Fungal assemblages in forest trees

Influence of internal and external conditions

Marta Agostinelli
Faculty of Forest Sciences
Department of Southern Swedish Forest Research Centre
Alnarp

Doctoral thesis
Swedish University of Agricultural Sciences
Alnarp 2018

Acta Universitatis agriculturae Sueciae
2018:77

Cover: Endophytic fungi isolated from twigs of pedunculate oak (*Quercus robur* L) growing on malt extract agar medium
(Photos: M. Agostinelli)

ISSN 1652-6880
ISBN (print version) 978-91-7760-296-5
ISBN (electronic version) 978-91-7760-297-2

Print: SLU Service/Repro, Alnarp 2018

Fungal assemblages in forest trees. Influence of internal and external conditions

Abstract

Forest trees host a plethora of microorganisms (bacteria, viruses, fungi) whose roles and diversity are still poorly understood despite the increased scientific interest for the past decades. The thesis focuses on the diversity of endophytic and epiphytic fungi in the aerial tissues of broadleaved trees. It tests a basic hypothesis that the diversity and frequency of endophytic and epiphytic fungi vary depending on the vitality and disease susceptibility of the host. Additionally the thesis explores if the chemical variation in trees may relate to differences in fungal community. Particularly, the aim was to describe how the fungal communities of three broadleaved species relate to the general vitality or specific pathogen resistance of the trees, and if herbivory or fertilization influence the fungi through altered levels of potentially antimicrobial metabolites, condensed tannins. Culture-based and culture-independent (NGS) techniques were used to capture the fungal community in the twigs of pedunculate oak (*Quercus robur* L), in the leaves of aspen (*Populus tremula* L), and in the leaves and twigs of European ash (*Fraxinus excelsior* L). The secondary metabolites were studied with HPLC, LC-MS, and GC-MS analyses.

The results showed that the fungal assemblages are influenced by a complex network of factors related to the status of the host (internal factors: health, chemotype) and its environment (season, site, nitrogen, herbivory). Trees with different vitality or different phenotypic response to pathogen hosted quantitatively and qualitatively diverse fungal communities. Tissue type and seasonal variation were confirmed to be highly selective factors in shaping fungal communities of forest trees. The endophytic communities associated with xylem seemed to be shaped by the tree vitality more readily than the fungi associated with leaf or bark. Condensed tannins, nitrogen fertilization, and herbivory did not explain the structure of fungal communities in aspen leaves. Leaf phenolic metabolites reflected well the general vitality phenotype of the trees, but the relationship between fungi and phenolics may not be straightforward.

The technological advances and the use of different methods to survey fungal communities may help disclosing the unknown fungal biodiversity hosted by forest trees. Further studies on fungal communities are needed to reveal the ecological relevance that fungal assemblages have in the regulation of major ecological cycles. Understanding the mechanisms regulating the establishment of fungal communities may contribute to the possibility of using fungal assemblages in forest practices to help forest coping with sudden changes and be able to provide different ecosystem services.

Keywords: endophyte, epiphyte, broadleaves mycobiome, fungal diversity, chemotype, fungal assemblages, phenolics, forest trees

Author's address: Marta Agostinelli, SLU, Department of Southern Swedish Forest Research Centre,
P.O. Box 49, 230 53 Alnarp, Sweden
E-mail: marta.agostinelli@slu.se

to *Adelina*

Aliquid amplius invenies in silvis, quam in libris. Ligna et lapides docebunt te, quod a magistris audire non possis (Bernard of Clairvaux).

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Agostinelli Marta, Cleary Michelle, Martín Juan Antonio, Albrectsen Benedicte Riber, Witzell Johanna (2018). Pedunculate Oaks (*Quercus robur* L.) Differing in Vitality as Reservoirs for Fungal Biodiversity. *Frontiers in Microbiology*, 9:1758, doi: 10.3389/fmicb.2018.01758

II Decker Vicki Huizu Guo, Agostinelli Marta, Witzell Johanna, Chen Sylvia, Cleary Michelle, Albrectsen Benedicte Riber. Foliar endophytes in relation to nitrogen and herbivory treatments in aspen genotypes (manuscript).

III Agostinelli Marta, Nguyen Diem, Witzell Johanna, Cleary Michelle. Diversity and composition of European ash (*F. excelsior* L.) mycobiome affected by phenotypic susceptibility to *Hymenoscyphus fraxineus* (manuscript).

The contribution of Marta Agostinelli to the papers included in this thesis was as follows:

I Marta Agostinelli planned the experiment with Johanna Witzell. She collected the samples and performed the laboratory work. She analysed the data with the contributions of Juan A. Martín, Benedicte R. Albrectsen, and Johanna Witzell. She wrote the manuscript in collaboration with the co-authors.

II Marta Agostinelli performed part of the laboratory work. She analysed part of the data and wrote the manuscript in collaboration with the co-authors.

III Marta Agostinelli was involved in planning of the field and laboratory work. She collected the samples and performed most of the laboratory work. She analysed the data with contributions from Diem Nguyen and wrote the manuscript in collaboration with the co-authors.

Abbreviations

C	Control
CCA	Canonical Correspondence Analysis
CICES	Common International Classification of Ecosystem Services
CF	Colonization Frequency
CTs	Condensed Tannins
DNA	Deoxyribonucleic Acid
EEA	European Environment Agency
GC-MS	Gas Chromatography–Mass Spectrometry
H	High
He	Herbivory
H-MCR	Hierarchical Multivariate Curve Resolution
HPLC	High-Performance Liquid Chromatography
I	Intermediate
ITS	Internal Transcribed Spacer
L	Low
LC-MS	Liquid Chromatography-Mass Spectrometry
M	Medium
MEA	Malt Extract Agar
MT	Morphotype
N	Nitrogen
NGS	Next Generation Sequencing
NHe	Nitrogen*Herbivory
NMDS	Non-metric Multidimensional Scaling
OPLS-DA	Orthogonal Partial Least Squares Discriminant Analysis
OTU	Operational Taxonomic Unit
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PERMANOVA	Permutational Multivariate Analysis of Variance
R	Resistant

S	Susceptible
S^{MT}	Morphotype Richness
SwAsp	Swedish Aspen collection
WA	Water Agar

1 Introduction

Most people think of a plant as one organism, however, just like the human body hosts a plethora of external and internal microorganisms, so do plants. The suite of microorganisms hosted by an individual is called *microbiome*. While the past years have seen an increase in research on the diverse roles of human microbiome (Turnbaugh et al., 2007), research on plants microbiome has only recently started to attract more interest in the scientific community. Understanding the role of microbiome in plants and forecasting microbiome responses to changes in host and environment may help predicting the effects of these changes on ecosystems and ecosystem cycles.

Fungi, bacteria, and viruses that constitute plants microbiome occupy all parts of plants, from the rhizosphere to the phyllosphere. The knowledge of the roles and diversity of plants microbiome has greatly increased in recent years, largely due to technological advances. The plant microbiome was originally studied with traditional techniques that allowed researchers to analyse only the culturable fraction of the microbiome (Müller and Hallaksela, 1998; Sieber, 1989; Toti et al., 1992). During the recent decades, the development of new and affordable sequencing technologies, including next generation sequencing (NGS), has helped researchers unrevealing a higher, hidden diversity of the microbiome (Porras-Alfaro and Bayman, 2011; Tedersoo and Lindahl, 2016).

Fungi are a main group of microbes forming the plant microbiome and the assemblages of fungi that share the same host are referred to as *fungal community* or *mycobiome*. The mycobiome of plants is composed of a community of ubiquitous and specific fungi which have different roles and colonize different parts of the trees, i.e. mycorrhizas, epiphytes, and endophytes. This thesis focuses on *fungal endophytes* that are fungi living for at least part of their life within the tissue of plants without causing apparent harm to the host (Sieber, 2007) and on *fungal epiphytes* that are instead living on the surfaces of the plants where they occupy a different niche than endophytes (Santamaría and Bayman, 2005). The roles of most fungal endophytes and epiphytes are still not

well known but the potential they have in for example enhancing plant health and fitness (Porras-Alfaro and Bayman, 2011; Rodriguez et al., 2009) has awaken an interest in studying them. A threat to microscopic fungal biodiversity is posed by the increased diffusion of invasive alien pathogens. Tree breeding for resistance is often seen as the long-term solution to overcome tree extirpation from the ecosystem as a consequence of invasive alien species. However, the improved resistance mechanisms in trees bred for resistance may also have unintentional consequences on the fungal biodiversity. For example, elm (*Ulmus* spp.) trees showing low susceptibility to Dutch elm disease pathogen have been found to host in xylem a less diverse fungal community than the highly susceptible trees (Martín et al., 2013). Consequently, the solution of introducing trees with improved resistance mechanisms in the ecosystems, could negatively affect part of the fungal biodiversity, demanding compensating management measures.

The research presented in this thesis aims to add knowledge to the current *state-of-the-art* of structures and functions of tree fungal communities, exploring their variation in response to certain tree internal and external factors. Throughout the thesis, the term **endophyte** and **epiphyte** will be used to refer only to the fungal fraction of the endophytic and epiphytic communities (bacteria and viruses are disregarded). **Fungal community**, **fungal assemblages**, and **mycobiome** will refer to the group of endophytes and epiphytes hosted by trees.

1.1 The relevance of trees in urban and forest ecosystems

Ecosystem services are defined as the benefits that natural environment and properly functioning ecosystems provide to human. Recently, the European Environment Agency (EEA) has released the Common International Classification of Ecosystem Services (CICES), a tool aiming to standardize the description of ecosystem services. According to this standardization, ecosystem services are described based on the provision, regulation and maintenance, and cultural biotic and abiotic services provided. Trees and forests have prominent roles as providers of several crucial ecosystems services, such as provision of oxygen, clean air and water, sequestration of carbon, amelioration of the climate, and stabilization of soil (Livesley et al., 2016). In urban contexts, trees can, besides improving human health, e.g., through positive effects on mental health (Lee and Maheswaran, 2011), provide the society with aesthetic, physiological, environmental, and economic benefits (Barrios et al., 2018; Endreny et al., 2017). The urban and peri-urban trees may also be important reservoirs and

stepping stones for biodiversity (Agostinelli et al., 2018; Dearborn and Kark, 2009).

In the changing climate and globalized world, the ability of trees and forest to provide multiple ecosystem services may be at increasing risk. To be able to provide continuity to ecosystem services, trees and forests need to remain functional, i.e., vital and healthy. Yet, the health of the trees and forests is continuously threatened by endemic and alien pests and pathogens. Especially the alien species may cause major disturbances in naïve tree populations (Jacobs, 2007; Newcombe and Dugan, 2010; Potter et al., 2011). Mainly due to increasing international trade, travels, and tourism, a plethora of invasive alien pests and pathogens have been moved across continents, leading to negative ecological, economical, and social consequences (Aukema et al., 2011). The introduction, establishment, and spreading of invasive alien species may pose a risk to tree species and the biodiversity associated with them, with possible cascade effects on the ecosystem, as illustrated by the case of Dutch elm disease that has decimated globally elm (*Ulmus spp.*) populations (Brasier, 1991; Mitchell et al., 2014; Pautasso et al., 2013). Another external factor that affects trees and forest health, thus interfering with the provision of ecosystem services, is climate change. Population dynamics and pathogenicity of fungal disease may be highly affected by the alterations in environment (temperature, humidity) due to climate change (Lindner et al., 2010). The highly diverse and dynamic microbial communities associated with trees, and their ability to influence plants response to abiotic and biotic factors, may help trees to respond and cope with the changing environment (de Assis Costa et al., 2018; Porras-Alfaro and Bayman, 2011; Rudgers et al., 2004).

