	13.285	<0.001	27.125	<0.001	31.297	<0.001	26.486	<0.001
	White D	-2.535	0.772	-10.948	0.622	-3.306	0.538	2.106	0.882	-16.13	0.305	4.518	0.415	-4.896	0.281	-1.876	0.87
	Intercept	15.445	<0.001	22.202	<0.001	21.539	<0.001	14.864	<0.001	11.322	<0.001	29.454	<0.001	28.395	<0.001	28.871	<0.001
	Yellow D	46.433	**0.011**	-11.906	0.464	2.864	0.546	0.99	0.926	20.362	**0.014**	-7.496	0.211	12.632	0.198	-6.493	0.294

Table 5 Linear mixed effect model results at the *fungus species level* for effects of tree clone richness, tree clone diversity and tree clone density in the local neighbourhood on pathogen load (%) encountered on all target individuals, separately for all fungus species (n = 425). Significant results are indicated in bold fonts.

	Discula betulina		***Venturia ditricha***		***Atopospora betulina***	
Predictor	Estimate	P	Estimate	p	Estimate	p
Intercept	19.143	<0.001	2.975	<0.001	0.011	0.325
Neighbourhood richness	0.31	0.609	-0.087	0.666	0.002	0.587
Intercept	19.415	<0.001	2.853	<0.001	0.01	0.316
Neighbourhood diversity	0.702	0.691	-0.128	0.826	0.007	0.438
Intercept	20.677	<0.001	2.902	<0.001	0.016	0.006
Blue density	-8.203	**0.025**	-1.375	0.286	0.004	0.883
Intercept	19.663	<0.001	2.94	<0.001	0.018	0.002
Green density	2.187	0.549	-1.754	0.146	-0.017	0.542
Intercept	19.85	<0.001	2.068	<0.001	0.016	0.013
Orange density	0.181	0.954	4.19	**<0.001**	0.003	0.902
Intercept	20.212	<0.001	2.815	<0.001	0.019	0.001
Pink density	-4.217	0.295	-0.598	0.659	-0.028	0.318
Intercept	20.456	<0.001	3.027	<0.001	0.019	0.001
Red density	-5.049	0.151	-2.268	**0.048**	-0.027	0.29
Intercept	18.834	<0.001	2.665	<0.001	0.017	0.006
Violet density	8.423	**0.018**	0.825	0.51	-0.002	0.94
Intercept	19.538	<0.001	2.785	<0.001	0.013	0.032
White density	2.35	0.461	-0.126	0.908	0.026	0.26
Intercept	19.615	<0.001	2.578	<0.001	0.013	0.049
Yellow density	1.543	0.62	1.108	0.289	0.022	0.339

5.5 Discussion

Our study demonstrated that genetic diversity affected foliar fungal pathogen richness and pathogen infestation of *Betula pendula*. The main effects observed were based on different but clone-specific degrees of susceptibility to different foliar fungal pathogens. In addition, pathogen richness and infestation of a particular birch clone individual depended on the local neighbourhood of that individual, with either higher of lower pathogen richness and load, depending on the abundance of the other birch clones in the local neighbourhood.

Tree clone diversity effects

Tree clone richness, as determined by the number of different birch clones present in a plot, positively affected fungal species richness at *community level*, thus confirming the first part of the first hypothesis. There were large differences in pathogen richness and load between the different clones, which translated into different overall plot pathogen richness and load. These host-specific differences result in a higher fungal richness of host-rich communities (Hudson et al. 2006; Barrett et al. 2009). In contrast, tree clone richness and tree clone diversity in the local neighbourhood did not influence pathogen richness. Hence, we have to reject the second part of our first hypothesis. At the *community level*, pathogen richness and load was mainly

determined by the characteristics of the clones that were planted in the plot, rather than by the particular local neighbourhood composition.

However at a smaller scale, the *tree clone level*, we observed an increase in pathogen load of the clone Blue by both tree clone richness and tree clone diversity in the local neighbourhood, and a decrease in pathogen load of the clones Green and Violet with increasing tree clone richness. For example, pathogen infestation of *Golovinomyces cichoracearum* (≡ *Erysiphe cichoracearum*) within mono- and polyclonal patches of *Solidago altissima* was also reduced with increasing stand diversity (Schmid 1994). In addition, rice blast infestation was lowest in mixtures of resistant and susceptible rice varieties compared to susceptible rice monocultures (Zhu et al. 2000). Similar results have been received for stem rust infestation on oats (Browning & Frey 1969).

At the finest scale, the *fungus species level*, we encountered strong clone-specific differences on pathogen load of the three fungus species observed in our study. This highly clone-specific susceptibility to a particular fungus species confirms the results of preceding studies on foliar fungal pathogens on birch, for instance for *Venturia ditricha*, which showed a different degree of infection on different birch genotypes of *Betula pubescens* and *B. pendula* (Poteri et al. 2001; Ahlholm et al. 2002). However, as Ahlholm et al. (2002) pointed out, the susceptibility of a particular birch genotype may additionally depend on environmental conditions. Thus, further biotic or abiotic impacts might shape the observed pathogen-host relationships (Warren & Mordecai 2010), as was shown, for example, for the impact of particularly warm or cold years on the pathogen load on *Tilia cordata* and *Quercus petraea* (Hantsch et al. unpublished data). Further modifications can be expected with biotic interactions, such as different degrees of competition for resources or facilitation of pathogen infection through injuries brought about by herbivores (García-Gutmán & Dirzo 2001; Hall et al. 2009).