1.2 Endophytic and epiphytic fungi of aerial tissues of trees

The kingdom of fungi is represented by a large number of taxa that differ with respect to morphology, ecology, and life strategies. Fungi are eukaryotic organisms that can be microscopic (microfungi) or form bigger organisms that can be visible to the human eye in the “classic” mushroom shape (macrofungi). The current estimation of fungal species is set to 2.2 to 3.8 million. Considering that around 120k species have been officially described to date, only 3 to 8% of the estimated fungal diversity is known (Hawksworth and Lücking, 2017). Only limited and incomplete knowledge of the ecological role is available for most fungal species (Hawksworth, 1991; Mueller and Schmit, 2007). Fungi are key players in many crucial ecosystem functions such as decomposition, nutrient cycling, and nutrient transportation. Some species are important human and

plant pathogens and can cause significant economic losses through the spoilage and degradation of food supplies (Gherbawy and Voigt, 2010; Mueller and Bills, 2004; Tedersoo and Lindahl, 2016). Despite the undoubtful importance of fungi in forest ecosystems, they have often been largely neglected in discussions about biodiversity conservation, land-use planning and management (Berg et al., 1994; Mueller and Bills, 2004). Moreover, it is not easy to survey fungal species; especially the microfungi are challenging to survey as they cannot be detected with traditional surveys since they do not produce visible structures (Halme et al., 2012). Previous studies conducted with culture-based techniques (i.e., isolation of endophytes from surface sterilized tissues to growth-medium, usually agar) and microscopy have shown that fungal communities of forest trees are highly diverse and dynamic (Saikkonen, 2007; Sieber, 2007; Unterseher, 2011). Nowadays, some of the limitations related to culture-based techniques have been overcome by molecular techniques that allow an in-depth survey of microfungal communities (Tedersoo and Lindahl, 2016) and enable studies on the total fungal diversity hosted by trees (Porras-Alfaro and Bayman, 2011).

Fungal endophytes are microfungi colonizing intercellularly or intracellularly the internal tissue of plants where they form highly localized infections (Carroll, 1988; Saikkonen et al., 1998; Schulz and Boyle, 2005; Sieber, 2007). All plants studied to date have been found to be colonized by at least one endophytic fungal species (Arnold and Herre, 2003). Endophytes can be divided into two main groups based on how they colonize the trees. Herbaceous species are colonized by clavicipitaceous endophytes that are transmitted vertically to the new plant via seed from the mother plant (Rodriguez et al., 2009). Trees are instead infected horizontally with non-clavicipitaceous endophytes dispersed via spores from the surrounding environment (Rodriguez et al., 2009). Endophytes may establish mutualistic symbioses with plants and contribute to tree fitness and resistance (Hardoim et al., 2015; Meier and Hunter, 2018; Mejía et al., 2008; Miller et al., 2008; Sieber, 2007; Vega et al., 2008). They may enhance plant growth, or defend the host against pests and pathogens e.g. by producing toxic secondary metabolites (Hubbard et al., 2014; Pansanit and Pripdeevech, 2018; Romeralo et al., 2015; Tanney et al., 2016). On the other hand, some endophytes may be latent pathogens or saprotrophs that stay dormant in the host until a change in the environment or in the host triggers the pathogenic or saprophytic behavior of the fungus (Parfitt et al., 2010; Promputtha et al., 2007; Rai and Agarkar, 2014).

Fungal epiphytes are microscopic fungi that, similar to endophytes, are transmitted horizontally by wind dispersed spores (Arnold and Herre, 2003). They are less abundant than bacterial epiphytes, but have a worldwide distribution, with the most abundant communities found on leaves (Hongsanan

et al., 2016). Fungal epiphytes may be saprotrophs, plant or fungal parasites, and partners in lichens (Reynolds and Gilbert, 2005) and may affect plant physiology limiting the photosynthetic ability covering the leaves (Hongsanan et al., 2016). Fungal epiphytes may play important roles in shaping microbiome assemblages and may have antagonistic roles against diseases (Lindow and Leveau, 2002).

1.3 Interactions of abiotic and biotic factors with trees and fungal communities

Various biotic and abiotic factors (Fig. 1) can shape the fungal community composition of trees (Albrectsen et al., 2018; Bálint et al., 2013; Jumpponen and Jones, 2010; Martín et al., 2013). These factors can affect the fungal community directly, limiting the inoculum dispersal, or indirectly, causing changes in the host or other associated organisms that have repercussions on the fungal community.

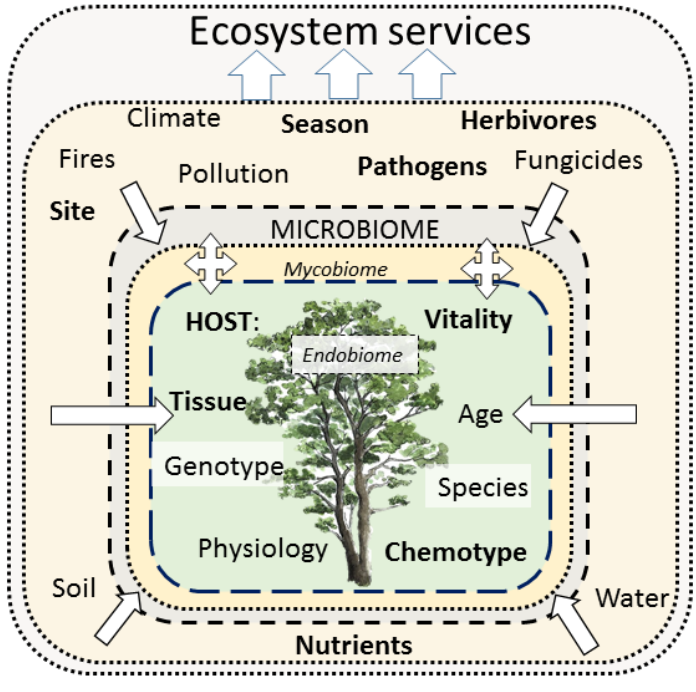

Figure 1. Interactions of mycobiome with the host and with selected biotic and abiotic factors. The crossed arrows represent the interactions between the fungal community and the host characteristics. The simple arrows represent the interactions that occur among the fungal communities, the host, and external biotic and abiotic factors. In bold are the biotic and abiotic factors that were studied in the thesis.

The dynamic interactions among fungal communities, host, and biotic and abiotic factors are still poorly understood, especially in trees (Baldrian, 2017; Porras-Alfaro and Bayman, 2011). Trees are long-living organisms and because of this, they are subjected to multiple infections and continuous changes in their surroundings during their entire life cycle. This longevity is challenging to capture in experiments and thus it is difficult to predict how fungal communities may change over time and react to the interactions with the host and other organisms that share the same habitat inside the trees. Yet, this information would be needed, because understanding the mechanisms behind the relationships of fungal communities, their host, and the influence of biotic and abiotic factors on their interactions, could contribute to the development of more sustainable practices to enhance trees and forest health (Agler et al., 2016; Koskella et al., 2017; Newcombe, 2011; Witzell and Martín, 2018).

The multidirectional interactions between host and fungal community may be affected by multiple factors that act in synergy. These include tissue type (Petrini and Fisher, 1990), host and tissue age (Sieber, 2007), physiology (Rajala et al., 2014), and genotype (Bálint et al., 2013) but host is the first factor discriminating fungal composition (Hoffman and Arnold, 2008). Co-evolution of fungal communities with their host has been dated back to the divergence of angiosperms and gymnosperms with some orders of endophytes being more related to conifers or broadleaves (Sieber, 2007). Among the external biotic and abiotic factors that play a role in fungal-host interactions are e.g., pathogens and pests (Albrectsen et al., 2018; Mejía et al., 2008), geography (Zimmerman and Vitousek, 2012), climate (Giauque and Hawkes, 2016), soil nutrient and fertilization (Lehtonen et al., 2005), fungicide applications (Karlsson et al., 2014), water stress and flooding (Kwaśna et al., 2016; Linaldeddu et al., 2011), pollution (Helander, 1995), and fires (Huang et al., 2016). Of the multitude of internal and external factors that may influence the dynamic relationships between fungal communities and their hosts, this thesis focuses on a set of specific aspects (highlighted in Fig. 1) that are briefly presented below.

1.3.1 Trees as habitat for endophytic and epiphytic biodiversity

Fungi colonize a wide array of habitats in the above- and below-ground parts of the trees (leaves, bark, wood, roots). The different spatial structure and nutrients in these habitats provide different niches where diverse species can thrive. According to the *trade off-based niche theory* (Tilman, 2004) and the *competitive exclusion principle* (Chase and Leibold, 2003), two species may co-exist in the same niche only if they utilize the resources in substantial different ways (Ernst et al., 2011; Wennekes et al., 2012). This process is based on the

trade-off between resource competition and species accumulation (Tilman, 2004). The *neutral theory* of biodiversity suggests instead that biodiversity is random and that species diversity follows unpredictable patterns (Hubbell, 2001). In this theory Hubbell (2001) affirmed that all species, regardless of their identity, have the same probability of surviving or dying. The problem with the two aforementioned theories is that the classical trade-off niche theory does not consider the relative abundance of species (Hubbell, 2001) and the neutral theory disregards the relation among species characteristics, community structure, and ecosystem conditions (Tilman, 2004). To overcome these limitations, Tilman (2004) proposed the stochastic niche theory that includes colonization dynamics and environmental conditions as factors shaping species diversity and composition.

The colonization process and consequently community composition are also influenced by the *priority effect* that states that species assemblages are strongly affected by the first species that colonizes the niche (Fukami et al., 2005; Weidlich et al., 2017). In order to better predict the priority effect on species composition, Vannette and Fukami (2014) proposed to divide the niche in three components identified as overlap niche, impact niche, and requirement niche. The priority effect is then analysed based on the similar use of resources (overlap niche), the influence of a species in modifying the environment (impact niche), and the environmental conditions that affect the survival, growth, and reproduction of a species (requirement niche). Detecting the niche component(s) that mostly affects the priority effect would help to understand the mechanisms behind fungal colonization success in plants. This information could assist in designing specific inoculation strategies that promote colonization of selected fungi to be used in tree protection and biological control (Vannette and Fukami, 2014).

Fungi that compose the mycobiome of a tree may be ubiquitous, generalist species (Baldrian, 2017; Sieber, 2007; Zabalgogeazcoa, 2008) that thrive in different environments and on different hosts. However, some fungal species may be highly selective and specific to a host or host tissue. For example, fungal assemblages of angiosperms and gymnosperms are very different. Fungi co-evolved together with their main host and when broadleaves and conifers evolution diverged around 300 million years ago, so did the fungal communities (Sieber, 2007). Diversity in fungal species composition is observed also within species belonging to the same family, i.e. *Cupressaceae* (Hoffman and Arnold, 2008) and the same genera, i.e. *Quercus* (Ragazzi et al., 2003; Sieber, 2007).

Fungal communities may vary greatly within the same host, as some fungal species are highly organ-specific. Highly dynamic communities have been observed in leaves of deciduous species, which annually produce new leaves that

are infected anew every year (Peršoh, 2015). Phyllosphere fungal communities may also depend on the age of the leaf (Arnold and Herre, 2003; Peñuelas et al., 2012). Moreover, phyllosphere communities, more than bark or wood communities, are exposed to multiple stressors and dynamic changes (i.e. temperature fluctuation, desiccation) that may strongly shape fungal assemblages (Lebeis, 2015). The bark of trees consists of a protective tissue outside the vascular cambium that is often rich in lignin but can contain rather low levels of carbohydrates, which may limit microbial growth (Baldrian, 2017). However, a rich mycobiome is often found in the bark of trees, with higher colonization rate usually recorded in bark than in xylem and leaves (Collado et al., 2000; Fisher and Petrini, 1990; Wang and Guo, 2007). Xylem community is still one of the least studied tissue types in forest trees (Baldrian, 2017). With relatively low nutrient composition and lower oxygen concentration, xylem is a highly selective substrate for microbial communities (Baldrian, 2017).

Host genotype is an important component of tree resistance to pathogens (Busby et al., 2013; McKinney et al., 2012). Selecting highly pathogen resistant trees is generally considered as the most sustainable way to preserve vital populations of tree species threatened by invasive alien pathogens (i.e. *Ulmus spp.*, *Fraxinus excelsior*). However, the mechanisms of genotypic resistance against pathogen may also select against other fungal species (Busby *et al.*, 2013; Martín et al. 2013). Since some fungal endophytes may enhance plant fitness and resistance against pathogens, an improved understanding of the mechanisms of endophyte selection by host genotype could help to predict how tree breeding can influence composition of fungal communities, and if endophytes would enhance the resistance or play a role in it. Tree phenotype is the characteristic of a tree that results from the interaction between the genotype and environmental factors. Tree genotype and biotic and abiotic factors have been shown to affect fungal community composition in different hosts (Albrectsen et al., 2018; Gonthier et al., 2006; Mejía et al., 2008).The possible reciprocal influences between tree phenotype and its plasticity and the endophytic communities are still poorly understood (Newcombe, 2011).

A special phenotypic character is the chemotype, which refers to the chemical profile of an organism. Trees may be grouped together based on the chemotype of specific chemical compounds (Keefover-Ring et al., 2014). Fungal endophytes are known to be able to alter chemical defenses in the host (White and Torres, 2010) but little is known about how the chemotype of a host may affect the fungal community.

1.3.2 Interactions of pests and pathogens with trees and fungal communities

During their long lifecycle, trees are continuously and simultaneously attacked by pests and pathogens. Trees have evolved different defense mechanisms to resist those attacks. The interactions between a tree and its natural enemies are multiple in time and space, and the final outcome, measured e.g. as tree vitality, will depend on many factors (Bonello et al., 2006). Trees possess defensive mechanisms that can be active at different time scales in relation to pathogen or pest attacks. The physical and chemical barriers present in the tissues and cells of trees before attack form the constitutive defenses. Induced defenses instead are triggered when the tree is attacked and the induction may occur locally, i.e. at the point of insect feeding, or systemically, in undamaged parts (Bonello et al., 2006; Karban and Baldwin, 1997). After an attack, biochemical responses, such as activation of phenolic metabolisms, may occur and the chemical composition within tissues may change quantitatively and qualitatively (Brignolas et al., 1995; Klepzig et al., 1995). The induced responses in cell metabolism (Bonello et al., 2006) may cause variation in fungal communities. The fungal endophyte community may also be altered by herbivory, e.g. if new fungi are transported to plant tissues by the herbivores, or if the herbivores alter the quality of plant tissues as a substrate for the fungi (Albrectsen et al., 2018). On the other hand, the fungal inhabitants of tissues may also influence herbivores (Coblentz and Van Bael, 2013; Wilson, 1995). For instance, the secondary metabolites produced by endophytes (Mousa and Raizada, 2013; Pansanit and Pripdeevech, 2018; Tanney et al., 2016) may affect the feeding preferences of herbivores (Coblentz and Van Bael, 2013). It has been reported that fungal metabolites in the host plant may even kill the larvae of gall-forming insects (Wilson, 1995). In general, the interactions between herbivores, fungi, and trees are complex and the outcomes (e.g. for the vitality of the trees) are often difficult to predict (Eyles et al., 2010).

1.3.3 Interactions between nitrogen fertilization, season and site with trees and fungal communities

The availability of nutrients, particularly nitrogen, is one of the limiting factor of tree growth and thus of great importance in productive forests (Högberg et al., 2017). With an increase of nitrogen availability in the ecosystems as a consequence of human activity (Vitousek et al., 1997), nitrogen limitation in forests may not be a concern for tree growth in future. However, nitrogen fertilization is a practice that may profoundly alter the microbial communities of soil and plants (Gallart et al., 2017; Paungfoo-Lonhienne et al., 2015; Siddikee

et al., 2016). The host internal chemical composition may change in response to nitrogen fertilization (Bailey et al., 2005; Decker et al., 2016). Among the compounds affected by higher nitrogen fertilization, are condensed tannins (CTs) which in high concentrations may affect negatively endophytic fungi (Bailey et al., 2005). High concentration of CTs have been found to have a toxic effect limiting the growth *in vitro* of most fungal species. There are however some fungal species of *Penicillium* and *Aspergillus* that are able to survive to high CTs concentration (Scalbert, 1991). An excessive nitrogen fertilization may reduce tree vitality (Dobbertin, 2005) and lead to a higher colonization success of opportunistic fungi. Moreover, the synthesis of fungal primary and secondary metabolites may be altered when nitrogen fertilization is applied to the host (Ibrahim et al., 2011).

Because the endophytic and epiphytic fungal communities of trees are composed by horizontally dispersed spores, the spatial (environmental) variation in fungal communities may be high (Christian et al., 2016; Helander et al., 2007; Pérez-Izquierdo et al., 2017). Distance among hosts and landscape fragmentation may affect the dispersal capacity of certain fungi (Fahrig, 2003; Peay et al., 2012; Vaz et al., 2014). In some cases, air-dispersed spores of the phyllosphere may be able to spread also in fragmented areas but the fragmentation may influence the frequencies with which the species are found (Helander et al., 2007). However, contrasting results have been found suggesting that site effect may be a consequence of localized climatic variations (Wang and Guo, 2007).

In perennial parts of trees, fungal community composition is a result of multiple accumulative infections that occur seasonally and throughout the life of the tree (Fort et al., 2016; Saikkonen et al., 1998; Scholtysik et al., 2013). The accumulation pattern of endophytic infection is particularly evident in leaves. Newly flushed leaves of forest trees are almost endophyte-free but accumulate a rich diversity of fungi by the end of the season (Faeth and Hammon, 1997; Fort et al., 2016). Effect of the seasonality on fungal communities has also been detected in perennial tissues of trees, e.g. Beck et al. (2014) found that there is variation in bark- and lichen-associated fungal communities between spring and autumn.

2 Objectives

The microbiome of trees is highly diverse and dynamic, consisting of a continuum of interactions between fungi and other microbes, the host, and multiple biotic and abiotic factors whose individual and synergic effects are poorly understood (Agler et al., 2016; Rai and Agarkar, 2014). The potential role of certain fungal endophytes as enhancers of plant fitness against biotic and abiotic stressors and resistance against pests and pathogens, have attracted the interest of researchers. A better understanding of the mechanisms behind fungal community assemblage and selection is needed if we want to enhance beneficial fungal communities in trees, or develop fungal tools for sustainable tree care or forestry practices (Agler et al., 2016; Koskella et al., 2017; Witzell and Martín, 2018). Moreover, understanding the role of mycobiome and microbiome in plants and forecasting their variations as consequences of host and environmental changes may help predicting the effects of these changes on ecosystems and ecosystem cycles.

In earlier studies with elms (*Ulmus* spp.), a negative relation was found between the diversity and frequency of xylem bound endophytes and low susceptibility of trees to the Dutch elm disease, caused by *Ophiostoma*-fungi (Martín et al. 2013). It was hypothesized that this could be due to the stronger resistance mechanisms (e.g. accumulation of phenolic metabolites) in trees that were less susceptible to the vascular pathogen, and that these mechanisms might also suppress the colonization by all other fungi (Martín et al. 2013). Should such a relation be common, it could mean that by promoting less susceptible trees in our forests, we might reduce habitats for fungal biodiversity. The underlying hypothesis of this thesis was therefore to test whether the negative relation between fungal biodiversity and tree vitality/resistance would be more commonly found in trees. The general goal of this thesis was to add to our understanding how the structure of fungal communities of broadleaved trees varies in response to different conditions (e.g., chemical composition) and

external factors (biotic and abiotic stress due to e.g. herbivory and fertilization). The studies presented in this thesis explore if the abundance and diversity of fungal communities reflect the tree vitality and degree of disease susceptibility and if this is stable across different tissue types, time of the season, and site. Moreover, the studies presented in this thesis examine if fungal communities respond to profiles in trees representing a specific chemotype that were subjected to nitrogen fertilization and insect herbivory. The specific objectives were the following:

1 Study the relation between tree vitality (Paper I) and tree resistance to pathogen (Paper III) and fungal communities in pedunculate oak and European ash, and to clarify how the community structure differs among different tissues (leaves, bark, xylem), time of the season, and sites;
2 Assess the effect of insect herbivory and nitrogen fertilization on fungal communities in aspen trees with known chemotype (Paper II);
3 Relate the tree fungal communities to the secondary metabolites composition of trees (Paper I and II).

3 Materials and methods

This thesis focuses on fungal endophyte and epiphyte communities associated with pedunculate oak (*Quercus robur,* L.), aspen (*Populus tremula* L.), and European ash (*Fraxinus excelsior* L.) growing in peri-urban area (Paper I), greenhouse chamber (Paper II), and seed orchards (Paper III), respectively. Additional analyses not reported in the manuscripts were added and marked in the thesis.

3.1 Sampling site and sample collection

3.1.1 *Quercus robur* L.

Pedunculate oak (*Quercus robur* L.) samples were collected from 15-year-old trees planted at the edge of an agricultural field in Alnarp, in the outskirt of the city of Malmö, Sweden (55°39’22’’N, 13°05’35’’E) at the end of September 2014 (Paper I).

Oak trees were assigned to three vitality classes: high (H), medium (M), and low (L). The identification of the different vitality classes was based on the visual assessment of the general conditions of shoots, twigs, and leaves, and on the assessment of crown transparency (Müller and Stierlin, 1990). Signs of pathogen or pest attacks were not found on samples with low vitality. Eight trees per vitality class (n=24 trees) were sampled. Four asymptomatic twigs, one per each cardinal point, were detached from each tree and transported in a plastic bag to the laboratory. The samples were processed within 24 h from collection. Four leaves per tree were collected from the crown and air-dried for analysis of phenolic compounds.

3.1.2 *Populus tremula* L.

Twelve aspen (*Populus tremula* L.) genotypes representing the *tremuloides*-chemotype were selected from the Swedish Aspen collection (SwAsp) (Paper II) (Luquez et al., 2008). The SwAsp collection was created with the scope of compiling a collection rich in natural genetic, genomic, and physiologic variation to be used for research. Aspen trees of the SwAsp collection were originally collected in spring 2003 at 12 different localities in Sweden and propagated via cuttings (Luquez et al., 2008).

Eight aspen plants per genotype were potted and grown in greenhouse in 2016. Four treatments were applied to two plants per each genotype (n=96 plants): control (C), herbivory (He), fertilization with nitrogen (N), and herbivory*fertilization (NHe). Nitrogen was applied weekly for four weeks for a total provision of N 44g/m^2. Herbivory treatment was performed with five adult aspen leaf beetles (*Chrysomela tremula* Fabricius, 1787). The insects fed for five days on three leaves enclosed in a mousseline net. An empty mousseline net was placed around the leaves of the plants not assigned to herbivory treatment for control.

The first fully expanded leaf was marked to be later processed for chemical analysis. The next three fully expanded leaves enclosed in the mousseline net were used for endophyte analysis. Leaves were collected and stored at 4°C for maximum three days prior processing.

3.1.3 *Fraxinus excelsior* L.

European ash (*Fraxinus excelsior* L.) trees were sampled at two seed orchards located in southern Sweden: Snogeholm (55˚32´N, 13˚32´E, 50 m) and Trolleholm (55˚57´N, 13˚12´E, 100 m). The seed orchards were established in the 1990s' with 106 clones of *F. excelsior* plus-trees (ash trees with good stem quality and growth) selected at 27 locations in southern Sweden (Stener, 2013). The scions collected from the plus-tree clones were grafted in root stocks at Snogeholm and Trolleholm seed orchards.

After the arrival of *Hymenoscyphus fraxineus* in Sweden (2001), none of the clones present at the two locations showed complete resistance to the pathogen. However, not all clones were equally affected by the disease expressing high variation. Stener (2013) ranked the clones with a scoring system based on the damage caused by *H. fraxineus*. Based on the ranking (Stener, 2013) the clones were divided in three susceptibility classes: resistant (R), intermediate (I), and susceptible (S). Up to seven ash trees per susceptibility class were sampled at both sites. From each tree, two visually healthy 2-3-year old twigs with leaves were selected depending on available material at each site at four time points

during growing season 2015. Leaves were detached and placed in a different bag than the twig. Samples were then transported to the laboratory on dry ice and stored at -20°C until further processing.

3.2 Analysis of endophytes and epiphytes

3.2.1 Culture-based method

Oak twigs and aspen leaves were surface sterilized following the protocols described by Helander et al. (2007) and Albrectsen et al. (2010) (Paper I and II, respectively). Bark and xylem of oak twigs were separated with a sterilized scalpel and four pieces per tissue (appr. 3x3x1mm) were plated on water agar (WA) Petri dish (Paper I). Three leaves per aspen tree were collected and ten pieces (1x1 cm) were cut from each leaf and plated individually on potato dextrose agar (PDA) Petri dish (Paper II). Petri dishes were stored in darkness at room temperature for a month and checked every second day for fungal growth. Emerging fungal hyphae were plated onto new malt extract agar (MEA) (Paper I) or potato dextrose agar (PDA) (Paper II) Petri dish. Emerged colonies were classified into morphotypes (MTs) based on their macro-morphological characteristics (i.e. colony colour, shape, texture). An isolate that was not possible to assign to any MT was classified as singleton.

Representative specimens for each MT of the oak and aspen fungal collection were selected for DNA extraction. The selected isolates were grown in malt extract broth for two to four weeks at room temperature in dark. Mycelium was harvested and lyophilized for 48h. Samples were then homogenized and genomic DNA was extracted with E.Z.N.A. SP Plant DNA kit (Omega Bio-Tek, Inc., Norcross, USA). The fungal internal transcribed (ITS) region was amplified using the primers ITS1 and ITS4 (White et al., 1990). PCR products were purified and quantified before being sent for Sanger sequencing.