Density effects of the most compatible birch clone

Against expectation, the density of a particular birch clone genotype had no impact on foliar fungal pathogen infestation by the same birch clone genotype in the local neighbourhood. Thus, the second hypothesis has to be rejected. This was an unexpected result since several studies showed host-density dependent effects on pathogen infection and infestation (e.g. Mundt & Leonard 1985; García-Guzmán & Dirzo 2006). For instance, pathogen infestation of *Prunus grayana* seedlings was highest in the nearest neighbourhood of adult conspecifics (Seiwa et al. 2008). However, Mundt and Browning (1985) already postulated that density effects of most compatible genotypes were present mainly in the beginning of the season,

whereas the importance of barrier effects and induced resistance increased during the season. Thus, as we collected leaves at the end of the growing season, density effects that might have been present earlier in the season might have been missed.

Identity and density effects of birch clones

We observed identity and density effects of particular susceptible or less susceptible birch clones. At the *community level*, the presence of the less susceptible clone Red reduced overall pathogen richness in the plot, because admixing this clone reduced the number of susceptible host trees available for fungal pathogens in a plot. In addition to this identity effect, overall pathogen richness at the *individual level* decreased with increasing density of the clone Red in the local neighbourhood, which points to barrier effects of incompatible hosts, diminishing pathogen transmission among more susceptible host genotypes (Mundt & Browning 1985; Cobb et al. 2010; Calonnec et al. 2013). Similar barrier effects were observed for the highly susceptible clones Violet and Yellow, where pathogen richness decreased with increasing density of the less susceptible clone Green in the local neighbourhood. Conversely, an increasing density of the highly susceptible clone Violet increased pathogen richness and load of the less susceptible clones Orange and Red. These positive density effects indicate that highly susceptible clones, such as the clones Violet and Yellow, act as facilitators of fungal pathogen infestation by increasing pathogen transmission rates and by providing a reservoir for pathogen propagules (Schmidt & Ostfeld; Cronin et al. 2010). The clone Violet was not only susceptible to the three foliar fungus species observed in our study but also to rust infestation by *Melampsoridium betulinum* (Viherä-Aarnio & Velling 2001). Accordingly, the clones Violet and Yellow have also been reported to be less susceptible to this rust fungus (Poteri et al. 2001). These birch clone-specific effects support our third hypothesis that more or less susceptible birch clones positively or negatively contribute to pathogen richness, respectively. Other studies provided also evidence that the presence of a particular host genotype with a given susceptibility influenced the pathogen infestation of the community or of particular host genotypes (Akanda & Mundt 1997). In forests, genetic identity of *Fraxinus excelsior* has been observed to determine susceptibility to the aggressive ash dieback pathogen *Chalara fraxinea* (McKinney et al. 2011). Moreover, Grünwald et al. (2008) detected differences in susceptibility of different *Viburnum* genotypes to *Phytophthora ramorum* leaf infection. In grasslands, it has been shown that different clonal plants of the prairie grass *Spartina pectinata* varied in resistance to *Puccinia seymouriana* and *P. sparganioides* (Davelos et al. 1996). However, much less attention has been paid to the fact that one particular host genotype is not similarly resistant to different pathogen species. For

example, the clone Green showed a low pathogen infestation by *V. ditricha* but a high infestation by *D. betulina*. Although such contrasting degrees of host susceptibilities have been described before, the consequence for overall pathogen infestation in stands of different tree clone richness had not been fully realized before.

Conclusion

In conclusion, our study contributes new knowledge on biodiversity–ecosystem functioning relationships with respect to genetic diversity in mono-specific forest birch stands. We demonstrated that tree clone composition and richness matter at all spatial scales considered in our study. Thus, intra-specific genetic diversity affected the inter-specific diversity of foliar fungal diseases (Dinoor & Eshed 1984). Knowledge on such relationships is highly important for establishing and managing mono-specific forest plantations.

5.6 Acknowledgements

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SYNTHESIS

This thesis focused on particular aspects of forest biodiversity on ecosystem functioning and aimed at assessing the effects of tree species diversity, functional tree diversity and tree clone diversity on species richness and infestation of foliar fungal pathogens in young European tree diversity experiments. In addition, identity and density of tree species and tree clones as further aspects of tree diversity were tested for effects on both response variables. Employing a valuable complementary hierarchical approach, pathogen richness and infestation of a total of 18 different tree species were examined at several spatial, as well as temporal scales.

6.1 General discussion

Effects of plant diversity on pathogenic micro-fungi have previously been analyzed mainly in observational studies in natural ecosystems (e.g. Braun 1982; Hirsch & Braun 1992; Desprez-Loustau et al. 2010). For example, Faliński and Mułenko (1995, 1996, 1997) examined forest communities in the Białowieża National Park in Poland and discovered that fungal diversity strongly depends on plant diversity. However, observational studies did not provide any insight into the underlying mechanisms of host-pathogen interactions within such complex communities (Bell et al. 2006; Hector et al. 2007). Such fundamental mechanisms may be disentangled by utilising biodiversity–ecosystem functioning (BEF) experiments.

Foliar fungal pathogen infestation within BEF grassland experiments has been recorded in few, mainly recent studies, with the unanimous result of lower pathogen infestation in species-rich communities (Mitchell et al. 2003; Roscher et al. 2007; Scherber et al. 2010). This thesis now adds comprehensive knowledge from tree diversity experiments, thereby filling the gap with regard to host-pathogen interactions in forest BEF research.

Tree species diversity effects across different spatial scales

Tree diversity could affect foliar fungal pathogens basically by tree species diversity at the *community level*, the *individual level*, the *tree species level* and the *fungus species level*, which has been studied in the BIOTREE experimental site at Kaltenborn (Chapter 2) and in the Kreinitz experiment (Chapter 3; but see also Figure 1-1).