3.2.2 Culture-independent method

Leaflets, bark and xylem of ash samples were placed in 50mL Falcon tubes and lyophilized for at least 48 h (Paper III). Leaf samples were then homogenized in a FastPrep-24 homogenizer (MP Biomedicals, Santa Ana, USA) while bark and xylem samples were lyophilized in liquid nitrogen. Genomic DNA was extracted with E.Z.N.A. SP Plant DNA kit (Omega Bio-Tek, Inc., Norcross, USA). The ITS2 region of the rDNA was amplified using the tagged primers ITS4 (White et al., 1990) and fITS7 (Ihrmark et al., 2012). Amplified PCR products were

cleaned and pooled in equimolar mix before being sent to Illumina MiSeq sequencing.

3.3 Extraction of plant secondary metabolites

Four air-dried leaves per oak tree were pooled together and each sample was homogenized into a fine powder using a ball mill (MM301, Retsch Gmbh) (Paper I). The extraction and high-performance liquid chromatography (HPLC) was done following the procedure described in Romeralo *et al*. (2016).

Aspen leaves were flash-frozen in liquid nitrogen, lyophilized and homogenized to fine powder using a ball mill (MM301, Retsch Gmbh) (Paper II). Condensed tannins (CTs) were assessed using Porter's assay (acid: butanol method, Bandau et al., 2015; Porter et al., 1985). Absorbance was measured on a Spectra Max 190 microplate reader (Molecular Devices, Sunnyvale, CA) at λ 550 nm. Procyanidin B2 ($C_{30}H_{26}O_{12}$, Sigma- Aldrich®, St. Louis, MO, USA) was used as standard.

Targeted LC-MS was run using the standard compounds from the Swedish Metabolomics Centre (SMC, Umeå, Sweden) including the salicinoid standards salicin, tremulacin, salicortin, and tremuloidin. The Mass Feature Extraction (MFE) was acquired with the MassHunter™ Qualitative Analysis (B06.00, Agilent Technologies Inc., Santa Clara, CA, USA). Mass Profiler Professional™ 12.5 (Agilent Technologies Inc., Santa Clara, CA, USA) was used to align and match the extracted features between samples. Linear standard curves were used to quantify the salicinoids on peak area basis. Based on the molecular weights, the masses of either or both deprotonated ions and formate adduct were assessed and guided by retention times if available (Abreu et al., 2011; Keefover-Ring et al., 2014).

Global GC-MS analysis was performed in a Pegasus III time-of-flight mass spectrometer, GC/TOFMS (Leco Corp., St Joseph, MI, USA). The mass (mean of integrated peak areas) was quantified with MATLAB™ R2011b (Mathworks, Natick, MA, USA). Custom script according to Jonsson et al. (2005) were used to perform base-line correction, chromatogram alignment, data compression, and Hierarchical Multivariate Curve Resolution (H-MCR). In order to identify the extracted mass spectra, the extracted mass spectra retention indices were compared with libraries of retention time indices and mass spectra (Schauer et al 2005). Peak area was used to quantify the identified chemicals and were then assigned to metabolite class (phenolic, amino acid, fatty acid).

3.4 Data compilation

The number of emerging colonies and morphotypes per tissue samples was recorded (bark and xylem in Paper I, and leaves in Paper II). The colonization frequency (CF) was then calculated as the proportion (%) of all colonies yielded from the samples divided by the total number of samples in the experiment (Papers I). Morphotype richness (S^{MT}) was measured as the number of different MTs per twig (Paper I) or tree (Paper II). For individual morphotypes, relative abundance (%) was calculated as the number of emerging colonies per MTs divided by the total number of colonies emerged in bark and xylem samples (Paper I) or leaves (Paper II).

UNITE database was used for the taxonomic analysis in QIIME bioinformatics pipeline (Abarenkov et al., 2010; Caporaso et al., 2010) (Paper III). USEARCH, implemented in QIIME, was used to cluster the sequence reads into Operational Taxonomic Units (OTUs) at 97% sequence identity. One representative sequence from each clustered OTU was selected to assess the closest taxonomic affiliation in UNITE implemented in QIIME. Statistical analysis was performed on OTUs assigned to fungal taxa after removing global singletons and OTUs with less than 10 reads (Brown et al., 2015). The OUT richness per ash samples was then measured as the sum of OTUs detected per samples.

Data analysis was performed in R (R Development Core Team, 2013) unless otherwise specified.

3.5 Data analysis

3.5.1 Fungal community

Mann-Whitney U (W) and Kruskal-Wallis (KW) tests (Castillo Lopez et al., 2014) were used to examine the variation of CF and S^{MT} in tissue type and vitality classes, respectively (Paper I). In Paper II, the effect of CTs, nitrogen, and herbivory on endophytes abundance and richness were tested with generalized linear models with negative binomial and Poisson distribution, respectively. Due to herbivory treatment, the variation in morphotype richness was tested only between C and N treatment. The relative abundance of endophytic isolates per treatment was visualized with a heat-map (Paper II). Linear mixed model was performed to test the variation of the OTU richness in ash samples in the susceptibility class, tissue type, time points, and site.

The variation in fungal community composition (i.e. the variation of species abundance and species presence) was analysed with permutational multivariate

analysis of variance (Permanova, Anderson, 2001) based on Bray-Curtis dissimilarity matrix using 999 permutations (adonis function, vegan package, Oksanen et al., 2016). Permanova analysis was performed in all three papers to test the relations between fungal community composition and the investigated factors. Permanova analysis was performed only between C and N treatments as not all leaves survived the H treatment (Paper II). Permanova analysis was constrained to time points in the investigation of ash fungal community (Paper III).

The probability of finding a given MT in a given tissue and susceptibility class was then predicted with logistic regression analysis (JMP® Pro 13, SAS Institute Inc., Cary, NC, United States) (Paper I). The variation in the endophytic communities of the three vitality classes of oak were visualized with non-metric multidimensional scaling (NMDS) (Paper I).

Fungal diversity was also tested with Hill numbers that can incorporate richness, exponent of Shannon index, and inverse Simpson index (Legendre and Legendre, 1998) (Paper III). The variation of fungal communities of ash trees and the effect of susceptibility class, tissue type, sampling site, and time points was visualized with canonical correspondence analysis (CCA) (Paper III).

3.5.2 Chemical data

The differences in HPLC-derived phenolic profiles were tested with canonical discriminant analysis (JMP® Pro 13, SAS Institute Inc., Cary, NC, United States). The effect of nitrogen, herbivory and their interaction on condensed tannins were tested with generalized linear model. To visualize the effects of nitrogen fertilization on the metabolic composition from GC-MS and LC-MS on the leaves orthogonal partial least squares discriminant (OPLS-DA) was performed (SIMCA v14.0).

4 Results

The results presented here focus on the main findings from the three manuscripts (Paper I, II, and III). Data not presented in the manuscripts were added to this chapter.

4.1 Tree phenotype as discriminant of fungal communities (Paper I and III)

4.1.1 Taxonomic diversity

The endophytic community detected in pedunculate oak trees was grouped into 28 MTs (Fig. 2, Paper I), most of which were identified as belonging to four classes of the phylum *Ascomycota* (Table 1).

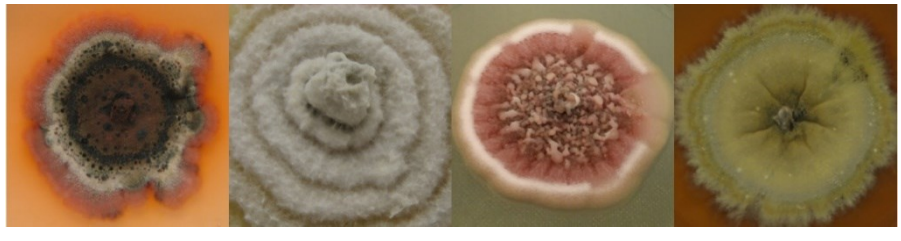

Figure 2. Endophytic fungi isolated from *Quercus robur* twigs growing on MEA. From left to right: MT22, MT3, MT27, MT4. Photo: Marta Agostinelli.

The fungal community of ash samples (272 ash samples and 19 neighbouring-tree samples) was composed by 4430 OTUs comprising 25,175,104 high quality reads (Paper III). The majority of reads were assigned to *Ascomycota* (59.3%) and *Basidiomycota* (38.5%). The molecular technique used with ash samples revealed a higher number of classes for both *Ascomycota* and *Basidiomycota* than what found with culture-based technique in oak and aspen (Table 1). The

reads assigned to *Chytridiomycota*, *Glomeromycota*, and *Zygomycota* represented the 0.03% of the total reads. The remaining 2.1% of reads belonged to unidentified fungi.

Table 1. *Relative abundance of isolates (oak and aspen) and reads (ash) per class. Only the classes with a relative abundance of 0.5% or higher are reported (not reported in Paper I, II, III).*

Phylum	Class	Oak	Aspen	Ash
Ascomycota	*Dothideomycetes*	17	41	34.2
	Eurotiomycetes	8.2	6.9	6.26
	Lecanoromycetes			7.5
	Leotiomycetes	20.3		1.2
	Orbiliomycetes			0.05
	Pezizomycotina			0.7
	Sordariomycetes	23.5	4.1	2.58
Basidiomycota	*Taphrinomycetes*			3.7
	Cystobasidiomycetes			7.6
	Exobasidiomycetes			5.1
	Microbotriomycetes			2.7
	Pucciniomycetes		0.5	0.3
	Tremellomycetes			19.8

4.1.2 Tree vitality and susceptibility

Tree vitality influenced the endophytic community of oak trees (Paper I). Both colonization frequency (CF) and morphotype richness (S^{MT}) were significantly different. This difference was mainly due to fungal communities in samples from low vitality trees deviating from those in samples from high and medium vitality trees. Venn diagram showed that 13 MTs were shared among all three vitality classes (Fig. 3). Low vitality classes hosted three morphotypes that were not found in other vitality classes, and only one MT was exclusively associated to high vitality class and none to medium vitality class (Fig. 3).

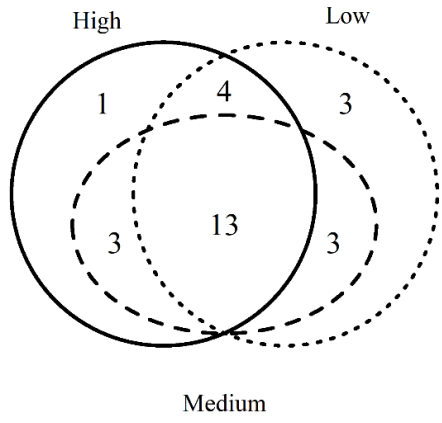

Figure 3. Venn diagram representing the unique and shared MTs for the three vitality classes of bark and xylem samples grouped together (modified from Paper I).

While the endophytic community of medium vitality class seemed to differentiate from the endophytic community of high and low vitality trees (Fig. 4), the Permanova analysis did not reveal difference in the endophytic community of the three vitality classes (Permanova: R^2=0.045, p=0.35). However, the logistic regression highlighted that vitality and tissue type correlated with MTs (Table 2). Particularly, MT14 was found to highly correlate only with vitality class (Table 2).

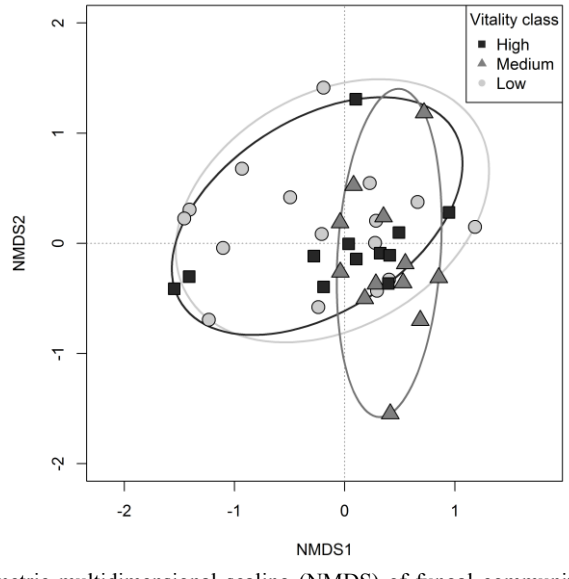

Figure 4. Non-metric multidimensional scaling (NMDS) of fungal communities of oak vitality classes (not presented in Paper I).

Table 2. *MTs relative abundance and logistic regression of oak MTs that were significantly correlated with vitality class or tissue type. The p-values are significant at α<0.05 (modified from Paper I).*

MTs	Relative abundance	Vitality - P>ChiSq	Tissue - P>ChiSq
MT3	14.1	0.92	0.0000
MT4	2.3	0.08	0.0000
MT5	3.6	0.038	0.0001
MT8	0.7	0.0004	0.0018
MT9	1.0	0.012	0.018
MT10	1.6	0.011	0.0000
MT11	3.6	0.022	0.0000
MT12	3.9	0.022	0.0000
MT14	5.9	0.0009	0.10
MT16	1.3	0.20	0.018
MT20	1.3	0.003	0.0083
MT22	18.6	0.26	0.0000
MT27	3.6	0.22	0.0000
MT28	2.3	0.37	0.05
MT29	4.9	0.07	0.0000

Tree susceptibility to ash decline had contrasting effects in shaping the endophytic and epiphytic community of ash samples (Paper III). The richness of OTUs detected per susceptibility class was not significantly different (lmer: $F=1.48$, $p=0.229$). The three susceptibility classes shared 992 OTUs (Fig. 5). The number of OTUs exclusively associated to a susceptibility class was higher in resistant and intermediate trees than in susceptible trees (Fig. 5). In contrast to richness of OTUs, the composition of fungal community of the three susceptibility classes differed significantly among each other (Permanova: $R^2=0.019$, $p=0.001$).

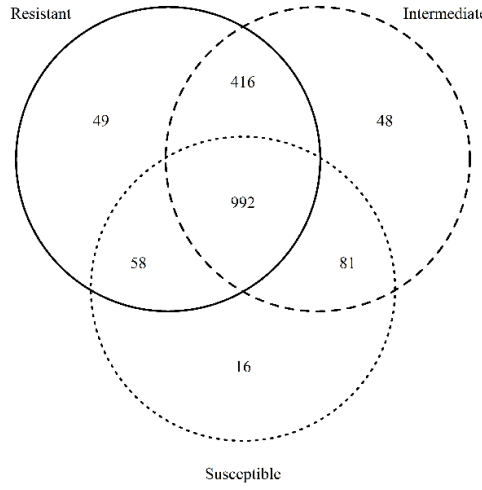

Figure 5. Venn diagram representing the unique and shared OTUs of the three susceptibility classes of ash trees.

4.1.3 Tissue type

Endophyte and epiphyte communities showed tissue-specific differences (Paper I and III). The endophytic community of oak bark samples had a higher colonization frequency and MTs richness than xylem (Mann–Whitney: U = 574.5, p=0.0001). The logistic regression identified tissue as the main discriminant factor in selecting endophytic morphotypes of oak (Table 2). The fungal communities were highly diverse across the leaf, bark, and xylem samples of ash trees (Paper III). The OTU richness was different among the susceptibility classes in leaves but not in bark, nor in xylem. On the other hand, the fungal community composition differed among the susceptibility classes of all three tissues (Table 3, Fig. 6).

Table 3. *Response of richness of OTUs and fungal community composition of ash tissues at susceptibility class. The p values are significant at $\alpha<0.05$*

Tissue	Richness of OTUs		Community composition	
	F value	lmer	R^2	Permanova
Leaf	5.21	p=0.007	0.034	p=0.009
Xylem	3.46	p=0.051	0.030	p=0.002
Bark	0.89	p=0.41	0.084	p=0.006

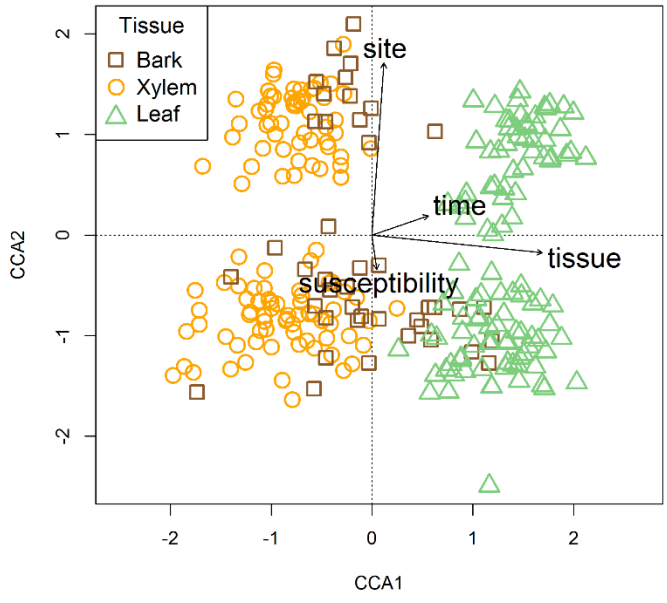

Figure 6. Canonical correspondence analysis (CCA) plot representing the variation in fungal community composition. The samples are shaped- and colour-coded according to the tissue type they belong to. The length of the arrows represents the relevance of a factor in driving the difference in the fungal community (modified from Paper III).

The difference in community composition observed among ash susceptibility classes was however not reflected in all tissue types (Paper III). While xylem fungal communities were statistically different between all three classes, leaf fungal communities were statistically different only between resistant-susceptible and intermediate-susceptible classes, and bark fungal communities only between intermediate-susceptible classes.

4.1.4 Season and site

Ash fungal community was affected by the time of collection with however some differences at tissue level (Paper III). The overall community composition (all ash trees studied together) was different between time points 1 and 3 (Hill's number, Tukey's HSD: p= 0.001). The richness of OTUs in leaf samples was statistically different between time point 1 and time points 2, 3, and 4 (Fig. 7). Richness of OTUs in xylem samples was different between time point 1 and time points 2 and 4 (Fig. 7). No difference in OTU richness was observed among the bark samples at the four time points (Fig. 7). According to Permanova analysis, the fungal community composition of ash trees differed among all four time points (Permanova: R^2=0.041, p=0.001). This same pattern was observed in all

tissue types with the exception of the fungal communities of bark samples at time points 2 and 3 that were not diverse.

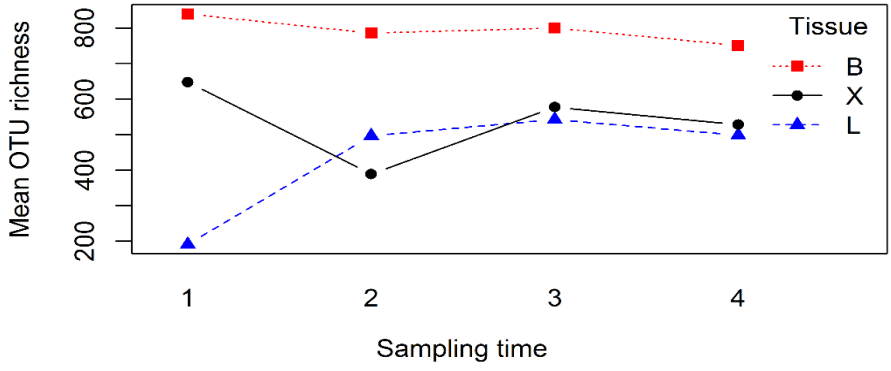

Figure 7. Interaction plot representing mean OTU richness for bark (B), xylem (X), and leaves (L) by time point (1, 2, 3, and 4) (not presented in Paper III).

The sampling sites did not have an effect on OTU richness detected in the samples. Fungal community composition was instead different between the two sites (Permanova: R^2=0.036, p=0.001) (Fig. 8).

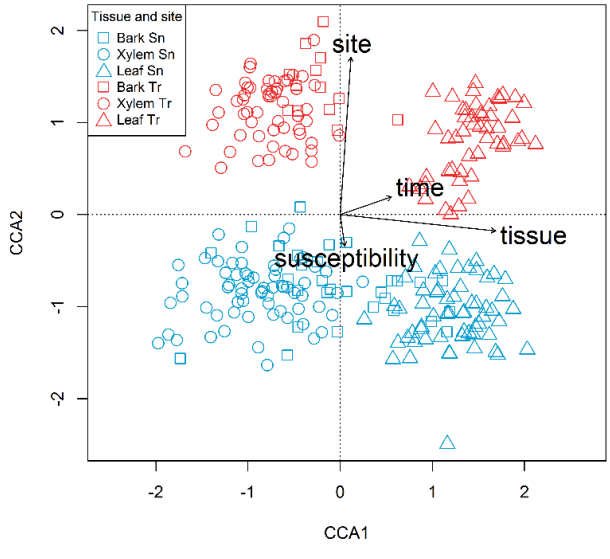

Figure 8. Canonical correspondence analysis (CCA) plot representing the variation in fungal community composition. The samples are shaped- and colour-coded according to the tissue type and sampling site (Sn-Snogeholm, Tr-Trolleholm). The length of the arrows represents the relevance of a factor in driving the difference in the fungal community (modified from Paper III).

4.2 Tree chemotype as discriminant factor of fungal communities (Paper II)

4.2.1 Taxonomic diversity

The isolates were grouped into 30 MTs (Paper II). The identified MTs belonged mainly to classes of the *Ascomycota* and one class of *Basidiomycota* (Table 1).

4.2.2 Nitrogen and herbivory

Differences in abundance of isolates were tested for the four treatments (C, N, He, and NHe) while differences in the richness of MTs were tested only in the two treatments where trees retained all three leaves (C and N) (Paper II). Neither the abundance nor the richness of endophytes outgrown from aspen leaves were significantly different among the treatments. However, there were MTs exclusively associated with a specific treatment (Fig. 9).

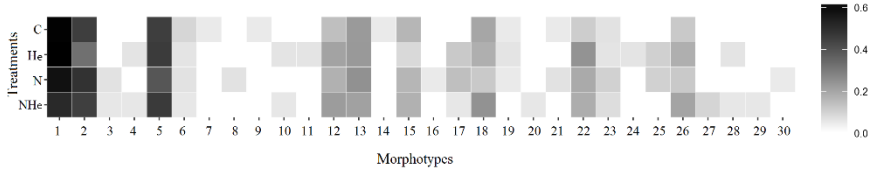

Figure 9. Heat map representing the relative abundance of endophyte detected per MT and treatment (C-control, He-herbivory, N-nitrogen, NHe-nitrogen*herbivory). The darker the colour, the higher the relative abundance of endophyte isolates (not presented in Paper II).

The endophytic community composition of aspen leaves was not significantly different among C and N treatments (Permanova: R^2=0.03, p=0.19).

4.3 Phenolic compounds relation to fungal communities and trees phenotype (Paper I and II)

4.3.1 Leaf phenolics in oak

The canonical discriminant analysis based on oak leaf phenolics showed a clear division among trees belonging to different vitality classes (H, M, and L) (Fig. 10) (Paper I). The highest level of phenolics were detected in low vitality trees, while medium vitality trees had the lowest level of phenolics (Paper I).

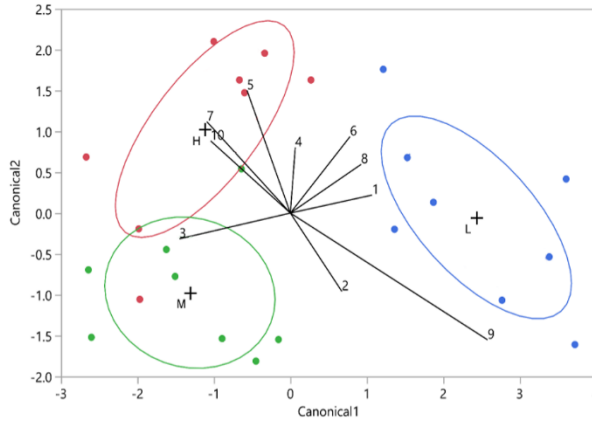

Figure 10. Canonical discriminant analysis of HPLC leaf data (10 UV-absorbing peaks) for the three vitality classes of oak (H-high: red; M-medium: green; L-low: blue). The ellipsoid lines represent the confidence region (95%) containing the true mean value of each vitality class. The black lines identified by the numbers represents the phenols from the ten biggest phenolic peaks that mostly discriminate between the vitality classes.

4.3.2 Leaf phenolics of aspen

Nitrogen fertilization significantly affected the concentration of CTs in aspen while herbivory and the interaction of nitrogen with herbivory did not affect CTs concentration (Paper II). Targeted metabolites (phenolics) of aspen leaves were affected by nitrogen fertilization (Fig. 11).