As foliar fungal pathogens are often host-specific specialists, they rely on the presence of a particular host species during their whole life cycles (Prell 1996). Thus, the first hypothesis predicted that increasing tree species diversity increases foliar fungal pathogen species richness (H1, Figure 1-1). In contrast to this hypothesis, neither tree species richness at the

community level nor the Shannon diversity of the local neighbourhood at the *tree species level* affected foliar fungal pathogen richness (Chapter 2). On the contrary, foliar fungal pathogen richness of *Tilia cordata* decreased with increasing Shannon diversity (Chapter 3). Hence, pathogen species richness of *T. cordata* accordingly responded similar as pathogen load to increasing Shannon diversity, indicating that generally lower values of pathogen load might be attended by the loss of some fungus species. Different neighbour tree species appear to impair development of particular fungus species, pointing out that results are highly tree species-specific. However, the absence of the supposed positive relationship could be also due to the spatial scale of the experiments since plot size and local neighbourhood were certainly smaller than natural forest communities. Hence, tree species diversity is assumed to affect particular foliar fungal pathogens at different spatial scales, which indeed depends on the dispersal ability of the pathogens (Burdon et al. 2006; Johnson & Thieltges 2010; Moore & Borer 2012). However, a greater rational might be that plot's tree species richness and Shannon diversity of the local neighbourhood were of minor importance at least for foliar fungal pathogen richness.

Furthermore, increasing plant species diversity has been demonstrated to reduce pathogen load of foliar fungal pathogens in grassland communities of the Cedar Creek and the Jena experiments (Mitchell et al. 2003; Roscher et al. 2007; Allen et al. 2013). Thus, the second part of the first hypothesis assumed a negative relationship between tree species diversity and foliar fungal pathogen load (H1, Figure 1-1). At the *community level*, effects of tree species richness on the overall foliar fungal pathogen load were absent (see Chapter 2). This was similar to the absence of tree species diversity effects on foliar fungal pathogen species richness and might be due to the short overall diversity gradient of only four tree species and the low number of plot replicates (16) (Naeem & Li 1997). Moreover, two of the tree species, *Picea abies* and *Pseudotsuga menziesii*, were uninfected by foliar fungal pathogens, and hence, did not contribute to the overall community's pathogen load. At the *tree species level* and with regard to the local tree neighbourhood, contrasting results were discovered. On the one hand, effects of Shannon diversity on overall foliar fungal pathogen load were neither encountered for any of the four tree species at the Kaltenborn experiment (see Chapter 2). These results reflected findings of a study in the Californian grasslands, where a generalist virus was also unaffected by local host species richness (Borer et al. 2010). On the other hand, overall foliar fungal pathogen load of *Tilia cordata* and *Quercus petraea* decreased with increasing Shannon diversity at the Kreinitz experiment (see Chapter 3), thereby confirming the assumed negative relationship (Jiang et al. 2008). Further support was provided by results

from the *fungus species level*, where mainly the dominant fungus species were affected by the Shannon diversity of the local tree neighbourhood, whereas low abundant or rare fungus species tended to be less influenced (see Chapters 2 & 3). In particular, the pathogen load of the common powdery mildew species *Erysiphe alphitoides* and *Erysiphe hypophylla* on *Q. petraea* and *Phyllactinia orbicularis* on *Fagus sylvatica*, as well as of the leaf spot fungus *Passalora microsora* and a sterile unidentified ascomycete on *T. cordata* decreased with increasing Shannon diversity (see macroscopic images on pages 23–27). All negative relationships of foliar fungal pathogen load at the *tree species level* and the *fungus species level* are due to a dilution of the particular host individuals in the local neighbourhood and supported hints from specialist insect herbivores (Otway et al. 2005; Castagneyrol et al. 2013). Thus, low host abundance appeared to be notably responsible for reduced transmission and infestation success of commonly high abundant and frequent foliar fungal pathogens (Keesing et al. 2010; Haas et al. 2011). These results highlight the importance of tree diversity in forest communities to maintain low levels of foliar fungal pathogen infection and infestation already at a small and local scale (Keesing et al. 2006).

Impact of tree species diversity on inter-annual variation

Variance in disease severity of foliar fungal pathogens is hypothesised to be lower in species-rich tree communities than in monocultures by the second hypothesis (H2, Figure 1-1; Yachi & Loreau 1999). This assumption is related to hints from grassland ecosystems, where year-to-year variance of above-ground biomass production has been observed to be higher in monocultures than in species-rich communities (Tilman 1996; Tilman et al. 2006; Eisenhauer et al. 2011). Moreover, ecosystem stability across different trophic levels of producers and consumers, including also foliar fungal pathogens as one group of consumers, was higher in species-rich communities (Proulx et al. 2010). Results from the Kreinitz experiment illustrated strong inter-annual variances of foliar fungal pathogen species richness and pathogen load of *T. cordata* and *Q. petraea* among three subsequent years. However, in contrast to the hypothesis and results from grassland studies, inter-annual variance of both response variables was unaffected by the Shannon diversity of the local tree neighbourhood (see Chapter 3). One explanation might have been covariates of the diversity–stability relationship, such as the abiotic environment (Loreau 2010). With respect to foliar fungal pathogens, weather conditions such as temperature, air humidity and precipitation have a high potential to affect disease risk and severity (Bourke 1970; McDonald et al. 2008; Makowski et al. 2011). In consequence, the strong inter-annual variance of foliar fungal pathogen richness and load, as observed for *T. cordata* and *Q. petraea* and their associated fungi, might have

masked a significant relationship with the Shannon diversity. This highlights the importance of long-term studies to moderate outlier in inter-annual variance through time, increasing the possibility to discover more strength in this BEF relationship (Yachi & Loreau 1999; Cardinale 2012).