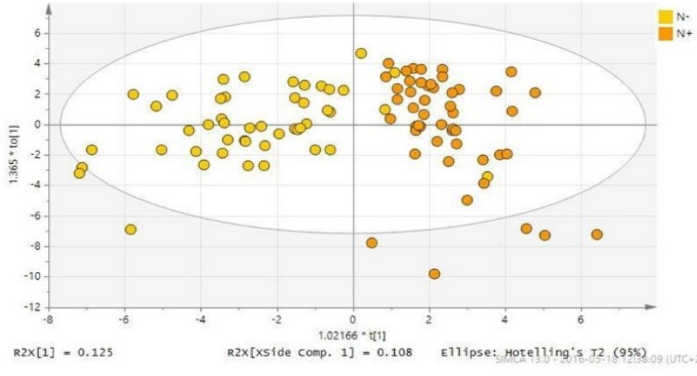

Figure 11. OPLS-DA plot of phenolics coloured after nitrogen fertilization (N+ corresponds to addition of 1.5kg/ha/yr of NH_4NO_3).

5 Discussion

The overall objective of my thesis was to contribute to the understanding of how fungal communities of the aerial tissues of broadleaved trees vary in relation to the phenotypic and chemotypic characteristics of the host. The results indicated that the regulation of tree mycobiome structure is influenced by a complex network of internal and external factors.

5.1 Fungal taxonomy

The fungal taxonomy found in the three species was overall comparable with the taxonomy described by previous studies conducted with similar methods on the tree species of the thesis (Arnold and Lutzoni, 2007; Higgins et al., 2007; Rodriguez et al., 2009). *Aureobasidium pullulans*, a common endophyte and epiphyte described on different hosts from temperate and tropical areas (Fröhlich et al., 2000; Martín et al., 2013; Osono, 2008; Sanz-Ros et al., 2015; Unterseher and Schnittler, 2009) was detected in all three hosts. Similarly to the findings of Unterseher and Schnittler (2009), *A. pullulans* was only rarely isolated in oak and aspen tissues but was one of the most common species found in ash, as also found by Fort et al. (2016). Other genus such as *Alternaria*, *Phomopsis*, and *Trichoderma* that have been commonly found on a variety of hosts (Crous and Groenewald, 2013; Ragazzi et al., 2003; Sanz-Ros et al., 2015; Sieber, 2007; Sun et al., 2012) were also not detected with cultivation-based techniques (Paper I, II) but were found with cultivation-independent techniques (Paper III). The fact that these commonly found genera were not recovered in oak and aspen may have different explanations. In the oak study, not all MTs were successfully identified via DNA, and in aspen the early sampling at the beginning of the season and the greenhouse condition may have selected for certain species. Cultivation-based technique is needed to recover endophytes but is known to be highly selective (Guo, 2010; Promputtha et al., 2007).

The fungi recovered from oak included generalist species (ex: *A. pullulans*) and host specific species such as *Amphiporte leiphemia* and *Colpoma quercinum* that are specific to pedunculate oak and to the *Quercus* genus, respectively (Ragazzi et al., 2003; Sieber, 2007). The community recovered in aspen was formed mainly by generalist species. *Fusarium*, *Ramularia*, *Penicillium*, *Cladosporium*, *Physalosporta* are indeed genera found on different hosts (Dulymamode et al., 2001; Fort et al., 2016; Guimarães et al., 2011; Sanz-Ros et al., 2015; Sieber, 2007; Tadych et al., 2012). Fungal endophytic genera such as *Cladosporium*, *Ramularia*, *Colpoma*, *Phoma*, are known to have pathogenic traits (Kehr, 1992; Thomma et al., 2005; Videira et al., 2016) and have been recovered both from oak and aspen tissues. However, in both studies, no signs of known pathogens were detected on the investigated tissues. In ash, the fungal community was characterized by few species found in all samples and many rare species. The most abundant species found in ash samples (Table 5 in Paper III) are ubiquitous and generalist species that can be found on different hosts (Cordier et al., 2012; Cross et al., 2017; Ibrahim et al., 2017; Jumpponen and Jones, 2010; Karlsson et al., 2014; Pawłowska et al., 2014; Power et al., 2017). This findings are in line with previous studies where the fungal communities of different hosts are composed by generalist and specialized species (Sanz-Ros et al., 2015; Schulz and Boyle, 2005; Sieber, 2007).

5.2 Tree phenotype and fungal communities

5.2.1 Fungal communities in relation to phenotypic vitality or pathogen resistance

The results of this thesis demonstrate that the fungal communities of trees vary qualitatively and quantitatively among trees showing different vitality or pathogen resistance phenotype (Paper I and Paper III). These results are in accordance with results of earlier studies that have shown a relation between the phenotypic responses to pathogenic attack or decline in vitality and the fungal diversity or abundancy in trees (Giordano et al., 2009; Martín et al., 2013). For instance, the xylem of declining Scots pine (Giordano et al., 2009) or elms that are susceptible to Dutch elm disease (Martín et al., 2013) have earlier been found to host a richer and more abundant endophytic communities than the vital or resistant conspecific trees.

In the xylem of ash trees, on the other hand, the richness of OTUs (presumably species) was not different among the three susceptibility classes. Instead, a difference in species richness was found in leaves, with low vitality

samples yielding a lower number of species than resistant and intermediate classes. The lower number of species detected in susceptible trees is in agreement with the previous findings by Koskella et al. (2017), who showed that bacteria communities of horse chestnuts susceptible to the bleeding canker agent *Pseudomonas syringae pv aesculi* had lower species richness than more resistant trees.

The contrasting results found in oak and ash studies (Paper I and III) may relate to the different causes of the reduced health condition of the trees. The low vitality in the studied oaks was not related to specific pathogens, while ash trees were under the attack of a highly pathogenic and invasive fungus. Thus, the tree responses that were active against fungal colonizers may have been different in the two studies. Moreover, the techniques used in the two studies are different. The culture-based approach captures the rather fast-growing fungi, which were thus likely to dominate in the oak. Moreover, the available inoculum from the surrounding environment may have influenced the results. The oak trees growing in the peri-urban setting where likely to be exposed to a different inoculum than the ash trees growing in the seed orchards. Interestingly, *Q. robur* trees showing low, intermediate, or high loss of vitality in the presence of soil-borne *Phytophthora* in a forest setting were found to harbour the highest diversity of culturable endophytes in the intermediate group (J. Witzell, unpublished data). Thus, the relation between fungal communities and tree vitality or pathogen resistance is likely to be under a complex regulation of internal and external factors that work in synergy across time and space.

5.2.2 The importance of tissue type

Throughout the studies, it was obvious that the type of tissue is a highly selective factor for fungal communities. The community composition differed clearly among the different tissues (Paper I and III), confirming the tissue-specificity of certain fungal species (Petrini and Fisher, 1990; Santamaría and Diez, 2005). Similar to other studies (Collado et al., 2000; Fisher and Petrini, 1990; Wang and Guo, 2007), the bark tissue was found to host the highest taxonomic richness, in both oak (Paper I) and ash (Paper III). Xylem, on the other hand, being the inner tissue, is likely to be more difficult to penetrate for the horizontally spreading fungal species. Moreover, the different structure and chemistry between bark and xylem may select for certain fungal species (Lourenço et al., 2016). Martín et al. (2013), studying elm (*Ulmus* sp.) trees that were selected based on their susceptibility to the vascular Dutch elm disease (DED) pathogen, found that the colonization frequency and morphotype richness of endophytes did not differ in the bark tissue of differently susceptible

trees, suggesting that in these trees the mechanisms behind low susceptibility were more strongly expressed in xylem than in bark. The lower taxonomic richness observed in leaf compared to bark (Paper III) is likely to relate to the fact that bark is exposed to new infection every year, while leaf communities of broadleaved trees are formed newly every year (Faeth and Hammon, 1997; Saikkonen et al., 1998; Scholtysik et al., 2013) and show a dynamic development within the growing season (Fort et al. 2016).

5.2.3 Temporal and spatial variation in tree fungal communities

Temporal and geographical effects influenced the fungal community composition. However, while seasonal changes have generally been found to statistically affect the fungal composition (Jumpponen and Jones, 2010; Unterseher et al., 2013), sites have been found to give contrasting results (Pérez-Izquierdo et al., 2017; Wang and Guo, 2007; Zimmerman and Vitousek, 2012). Wang and Guo (2007) suggested that the effect of site may be related to local variation. In the ash study, there was a strong response of the fungal community to both season and site (Paper III). The seasonal pattern observed in leaf samples was not seen in bark nor in xylem. While the fungal community composition changed in both perennial tissues, the species richness remained similar suggesting that variation in species abundance may have stronger effect than shift in species composition in xylem and bark. However, species richness in the xylem samples from ash had a drop between time point 1 and time point 2 that was not possible to relate to any particular event in the sampling. Even if not statistically different, species richness in all three tissue types tended to decrease at the end of the growing season from time point 3 to time point 4, suggesting that fungal species with a more saprotrophic or pathotrophic life style may take over some niche and displace some fungi. A similar pattern was detected by Fort et al. (2016) in foliar communities of grapevine, but not in forest trees (oak, hornbeam, chestnut) growing closely to the vineyard. The change observed in ash leaf may relate to alteration in leaf physiology as a consequence of senescence, increased pressure from *H. fraxineus*, and competition dynamics (Cross et al., 2017; Jumpponen and Jones, 2010). According to the propagule pressure hypothesis (Lockwood et al., 2005), the higher the propagule present, the higher the possibility of success in the establishment of an invasive species. Cross et al. (2017) suggested that the success of *H. fraxineus* in colonizing ash leaves may relate to the higher propagule pressure found towards the end of the vegetative season than at the beginning. The temporal variation observed in bark and xylem may be related to the physiological changes in trees as consequence of accumulated pathogen infections (Cross et al., 2017). According to Wong et

al. (2009), there is a variation in the carbohydrate metabolism between season. This could lead to decreasing availability of carbohydrates for the fungi in the end of the season. At the last sampling point, the leaves had started to senesce. Thus, the altered translocation of nutrients in the tree may have changed the availability of nutrients for the fungi, influencing their abundance. Fungi use substrates in different ways (Blumenstein et al., 2015), thus a change in nutritional niche characteristic may favour species better adapted to a substrate and displace those not fitting with the new substrate regime.

5.3 Tree chemotype and fungal communities

Host species has been considered as one of the major factors shaping the mycobiome of plants (Schulz and Boyle, 2005; Stone et al., 2000). Recent studies have shown that the genetic variation within a species can also have a strong selective effect on fungal communities (Albrectsen et al., 2018; Bálint et al., 2013; Christian et al., 2016; Lamit et al., 2014; Pérez-Izquierdo et al., 2017). However, it is still unclear whether the chemotypic characters influence the composition of fungal community. A previous study also done in greenhouse conditions using aspen genotypes from the SwAsp collection (Luquez et al., 2008), showed that different chemotypes shaped the endophytic communities of the leaves of aspen (Albrectsen et al., 2018). In contrast to Albrectsen et al. (2018), the aspen genotypes from the SwAsp collection selected for this study were selected to represent similar salicinoid chemotype (Luquez et al., 2008) and no effect was observed in the endophytic community. These results suggests that there is a connection between the salicinoid metabolism and fungal communities in the leaves of aspen. Whether the determinants of the chemotype influence fungi directly or through indirect mechanism(s) remains to be studied.

Fertilizer addition and insect herbivory have been previously reported as factors shaping fungal communities (Albrectsen et al., 2018; Coblentz and Van Bael, 2013; Eschen et al., 2010; Lehtonen et al., 2005). Nitrogen is an important nutrient for tree growth (Högberg et al., 2017) but an excessive fertilization may have counteractive effects, increasing the susceptibility of the trees to diseases and pests (Veresoglou et al., 2013). Several studies connected nitrogen fertilization with the alteration of fungal communities in soils and rhizosphere of agricultural and forest environments. Fertilization with nitrogen has been associated with reduction in fungal abundance and fungal diversity in soil and rhizosphere in agricultural and forest environments (Chen et al., 2017; Ishida and Nordin, 2010; Paungfoo-Lonhienne et al., 2015). On the other hand, nitrogen addition has been connected with an increased abundance of saprotrophic and pathotrophic fungal species in arbuscular and ectomycorrhizal

fungi (Jiang et al., 2018; Morrison et al., 2016). While there are many studies focusing on the effects of nitrogen on soil fungal communities, the effect of nitrogen fertilization on fungal endophyte communities of aerial tissues of forest trees is less well investigated. Eschen et al. (2010) investigated this aspect in *Cirsium arvense* and found that nitrogen was involved in the variation of foliar fungal communities. In the aspen study, nitrogen did not affect the endophytic abundance or diversity (Paper II).

The endosymbionts living in the plant tissues may provide their hosts with an additional defensive mechanism against insect pests. Endophytes may directly affect the herbivores by producing secondary metabolites that reduce the attractiveness or palatability of the plant tissues to herbivores (Estrada et al., 2013). Earlier, herbivory treatment by *Chrysomela* beetles was reported to be responsible of enriching leaf fungal communities and increasing the abundance of fungi in aspen clones selected from the SwAsp collection (Albrectsen et al., 2018). Our results (Paper II) indicate that the impacts of herbivory on tree endobiome may not be straightforward. There was, however, a tendency to higher endophyte richness in herbivory treatment than in control, suggesting that herbivory may promote endophyte richness.

5.4 Chemical profile and fungal communities

Previous studies have demonstrated that phenolics are good indicators for pathogen resistance in trees (Conrad et al., 2017; McPherson et al., 2014; Witzell and Martín, 2008), but less is known about the correlation between phenolic levels and endophytic infections. Phenolics have antifungal properties that could affect the endophytic communities, or their levels may be indirectly related to tree resistance (Witzell and Martín, 2008). On the other hand, fungi may be able to utilize the carbon-based phenolics as a source of energy. For instance, Blumenstein et al. (2015) found that some endophytes were able to effectively utilize phenolics as a substrate. Phenolics have been found to accumulate in plant tissues when the plant is in a situation of stress as result of wounding (Eyles et al., 2010; Lattanzio et al., 2006; Sherwood and Bonello, 2013). It is also possible that endophytes induce changes in phenolic metabolism of their hosts (Yang et al., 2016).

The results from oak and aspen experiments (Paper I and II) reveal an ambiguous relation between the phenolic pool and endophytic status of trees. Oak leaves are known to be rich in phenolics (Covelo and Gallardo, 2001) and the canonical discriminant analysis on oak leaf phenolics showed that the phenolic profiles discriminated well among the three vitality groups (Paper I). The higher concentration of phenolics found in low vitality trees (Paper I) may

relate to accumulation of phenolics in leaf tissue as trees respond to stress. Since endophytic infections may induce phenolic metabolism, the higher levels of phenolics in less vital trees (with rich endophytic flora) could also be explained by the more frequent and diverse endophytic infections. Phenolic production may also depend on plant genetic traits (Schweitzer et al., 2008) but since the genetic background of oak trees is unknown, it was not possible to relate the phenolic production to it.

The analysis of total phenolics revealed that phenolics decreased with nitrogen fertilization, clearly separating fertilized trees from non-fertilized ones (Paper II). The increased nitrogen availability in plant tissue may then affect the fungal composition via changing the substrate characteristic and favouring species better adapted to nitrogen utilization. Condensed tannins are known to have defensive properties against herbivores, but there was no indication of CTs being induced by herbivory in aspen leaves (Paper II). In fact, CTs concentration decreased in aspen plants subjected to herbivory compared to control treatment. The concentration of CTs was more strongly affected by nitrogen fertilization, confirming what found previously by Decker et al. (2016). Nitrogen fertilization, by reducing the concentration of antifungal CTs, may have made the leaf a more suitable substrate for endophytic fungi. Bailey et al. (2005) found that higher amount of CTs were correlated to lower endophytic infection and diversity that was however not found in Paper II. The negative correlation between lower CTs concentration and endophytic richness needs however to be further investigated. Future studies could focus on investigating which endophytic groups are mainly affected by high levels of CTs, and whether there is a three-way interaction between endophytes, trees and herbivores.

6 Concluding remarks and future prospect

In summary, the results of this thesis provided some support for the initial hypothesis that the fungal communities are more diverse in the less vital trees, and the most pronounced differences were observed in the communities of the xylem (cf. Martín et al., 2013). Fungal communities in leaves diversified more than those in bark or xylem during the growing season, showing a rapid increase in species richness and diversity from May to September, which is in line with earlier studies (Jumpponen and Jones, 2010; Scholtysik et al., 2013). Interestingly, nitrogen and herbivory did not have a clear effect on fungal communities of aspen leaves, although this could have been expected based on earlier studies (Albrectsen et al., 2018; Eschen et al., 2010) and especially considering that fertilization clearly reduced the concentration of potentially antifungal condensed tannins, changing the quality of the substrate. In general, the connection between host tree phenolics and fungal communities seems ambiguous and warrants more studies, which should particularly consider possible indirect relations (e.g., phenolics being indicators for physiological mechanisms that influence the fungi, rather than directly acting as antifungal compounds, which is traditionally assumed).

Studying microscopic fungal communities is challenging and fascinating because it reveals the unique and complex world hidden in front of us in every plant, while we still cannot grasp the totality of it. There are many complex and elusive mechanisms through which fungi and trees are continuously balancing their interactions. This thesis confirms that multiple factors are involved in shaping the variation of fungal communities of the aerial tissues of deciduous forest trees. Using both culture-dependent and culture-independent methods it was possible to add to the current knowledge which factors may or may not have a strong role in the variation of fungal communities. The results highlighted that phenotypically different trees, host species, and tissue type contribute to the variation of fungal communities in aerial tissues. The microbiome and host

interact in a complex matrix of seasonal and site-specific factors, adding to the dynamics.

The potential use of endophytes as one of the tools in integrated pest management in forest settings is hindered e.g. by the lack of understanding of how to ensure a continuous, stable, and reliable endophytic community over the time (Newcombe, 2011; Witzell and Martín, 2018). A profound complication in clarifying the dynamics of fungal communities in trees is the difficulty of studying fungal communities in vivo in large, old trees (Albrectsen and Witzell, 2012). Moreover, community studies still tend to focus only on one microbial category (Agler et al., 2016; Gomes et al., 2018; Laforest-Lapointe et al., 2016; Leff et al., 2015), thus providing new insights on only one component of a more complex microbial interaction. Our ability to include fungal communities as a forest management tool is thus not only limited to our understanding of how fungal communities are formed and maintained in different hosts, tissues, and environmental conditions, but also on how they interact with the microbial community (bacteria and viruses) sharing the same host. The cheaper and easier accessibility to high throughput sequencing techniques, combined with traditional culturing-based techniques may allow researcher to describe the hidden mycobiome and microbiome diversity to much greater detail than what was possible before (Campanile et al., 2007; Martín et al., 2015; Porras-Alfaro and Bayman, 2011; Pusztahelyi et al., 2015). Moreover, tools such as phenotype microarrays and FUNGuild[1] may help describing and characterizing the functional and ecological roles of fungi. Phenotype microarrays method has recently been used to study the in vitro substrate utilization by living cells of different fungi as a proxy of their enzymatic capacities, which supports interpretations of competitive relations among fungi (Blumenstein et al., 2015). FUNGuild is a flat database hosted by GitHub that assigns a trophic mode to the fungal species investigated (Nguyen et al., 2016). The fungi are categorized as pathotroph, saprotroph, or symbiotroph based on the existing information present in the database (Nguyen et al., 2016). Large sequence pools of data generated by NGS may be analysed with FUNGuild to describe the ecological characteristics and functional roles of fungal communities (Nguyen et al., 2016). Thus, in near future, we are likely to know much more about the functions of endophytes in forest trees.

To conclude, a better understanding of the mechanisms and roles of fungi, at the individual and community level, is needed in order to develop integrated and sustainable approaches to use fungal communities into forest management practices. The lack of ecological information of many fungal species is still high, and there is a need to employ multidisciplinary approaches to study not only the

[1]. https:// github.com/UMNFuN/FUNGuild

taxonomic diversity of a community but also its ecological role in the ecosystem. A crucial challenge for future research is to understand how the tree mycobiome may affect the capacity of forests to provide different ecosystems services for the needs of the human kind.

References

Abarenkov, K., Henrik Nilsson, R., Larsson, K.-H., Alexander, I.J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A.F.S., Tedersoo, L., Ursing, B.M., Vrålstad, T., Liimatainen, K., Peintner, U., Kõljalg, U., 2010. The UNITE database for molecular identification of fungi - recent updates and future perspectives. New Phytol. 186, 281–285. doi:10.1111/j.1469-8137.2009.03160.x

Abreu, I.N., Ahnlund, M., Moritz, T., Albrectsen, B.R., 2011. UHPLC-ESI/TOFMS determination of salicylate-like phenolic gycosides in *Populus tremula* leaves. J. Chem. Ecol. 37, 857–870. doi:10.1007/s10886-011-9991-7

Agler, M.T., Ruhe, J., Kroll, S., Morhenn, C., Kim, S.-T., Weigel, D., Kemen, E.M., 2016. Microbial hub taxa link host and abiotic factors to plant microbiome variation. PLOS Biol. 14, e1002352. doi:10.1371/journal.pbio.1002352

Agostinelli, M., Cleary, M., Martín, J.A., Albrectsen, B.R., Witzell, J., 2018. Pedunculate oaks (*Quercus robur* L.) differing in vitality as reservoirs for fungal biodiversity. Front. Microbiol. 9, 1758. doi:10.3389/fmicb.2018.01758

Albrectsen, B.R., Björkén, L., Varad, A., Hagner, Å., Wedin, M., Karlsson, J., Jansson, S., 2010. Endophytic fungi in European aspen (*Populus tremula*) leaves—diversity, detection, and a suggested correlation with herbivory resistance. Fungal Divers. 41, 17–28. doi:10.1007/s13225-009-0011-y

Albrectsen, B.R., Siddique, A.B., Decker, V.H.G., Unterseher, M., Robinson, K.M., 2018. Both plant genotype and herbivory shape aspen endophyte communities. Oecologia 1–11. doi:10.1007/s00442-018-4097-3

Albrectsen, B.R., Witzell, J., 2012. Disentangling functions of fungal endophytes in forest trees, in: Paz Silva, A., Sol, M. (Eds.), Fungi: types, environmental impact and role in disease. Huntington,NY: Nova Science Publishers, pp. 235–246.

Anderson, M.J., 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Can. J. Fish. Aquat. Sci. 58, 626–639. doi:10.1139/cjfas-58-3-626

Arnold, A.E., Herre, E.A., 2003. Canopy cover and leaf age affect colonization by tropical fungal endophytes: ecological pattern and process in *Theobroma cacao* (*Malvaceae*). Mycologia 95, 388. doi:10.2307/3761880

Arnold, A.E., Lutzoni, F., 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? Ecology 88, 541–549. doi:10.1890/05-1459

Aukema, J.E., Leung, B., Kovacs, K., Chivers, C., Britton, K.O., Englin, J., Frankel, S.J., Haight, R.G., Holmes, T.P., Liebhold, A.M., McCullough, D.G., von Holle, B., 2011. Economic impacts of Non-Native forest insects in the continental United States. PLoS One 6, 1–7. doi:10.1371/journal.pone.0024587

Bailey, J.K., Deckert, R., Schweitzer, J.A., Rehill, B.J., Lindroth, R.L., Gehring, C., Whitham, T.G., 2005. Host plant genetics affect hidden ecological players: links among *Populus*, condensed tannins, and fungal endophyte infection. Can. J. Bot. 83, 356–361. doi:10.1139/b05-008

Baldrian, P., 2017. Forest microbiome: diversity, complexity and dynamics. FEMS Microbiol. Rev. 41, 109–130. doi:10.1093/femsre/fuw040

Bálint, M., Tiffin, P., Hallström, B., O'Hara, R.B., Olson, M.S., Fankhauser, J.D., Piepenbring, M., Schmitt, I., 2013. Host genotype shapes the foliar fungal microbiome of balsam poplar (*Populus balsamifera*). PLoS One 8, e53987. doi:10.1371/journal.pone.0053987

Bandau, F., Decker, V.H.G., Gundale, M.J., Albrectsen, B.R., 2015. Genotypic tannin levels in *Populus tremula* impact the way nitrogen enrichment affects growth and allocation responses for some traits and not for others. PLoS One 10, e0140971. doi:10.1371/journal.pone.0140971

Barrios, E., Valencia, V., Jonsson, M., Brauman, A., Hairiah, K., Mortimer, P.E., Okubo, S., 2018. Contribution of trees to the conservation of biodiversity and ecosystem services in agricultural landscapes. Int. J. Biodivers. Sci. Ecosyst. Serv. Manag. 14, 1–16. doi:10.1080/21513732.2017.1399167

Beck, A., Peršoh, D., Rambold, G., 2014. First evidence for seasonal fluctuations in lichen- and bark-colonising fungal communities. Folia Microbiol. (Praha). 59, 155–157. doi:10.1007/s12223-013-0278-y

Berg, Åke, Ehnström, B., Gustafsson, L., Hallingbäck, T., Jonsell, M., Weslien, J., 1994. Threatened plant, animal, and fungus species in Swedish forests: distribution and habitat associations. Conserv. Biol. 8, 718–731.