No functional tree diversity effects

Negative selection effects of tree diversity on foliar fungal pathogens might be caused by particular functional traits, such as physical and chemical leaf defence traits (Nics & Rubiales 2002; Calo et al. 2006; Jiang et al. 2008). Thus, the third hypothesis assumed that increasing functional diversity (FD) reduces foliar fungal pathogen richness and infestation (H3, Figure 1-1), since low FD within a tree community is brought about by tree species of similar trait values, which concerns mainly to phylogenetically closely related tree species suffering from a higher risk of different fungus species.

Surprisingly, functional tree diversity turned out to be unimportant for foliar fungal pathogen species richness and pathogen load in the BIOTREE experimental site at Bechstedt (see Chapter 4). The absence of a general FD effect was beyond that independent of the type of traits on which FD was based, i.e. there was no difference in employing traits with respect to productivity, resource use and nutrient cycling or regarding physical and chemical leaf traits. Moreover, a differentiation of FD into functional richness, functional evenness and functional divergence also did not provide deeper insights. Similar results were found in grasslands by Zhang et al. (2011) who observed that the activity of microbial communities was independent of functional group diversity. Furthermore, development and survival of herbivores also did not depend on functional group diversity (Specht et al. 2006; Sonnemann et al. 2012). In contrast, functional group diversity has been shown to affect richness and abundance of Collembola (Sabais et al. 2011) and to influence herbivory resistance and regeneration (Scherber et al. 2010). However, the present study was conducted at an early stage of the Bechstedt experiment, hence using tree saplings of approximately 10 years and FD effects might still develop in the future (de Bello et al. 2009; Philpott et al. 2012). This assumption is supported by Reich et al. (2012) who reported a high redundancy in young BEF experiments and increasing functional diversity effects with time.

Furthermore, FD analyses might be biased by methodological choices, for instance by trait selection, indicating also the risk to miss the inclusion of important traits in a study (Petchey & Gaston 2006; Poos et al. 2009; Swenson et al. 2011). The finding that none of the selected physical or chemical defence traits affect the foliar fungal pathogens might be a hint that

important traits may not have been included in the study (see Chapter 4). However, it was unexpected that the selected leaf traits did not affect foliar fungal pathogens, as, for example, Valkama et al. (2005) demonstrated that foliar fungal pathogen load was related to physical and chemical leaf traits. However, in accordance to the results of the present thesis, a study from tropical forests provided evidence that leaf toughness and leaf chemistry had no effects on leaf endophyte infection, although leaf chemistry influences endophyte community assemblages (Arnold & Herre 2003). Unlike former expectations, among all tested leaf traits there was one significant relationship as foliar fungal pathogen species richness increased with rising phenolic content, which was mainly brought about by the genera *Acer* and *Carpinus* (see Chapter 4). The relationship between the high investment in phenolics, as an important defence compound, and the high number of foliar fungal pathogen species, which were able to overcome this host defence, suggests an evolutionary *arms race* especially in the three *Acer* species (Clay & Kover 1996a, b). Sanchez-Azofeifa et al. (2012) discovered a similar relationship for endophyte richness and leaf phenolic content. In contrast, Huang et al. (2008) encountered a reduction of endophyte richness with increasing phenolic content. However, the unexpected absence of negative relationships between foliar fungal pathogen load and phenolic content or tannin content revealed rather a diffuse than a pair wise co-evolution of the host-pathogen interaction (Morris et al. 2007). This assumption was further confirmed by a phylogenetic analysis, where high phylogenetic conservation of host defence traits (i.e. phenolic and tannin content) was detected, as well as strong phylogenetic patterns of foliar fungal pathogen richness and load (see Chapter 4). The latter effect was at least due to the disease-resistance of the two gymnosperm tree species, contrasting the disease-proneness of the angiosperm tree species. Accordingly, Tedersoo et al. (2013) recently determined that host phylogeny explains fungal species richness and community composition of symbiotic ectomycorrhizal fungi. Since such phylogenetic analyses of foliar fungal pathogen species richness and load are indeed very rare, further studies are required to enrich our knowledge on the role of phylogenetic relatedness of hosts on richness and infestation patterns by foliar fungal pathogens.

Importance of tree clone diversity

Genetic tree diversity of very closely related hosts, i.e. individuals of several clones of the same tree species, might be of importance for foliar fungal pathogen infection, because of host genotype-specific differences in resistance to foliar fungal pathogens (Clay & Kover 1996a). Such tree clone diversity effects have been studied in the Satakunta birch clone diversity experiment (see Chapter 5).

Since the compatibility between hosts and foliar fungal pathogens is highly specific, an increase in pathogen species richness, but a reduction in pathogen load is expected with increasing tree clone richness or the Shannon diversity of tree clones (H1, Figure 1-1). Tree clone richness effectively influenced foliar fungal pathogen richness at the *community level*, but tree clone richness and the Shannon diversity of the local neighbourhood appeared to be unimportant for pathogen richness. Hence, a community rich in tree clones is also rich in foliar fungal pathogen species, which was mainly due to birch clone-specific differences in pathogen richness among the different clones rather than to the particular neighbourhood composition (Hudson et al. 2006; Barrett et al. 2009). In contrast, foliar fungal pathogen load was affected at a smaller scale, as pathogen load of the birch clones Green and Violet was reduced by increasing tree clone richness of the local neighbourhood, indicating strong birch clone-specific differences in susceptibility to foliar fungal pathogens. In addition, variances in susceptibility of birch clones of *B. pendula* and *Betula pubescens* have already been observed for the common micro-fungi *Venturia ditricha* (see macroscopic images on pages 23–27; Poteri et al. 2001; Ahlholm et al. 2002). One reason for the absence of tree clone diversity effects might be the influence of further covariates, such as environmental conditions which could affect the susceptibility of birch clones (Ahlholm et al. 2002). Nevertheless, the results on the effects of tree clone diversity point to the importance of intra-specific diversity in mono-specific forests.

Role of host density

Since tree host diversity and density are highly correlated, communities with high tree diversity have only a low density of every particular tree host (Moore & Borer 2012). In consequence, resource and microhabitat availability for specialist foliar fungal pathogens is highest in susceptible monocultures (Root 1973). Thus, for tree species, increasing host species density is assumed to reduce foliar fungal pathogen richness, but to increase pathogen infestation, while for tree clones, foliar fungal pathogen infestation of a particular tree clone may increase with growing density of the same tree clone in the local neighbourhood (H4, Figure 1-1; Mundt & Browning 1985; Keesing et al. 2006; Moore & Borer 2012). Addressing the causality of tree diversity and host density, this PhD study aimed to disentangle both effects on foliar fungal pathogens, focusing on tree species (see Chapter 3) and tree clones (see Chapter 5).

In general, both host tree species proportion and host tree clone density did neither affect foliar fungal pathogen richness nor infestation of *T. cordata* or of *Q. petraea* (see Chapter 3)

or pathogen load of the silver birch clones (see Chapter 5). Thus, the fourth hypothesis has to be rejected. These unexpected results are in contrast to the findings in the grassland BEF experiments where negative plant species diversity effects were in accordance to host species abundance effects, indicating an increase in disease severity of foliar fungal pathogens by higher host density (Mitchell 2002; Mitchell et al. 2003). The facilitation of transmission of host-density dependent foliar fungal pathogens through high host abundance has also been demonstrated in several observational studies. For instance, the infestation of the leaf spot fungus *Phyllosticta* sp. increased with higher density of *Polygonatum biflorum* in the understory of deciduous forests (Warren & Mordecai 2010). Furthermore, that overall foliar fungal pathogen infestation of a particular tree clone was not affected by the density of the same tree clone in the local neighbourhood contrasts results from other studies. For instance, seedling mortality of *Prunus grayana* was host density-dependent, as foliar fungal pathogen infestation of seedlings was highest in the nearest neighbourhood of adult con-specifics (Seiwa et al. 2008). However, density effects of most compatible plant host genotypes appeared to be particularly present in the early growing season, whereas barrier effects and induced resistance increased to the end of the vegetation period (Mundt & Browning 1985), indicating that the time frame is short, where host density-pathogen infestation relationships are visible (Burdon 1993). Finally, that tree species and tree clone diversity did not operate through density effects clearly indicated that a community composition with non-host tree species and/or less susceptible tree clones play a role for foliar fungal pathogen infection and infestation, supporting the biodiversity–disease hypothesis (Elton 1958).

Strength of identity effects

In contrast to other ecosystem functions and processes, the infestation by highly specific foliar fungal pathogens allows two different types of identity and density effects, the effects of the host trees (which was described above) and of the non-host trees. There is a high potential that tree diversity effects are superimposed by non-host tree identity effects due to the presence of particular tree species or tree clones within a community. Such identity effects have been found to be stronger than tree diversity per se (Nadrowski et al. 2010; Mouillot et al. 2011). More generally, identity effects on foliar fungal pathogens are an indicator of functional uniqueness of host species or genotypes (Naeem et al. 2002).

Thus, the presence of disease-prone or disease-resistant tree species might be highly important for overall community disease risk and severity (H5, Figure 1-1). At the *community level*, where tree species diversity and functional tree diversity effects were absent (see Chapters 2

& 4), the presence of disease-prone tree species (e.g. *A. platanoides*, *Q. petraea*) increased foliar fungal pathogen species richness and pathogen load, whereas disease-resistant tree species (e.g. gymnosperm tree species) reduced the overall negative pathogen impact. These results confirm studies on endophytes where host tree identity was discovered to be a main predictor for endophyte community composition and abundance (Rajala et al. 2013). Similarly, higher foliar fungal pathogen load was observed in grassland communities with higher abundance of disease-prone species (Mitchell et al. 2003), and grass host identity was a key determinant for the abundance of yellow-dwarf virus in open meadows (Moore & Borer 2012).

Non-host trees in the local neighbourhood might affect the tree host individual's competitive ability and the micro-environmental conditions, such as light availability or humidity, and hence facilitate or hinder foliar fungal pathogen infection and infestation (H5, Figure 1-1; Bourke 1970; Cordier et al. 2012; Peñuelas et al. 2012). By analysing such effects in different tree diversity experiments at the *individual level*, the *tree species level* and the *fungus species level* (see Chapters 2 & 3), non-host tree identity effects were found to be absent at the Kaltenborn site when taking only the presence or absence of the neighbourhood species into account (see Chapter 2). In contrast, when growth traits, such as basal area, were included in addition to the presence/absence information of a neighbour tree species, then non-host tree proportion effects emerged both at the *tree species level* and the *fungus species level* (see Chapter 3). In the Kreinitz experiment facilitation of foliar fungal pathogen infestation was found for the overall pathogen infestation on *T. cordata* and *Q. petraea* by high proportions of *F. sylvatica* and *Fraxinus excelsior*, respectively. Similar effects have been encountered at the *fungus species level*. The underlying mechanisms are yet not fully clear, but most probably characteristics of the vertical canopy structure of the particular non-host neighbour tree species, which impacts microclimatic conditions, are involved (Cobb et al. 2010; Calonnec et al. 2013). In this respect, *F. sylvatica* exhibited the lowest, but *F. excelsior* tallest heights compared to the other tree species of the Kreinitz experiment. *Fraxinus excelsior*, indeed, was highly infested by ash dieback symptoms, inclusive early defoliation and crown thinning (Kowalski 2006). Thus, the micro-climate was affected by these tree species traits, resulting in higher temperatures and lower air humidity and consequently in higher fungal infestation (Calonnec et al. 2013). Conversely, the gymnosperm tree species *Pinus sylvestris* and *P. abies* exhibited high canopy density (see Chapter 3), resulting in lower temperatures and higher air humidity and, in addition, might be a physical barrier and accordingly a key factor for inhibition of pathogen dispersal (Cobb et al. 2010; Calonnec et al. 2013). An inhibition of

pathogen infestation at the *tree species level* and the *fungus species level* was observed through high neighbourhood proportions of both gymnosperm tree species, which translates into a facilitation of the tree host species *T. cordata* and *Q. petraea* (see Chapter 3). Such host facilitating identity effects through the presence of non-hosts were also determined in grasslands (Mitchell et al. 2002), indicating a potentially shielding of the target trees from compatible spores. As these neighbour identity effects were either positive or negative for the foliar fungal pathogen or the host in particular years (see Chapter 3), they represent a type of unspecific and idiosyncratic facilitation (Mitchell et al. 2003; Eisenhauer et al. 2011) and further long-term studies are required to provide more general conclusions.

Parallel to tree species identity effects and as expected in the fifth hypothesis, there were also tree clone identity effects as the presence and density of particular silver birch clones affected foliar fungal pathogen species richness and load (H5, Figure 1-1; see Chapter 5). At the *community level*, overall foliar fungal pathogen richness was reduced by the presence of the less susceptible clone Red in a community through diminished availability of susceptible tree host individuals. This identity effect appeared to be a density effect as foliar fungal pathogen richness of all clone individuals was reduced by increasing density of the clone Red in the local neighbourhood. Further negative density effects were discovered, for instance, for the high susceptible clones Violet and Yellow as foliar fungal pathogen richness and load was reduced by increasing density of less susceptible clones (e.g. Blue & Green). Thus, mainly the less susceptible clones mattered as they might present a physical barrier by hampering the foliar fungal pathogen transmission among susceptible clones (Mundt & Browning 1985; Cobb et al. 2010; Calonnec et al. 2013). However, there were also positive density effects, mainly by susceptible clones (e.g. Violet & Yellow) which increased foliar fungal pathogen richness and load of less susceptible clones (e.g. Blue, Orange & Red). For instance, higher percentages of the very susceptible clone Violet in the local neighbourhood increased the overall foliar fungal pathogen load of all clone individuals. In addition, foliar fungal pathogen richness of the less susceptible clones Orange and Red, as well as the pathogen load of the clone Red increased by the density of the clone Violet. Thus, the high susceptible clones facilitated foliar fungal pathogens by supporting pathogen transmission rates and by providing a pathogen reservoir (Cronin et al. 2010). Regarding also examples from literature (e.g. Davelos et al. 1996; Akanda & Mundt 1997), identity and density effects of particular tree host genotypes signify their important role for foliar fungal pathogen transmission and in consequence for the successful interaction of highly compatible tree hosts and foliar fungal pathogens (Ahlholm et al. 2002). According to this, a certain clone mixture might limit foliar

fungal pathogen transmission among susceptible tree clones in mono-specific communities and is, hence, required to diminish foliar fungal pathogen infestation.

Summary

The present PhD thesis provides important new knowledge on the relationship between tree diversity and foliar fungal pathogen richness and infestation from several tree diversity experiments.

Regarding the first hypothesis, one key result was that tree species richness effects were absent at the *community level*. In contrast, the Shannon diversity of the local neighbourhood affected at least foliar fungal pathogen richness and load of *T. cordata* and pathogen load of *Q. petraea* at the *tree species level*, as well as the pathogen load mainly of the most abundant fungus species on *F. sylvatica*, *Q. petraea* and *T. cordata* at the *fungus species level*. In particular, the dilution effects of the Shannon diversity on foliar fungal pathogen loads complemented the results of grassland experiments, and thus, contributed to closing the research gap in forest communities. Further support for the first hypothesis was provided by tree clone richness which has been demonstrated to increase foliar fungal pathogen richness at *community level*, but to decrease pathogen load at *tree clone level*. In contrast to the expectations of the second hypothesis, inter-annual variance of foliar fungal pathogen richness and infestation was not decreased by increasing community diversity, i.e. species-rich tree communities did not exhibit higher stability with respect to foliar fungal pathogen diseases. Contrary to the third hypothesis, functional tree diversity did not affect foliar fungal pathogens at the *community level*, but hinted at functional redundancy. Moreover, traditionally used leaf traits seemed to be without any influence on the foliar fungal pathogens, solely foliar fungal pathogen richness even grew with increasing phenolic content. This interesting positive relationship points to an evolutionary *arms race* of hosts and foliar fungal pathogens and was also supported by phylogenetic patterns. In contrast to the fourth hypothesis, both tree host species proportion and density of the most compatible tree clone in the local neighbourhood did not affect pathogen richness and infestation. The absence of such density effects accordingly pointed to the strength of the observed tree species diversity and tree clone diversity effects. Supporting the fifth hypothesis, tree species richness and functional tree diversity effects were superimposed by tree species identity effects at the *community level*. Moreover, tree species identity effects within the local neighbourhood probably operated through changes in competition intensity and microclimate, but were particularly idiosyncratic. In addition, identity and density effects of tree clones revealed

positive contributions of susceptible tree clones to foliar fungal pathogen richness and load and negative impacts by less susceptible tree clones. This hints to the importance of tree clone composition within mono-specific communities for foliar fungal pathogen transmission and infection.

In conclusion, results of the present PhD thesis explicitly revealed strong bottom-up effects by tree species diversity, tree clone diversity, as well as by tree species and tree clone identity on foliar fungal pathogen richness and infestation in young experimental forest communities in the temperate and boreal zone. As pathogen richness and infestation was affected at different spatial and temporal scales, forest community composition appears to be beyond that of highest importance for bottom-up control of foliar fungal pathogen diseases.

6.2 Outlook

The present PhD study was conducted as a comprehensive study in the forest BEF experiments to disentangle several aspects of tree diversity effects on foliar fungal pathogens. It was clearly demonstrated that on the one hand foliar fungal pathogens are feasible study organisms and on the other hand forest BEF experiments, similar to grassland BEF experiments, are a useful approach to study plant diversity effects on plant diseases. However, the observed differences in strength and direction of tree diversity and identity effects between the different spatial and temporal scales reveal the complexity of tree diversity effects. One future avenue of research would be a spatial-scale approach, increasing the local neighbourhood continuously until arriving at the plot scale. However, one limitation of the forest BEF experiments is the area available for such an analysis with plot size being often comparatively too small to reflect natural forest conditions. Accordingly, in natural forests a much larger number of tree individuals interact with each other than in experimental plots, hence, enhancing the exchange and the storage of foliar fungal pathogens in natural forests. Moreover, interspaces between plots in forest BEF experiments are rather small compared to distances between different natural forests. However, this spatial proximity could lead to inter-plot interference by facilitating foliar fungal pathogen transmission between proximate plots, and thus, community composition of neighbour plots might shape the effects of community composition of the target plot and vice versa (Paysour & Fry 1983). Thus, an interface between the experimental BEF studies and the observational studies in natural forests is required to gain more profound information concerning the influence of tree diversity on host-pathogen interaction.

Furthermore, natural forests comprise tree individuals of different ages caused by succession, whereas tree individuals in the BEF experiments are of the same age and generally within a sapling phase. As the ontogenetic stage of host individuals might affect the degree of infection, young forest BEF experiments solely allow insights into a particular ontogenetic phase of the host, pointing out the importance of a long-term perspective of the forest BEF experiments. Although one should be cautious to apply any conclusions from such young experimental platforms to natural forests, these results are certainly applicable for tree nurseries, as well as for afforestations and reforestations.

As microscopic and macroscopic analyses only cover a fraction of all foliar fungal pathogens on tree leaves, molecular analyses would provide valuable additional insight into hidden tree diversity, also including inconspicuous and non-fruiting fungus species.

The inter-annual idiosyncratic effects point to the importance of local weather conditions for the relationship of tree diversity and fungal disease severity. In particular, more information on the optimum and tolerated weather conditions of the different foliar fungal pathogen species would be desirable. So far, models for disease severity in forests with respect to species composition have low predictive power, in particular in the context of climatic change and its consequences at the local scale.

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APPENDIX

Curriculum vitae

Lydia Hantsch

Date and place of birth	27.05.1986, Löbau
Nationality	German
Address	Richard-Wagner-Str. 35, 06114 Halle, Germany

Education

10.2010 – present	PhD student, Martin Luther University Halle-Wittenberg
	Research topic: "Tree diversity effects on species richness and infestation of foliar fungal pathogens in different European tree diversity experiments", supervisors: Prof. Helge Bruelheide, Prof. Uwe Braun
	Funding: Scholarship of the federal state Sachsen-Anhalt (10.2010 – 12.2010), Scholarship of the German Federal Environmental Foundation (DBU; 01.2011 – present)
10.2004 – 01.2010	Studies in biology, Martin Luther University Halle-Wittenberg
	Major subject: Geobotany
	Minor subjects: Plant physiology, phytopathology, ecological biochemistry
	Diploma thesis: "Relationship between genetic diversity and invasion success of *Senecio vernalis* Waldst. & Kit. (Asteraceae)", supervisor: Dr. habil. Alexandra Erfmeier
	Final mark: 1.1
07.2004	University-entrance-diploma, Gymnasium Seifhennersdorf
	Final mark: 1.6

Practical and occupational experience

02.2013 – 03.2013	Research assistant, Martin Luther University Halle-Wittenberg, supervisor: Dr. habil Alexandra Erfmeier, DFG Research Unit 891, organization assignment

10.2012 – 11.2012	Research assistant, Martin Luther University Halle-Wittenberg, supervisor: Dr. habil Alexandra Erfmeier, DFG Research Unit 891, organization assignment
10.2011 – 07.2013	Internship/service contracts, NATURstiftung David Erfurt, supervisor: Dr. Sabine Kathke
06.2010 – 09.2010	Research assistant, Martin Luther University Halle-Wittenberg, supervisor: Prof. Helge Bruelheide, DFG Research Unit 891
02.2010 – 09.2010	Research assistant, Helmholtz Centre for Environmental Research Leipzig/experimental station Bad Lauchstädt, supervisor: Dr. Christoph Fühner, BMBF-project IWAS
07.2007 – 03.2008	Internship/student research assistant, Helmholtz Centre for Environmental Research Halle Topic: "Changes of phenology data in German flora – Indicators for climate change", supervisor: Dr. Stefan Klotz
02.2003 – 01.2004	Internship, International Institute Zittau Topic: "Isolation and physiological-enzymatic characterisation of basidiomycetes", supervisor: Prof. Martin Hofrichter

Teaching and supervised theses

06.2013	Excursion (vegetation of ruderal habitats), Halle/Trotha
2013	Master thesis – Steffen Bien, topic: "Relationship between tree diversity and foliar fungal pathogens in a Chinese subtropical tree diversity experiment" (preliminary title)
06.2012	Advanced practical course (geobotany), Martin Luther University Halle-Wittenberg
2012	Bachelor thesis – Steffen Bien, topic: "Relationship between tree diversity and foliar fungal pathogen infestation on *Quercus petraea* in the tree diversity experiment Kreinitz"
2012	Bachelor thesis – Stine Radatz, topic: "Relationship between tree diversity and foliar fungal pathogen infestation on *Tilia cordata* in the tree diversity experiment Kreinitz"
05.2011	Excursion (vegetation of xerothermal habitats), Lettin
06.2010	Advanced practical course (geobotany), Martin Luther University Halle-Wittenberg
05.2009, 09.2009, 05.2010, 05.2011, 05.2013	Several-day excursion "Faule Ort" (botany and ecology), Müritz National Park

List of publications

Publications of the dissertation

Hantsch L, Bien S, Radatz S, Braun U, Auge H, Bruelheide H (under review) Effects of tree diversity, host and non-host species proportions on foliar fungal pathogens. Journal of Ecology.

Hantsch L, Braun U, Durka W, Koricheva J, Bruelheide H (in preparation) Tree clone diversity effects on foliar fungal pathogens in a birch-clone experiment.

Hantsch L, Braun U, Haase J, Purschke O, Scherer-Lorenzen M, Bruelheide H (submitted) No plant functional diversity effects on foliar fungal pathogens in experimental tree communities. Fungal Diversity.

Hantsch L, Braun U, Scherer-Lorenzen M, Bruelheide H (2013) Species richness and species identity effects on occurrence of foliar fungal pathogens in a tree diversity experiment. Ecosphere 4:81.

Other publications by the author

Baeten L, Verheyen K, Wirth C, Bruelheide H, Bussitti F, Finer L, Jaroszewicz B, Selvi F, Valladares F, Allen E, Ampoorter E, Auge H, Avăcăriei D, Barbaro L, Bărnoaiea I, Bastias CC, Bauhus J, Beinhoff C, Benavides R, Benneter A, Berger S, Berthold F, Boberg J, Bonal D, Brüggemann W, Carnol M, Castagneyrol B, Charbonnien Y, Chećko E, Coomes D, Coppi A, Dalmaris E, Dănilă G, Dawud MW, de Vries W, De Wandeler H, Deconchat M, Domisch T, Duduman G, Fischer M, Fotelli M, Gessler A, Gimeno TE, Granier A, Grossiord C, Guyot V, Hantsch L, Hattenschwiler S, Hector A, Hermy M, Holland V, Jactel H, Joly F-X, Jucker T, Kolb S, Koricheva J, Lexer MJ, Liebergesell M, Milligan H, Müller S, Muys B, Nguyen D, Nichiforel L, Pollastrini M, Proulx R, Rabasa S, Radoglou K, Ratcliffe S, Raulund-Rasmussen K, Seiferling I, Stenlid J, Vesterdal L, von Wilpert K, Zavala MA, Zielinski D, Scherer-Lorenzen M (2013) A novel comparative research platform designed to determine the functional significance of tree species diversity in European forests. Perspectives in Plant Ecology, Evolution and Systematics, http://dx.doi.org/10.1016/j.ppees.2013.07.002.

Erfmeier A, Hantsch L, Bruelheide H (2013) The Role of Propagule Pressure, Genetic Diversity and Microsite Availability for Senecio vernalis Invasion. PLoS ONE 8:e57029.

Hantsch L, Bruelheide H, Erfmeier A (2013) High phenotypic variation of seed traits, germination characteristics and genetic diversity of an invasive annual weed. Seed Science Research 23:27–40.

Conference contributions

Hantsch L, Bien S, Radatz S, Auge H, Braun U, Bruelheide H (2013) Effects of neighbour diversity, host and neighbourhood density and inter-annual variation in weather conditions on foliar fungal pathogens. 3rd International Congress on Planted Forests – Scientific Workshop Vulnerability and Risk Management in Planted Forests, Bordeaux.

Hantsch L, Bien S, Radatz S, Auge H, Braun U, Bruelheide H (2013) Effects of neighbour diversity, host and neighbourhood density and inter-annual variation in weather conditions on foliar fungal pathogens. Annual Meeting FunDivEUROPE, Florenz.

Hantsch L, Scherer-Lorenzen M, Braun U, Bruelheide H (2012) Functional diversity effects on transmission of foliar fungal pathogens in the German tree diversity experiment BIOTREE. 42th Anniversary Conference, Gesellschaft für Ökologie, Lüneburg.

Hantsch L, Braun U, Bruelheide H (2011) Biodiversity-effects on resistance to foliar fungal pathogens in the German tree diversity experiment BIOTREE. Annual Meeting FunDivEUROPE, Helsinki.

Hantsch L, Braun U, Bruelheide H (2011) Biodiversity-effects on resistance to foliar fungal pathogens in different European tree diversity experiments. Pathogen-Herbivory-Workshop BACCARRA/FunDivEUROPE, London.

Hantsch L, Scherer-Lorenzen M, Braun U, Bruelheide H (2011) Biodiversity-effects on resistance to foliar fungal pathogens. 41th Anniversary Conference, Gesellschaft für Ökologie, Oldenburg.

Hantsch L, Bruelheide H, Erfmeier A (2010) The role of propagule pressure, genetic diversity and microsite availability for *Senecio vernalis* invasion. 40th Anniversary Conference, Gesellschaft für Ökologie, Gießen.

Eigenständigkeitserklärung

Hiermit erkläre ich, dass die Arbeit mit dem Titel „Tree diversity effects on species richness and infestation of foliar fungal pathogens in European tree diversity experiments“ bisher weder bei der Naturwissenschaftlichen Fakultät I Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt wurde.

Ferner erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst sowie keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Die den Werken wörtlich oder inhaltlich entnommenen Stellen wurden als solche von mir kenntlich gemacht.

Ich erkläre weiterhin, dass ich mich bisher noch nie um einen Doktorgrad beworben habe.

Halle, den 20.09.2013

Lydia Hantsch