Blumenstein, K., Macaya-Sanz, D., Martín, J.A., Albrectsen, B.R., Witzell, J., 2015. Phenotype MicroArrays as a complementary tool to next generation sequencing for characterization of tree endophytes. Front. Microbiol. 6, 1–14. doi:10.3389/fmicb.2015.01033

Bonello, P., Gordon, T.R., Herms, D.A., Wood, D.L., Erbilgin, N., 2006. Nature and ecological implications of pathogen-induced systemic resistance in conifers: A novel hypothesis. Physiol. Mol. Plant Pathol. 68, 95–104. doi:10.1016/j.pmpp.2006.12.002

Brasier, C.M., 1991. Ophiostoma novo-ulmi sp. nov., causative agent of current Dutch elm disease pandemics. Mycopathologia 115, 151–161. doi:10.1007/BF00462219

Brignolas, F., Lacroix, B., Lieutier, F., Sauvard, D., Drouet, A., Claudot, A.C., Yart, A., Berryman, A.A., Christiansen, E., 1995. Induced responses in phenolic metabolism in two Norway spruce clones after wounding and inoculations with *Ophiostoma polonicum*, a bark beetle-associated fungus. Plant Physiol. 109, 821–827. doi:10.1104/PP.109.3.821

Brown, S.P., Veach, A.M., Rigdon-Huss, A.R., Grond, K., Lickteig, S.K., Lothamer, K., Oliver, A.K., Jumpponen, A., 2015. Scraping the bottom of the barrel: are rare high throughput sequences artifacts? Fungal Ecol. 13, 221–225. doi:10.1016/j.funeco.2014.08.006

Busby, P.E., Zimmerman, N., Weston, D.J., Jawdy, S.S., Houbraken, J., Newcombe, G., 2013. Leaf endophytes and *Populus* genotype affect severity of damage from the necrotrophic leaf pathogen, *Drepanopeziza populi*. Ecosphere 4, art125. doi:10.1890/ES13-00127.1

Campanile, G., Ruscelli, A., Luisi, N., 2007. Antagonistic activity of endophytic fungi towards Diplodia corticola assessed by in vitro and in planta tests. Eur. J. Plant Pathol. 117, 237–246. doi:10.1007/s10658-006-9089-1

Caporaso, J.G., Lauber, C.L., Walters, W. a, Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. doi:10.1038/ismej.2012.8

Carroll, G., 1988. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 69, 2–9.

Castillo Lopez, D., Zhu-Salzman, K., Ek-Ramos, M.J., Sword, G.A., 2014. The entomopathogenic fungal endophytes *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) and *Beauveria bassiana* negatively affect cotton aphid reproduction under both greenhouse and field conditions. PLoS One 9. doi:10.1371/journal.pone.0103891

Chase, J., Leibold, M., 2003. Ecological niches: linking classical and contemporary approaches. University of Chicago Press, Chicago.

Chen, Y.-L., Xu, Z.-W., Xu, T.-L., Veresoglou, S.D., Yang, G.-W., Chen, B.-D., 2017. Nitrogen deposition and precipitation induced phylogenetic clustering of arbuscular mycorrhizal fungal communities. Soil Biol. Biochem. 115, 233–242. doi:10.1016/j.soilbio.2017.08.024

Christian, N., Sullivan, C., Visser, N.D., Clay, K., 2016. Plant host and geographic location drive endophyte community composition in the face of perturbation. Microb. Ecol. 72, 621–632. doi:10.1007/s00248-016-0804-y

Coblentz, K.E., Van Bael, S.A., 2013. Field colonies of leaf-cutting ants select plant materials containing low abundances of endophytic fungi. Ecosphere 4, 1–10. doi:10.1890/ES13-00012.1

Collado, J., Platas, G., Peláez, F., 2000. Host specificity in fungal endophytic populations of *Quercus ilex* and *Quercus faginea* from central Spain. Nov. Hedwigia 71, 421–430.

Conrad, A.O., McPherson, B.A., Wood, D.L., Madden, L. V., Bonello, P., 2017. Constitutive phenolic biomarkers identify naïve *Quercus agrifolia* resistant to *Phytophthora ramorum*, the causal agent of sudden oak death. Tree Physiol. 37, 1686–1696. doi:10.1093/treephys/tpx116

Cordier, T., Robin, C., Capdevielle, X., Desprez-Loustau, M.-L., Vacher, C., 2012. Spatial variability of phyllosphere fungal assemblages: genetic distance predominates over geographic distance in a European beech stand (*Fagus sylvatica*). Fungal Ecol. 5, 509–520. doi:10.1016/j.funeco.2011.12.004

Covelo, F., Gallardo, A., 2001. Temporal variation in total leaf phenolics concentration of *Quercus robur* in forested and harvested stands in northwestern Spain. Can. J. Bot. 79, 1262–1269. doi:10.1139/b01-109

Cross, H., Sønstebø, J.H., Nagy, N.E., Timmermann, V., Solheim, H., Børja, I., Kauserud, H., Carlsen, T., Rzepka, B., Wasak, K., Vivian-Smith, A., Hietala, A.M., 2017. Fungal diversity and seasonal succession in ash leaves infected by the invasive ascomycete *Hymenoscyphus fraxineus*. New Phytol. 213, 1405–1417. doi:10.1111/nph.14204

Crous, P.W., Groenewald, J.Z., 2013. A phylogenetic re-evaluation of *Arthrinium*. IMA Fungus 4, 133–54. doi:10.5598/imafungus.2013.04.01.13

de Assis Costa, O.Y., Tupinambá, D.D., Bergmann, J.C., Barreto, C.C., Quirino, B.F., 2018. Fungal diversity in oil palm leaves showing symptoms of Fatal Yellowing disease. PLoS One 13, e0191884. doi:10.1371/journal.pone.0191884

Dearborn, D.C., Kark, S., 2009. Motivations for conserving urban biodiversity. Conserv. Biol. 24, 432–440. doi:10.1111/j.1523-1739.2009.01328.x

Decker, V.H.G., Bandau, F., Gundale, M.J., Cole, C.T., Albrectsen, B.R., 2016. Aspen phenylpropanoid genes' expression levels correlate with genets' tannin richness and vary both in responses to soil nitrogen and associations with phenolic profiles. Tree Physiol. 37, 270–279. doi:10.1093/treephys/tpw118

Dobbertin, M., 2005. Tree growth as indicator of tree vitality and of tree reaction to environmental stress: A review. Eur. J. For. Res. 124, 319–333.

Dulymamode, R., Cannon, P.F., Peerally, A., 2001. Fungi on endemic plants of Mauritius. Mycol. Res. 105, 1472–1479. doi:10.1017/S0953756201004701

Endreny, T., Santagata, R., Perna, A., Stefano, C. De, Rallo, R.F., Ulgiati, S., 2017. Implementing and managing urban forests: a much needed conservation strategy to increase ecosystem services and urban wellbeing. Ecol. Modell. 360, 328–335. doi:10.1016/j.ecolmodel.2017.07.016

Ernst, M., Neubert, K., Mendgen, K.W., Wirsel, S.G., 2011. Niche differentiation of two sympatric species of *Microdochium* colonizing the roots of common reed. BMC Microbiol. 11, 242. doi:10.1186/1471-2180-11-242

Eschen, R., Hunt, S., Mykura, C., Gange, A.C., Sutton, B.C., 2010. The foliar endophytic fungal community composition in *Cirsium arvense* is affected by mycorrhizal colonization and soil nutrient content. Fungal Biol. 114, 991–998. doi:10.1016/j.funbio.2010.09.009

Estrada, C., Wcislo, W.T., Van Bael, S.A., 2013. Symbiotic fungi alter plant chemistry that discourages leaf-cutting ants. New Phytol. 198, 241–251. doi:10.1111/nph.12140

Eyles, A., Bonello, P., Ganley, R., Mohammed, C., 2010. Induced resistance to pests and pathogens in trees. New Phytol. 185, 893–908. doi:10.1111/j.1469-8137.2009.03127.x

Faeth, S.H., Hammon, K.E., 1997. Fungal endophytes in oak trees: experimental analyses of interactions with leafminers. Ecology 78, 820. doi:10.2307/2266061

Fahrig, L., 2003. Effects of habitat fragmentation on biodiversity. Annu. Rev. Ecol. Syst. 34, 487–515.

Fisher, P.J., Petrini, O., 1990. A comparative study of fungal endophytes in xylem and bark of *Alnus* species in England and Switzerland. Mycol. Res. 94, 313–319. doi:10.1016/S0953-7562(09)80356-0

Fort, T., Robin, C., Capdevielle, X., Delière, L., Vacher, C., 2016. Foliar fungal communities strongly differ between habitat patches in a landscape mosaic. PeerJ 4, e2656. doi:10.7717/peerj.2656

Fröhlich, J., Hyde, K.D., Petrini, O., 2000. Endophytic fungi associated with palms. Mycol. Res. 104, 1202–1212.

Fukami, T., Martijn Bezemer, T., Mortimer, S.R., Putten, W.H., 2005. Species divergence and trait convergence in experimental plant community assembly. Ecol. Lett. 8, 1283–1290. doi:10.1111/j.1461-0248.2005.00829.x

Gallart, M., Adair, K.L., Love, J., Meason, D.F., Clinton, P.W., Xue, J., Turnbull, M.H., 2017. Host genotype and nitrogen form shape the root microbiome of *Pinus radiata*. Microb. Ecol. doi:10.1007/s00248-017-1055-2

Gherbawy, Y., Voigt, K., 2010. Molecular identification of fungi. Springer Berlin Heidelberg, Berlin, Heidelberg. doi:10.1007/978-3-642-05042-8

Giauque, H., Hawkes, C. V., 2016. Historical and current climate drive spatial and temporal patterns in fungal endophyte diversity. Fungal Ecol. 20, 108–114. doi:10.1016/j.funeco.2015.12.005

Giordano, L., Gonthier, P., Varese, G.C., Miserere, L., Nicolotti, G., 2009. Mycobiota inhabiting sapwood of healthy and declining Scots pine (*Pinus sylvestris* L.) trees in the Alps. Fungal Divers. 38, 69–83.

Gomes, T., Pereira, J.A., Benhadi, J., Lino-Neto, T., Baptista, P., 2018. Endophytic and epiphytic phyllosphere fungal communities are shaped by different environmental factors in a Mediterranean ecosystem. Microb. Ecol. 1–12. doi:10.1007/s00248-018-1161-9

Gonthier, P., Gennaro, M., Nicolotti, G., 2006. Effects of water stress on the endophytic mycota of *Quercus robur*. Fungal Divers. 21, 69–80.

Guimarães, J.B., Pereira, P., Chambel, L., Tenreiro, R., 2011. Assessment of filamentous fungal diversity using classic and molecular approaches: case study – Mediterranean ecosystem. Fungal Ecol. 4, 309–321. doi:10.1016/j.funeco.2011.01.006

Guo, L.-D., 2010. Molecular diversity and identification of endophytic fungi, in: Gherbawy, Y., Voigt, K. (eds.), Molecular identification of fungi. Springer Berlin Heidelberg, Berlin, Heidelberg. doi:10.1007/978-3-642-05042-8

Halme, P., Heilmann-Clausen, J., Rämä, T., Kosonen, T., Kunttu, P., 2012. Monitoring fungal biodiversity - towards an integrated approach. Fungal Ecol. 5, 750–758. doi:10.1016/j.funeco.2012.05.005

Hardoim, P.R., van Overbeek, L.S., Berg, G., Pirttilä, A.M., Compant, S., Campisano, A., Döring, M., Sessitsch, A., 2015. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol. Mol. Biol. Rev. 79, 293–320. doi:10.1128/MMBR.00050-14

Hawksworth, D.L., 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycol. Res. 95, 641–655. doi:10.1016/S0953-7562(09)80810-1

Hawksworth, D.L., Lücking, R., 2017. Fungal diversity revisited: 2.2 to 3.8 million species. Microbiol. Spectr. 5. doi:10.1128/MICROBIOLSPEC.FUNK-0052-2016

Helander, M., Ahlholm, J., Sieber, T.N., Hinneri, S., Saikkonen, K., 2007. Fragmented environment affects birch leaf endophytes. New Phytol. 175, 547–553.

Helander, M.L., 1995. Responses of pine needle endophytes to air pollution. New Phytol. 131, 223–229. doi:10.1111/j.1469-8137.1995.tb05723.x

Higgins, K.L., Arnold, A.E., Miadlikowska, J., Sarvate, S.D., Lutzoni, F., 2007. Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from

three major plant lineages. Mol. Phylogenet. Evol. 42, 543–55. doi:10.1016/j.ympev.2006.07.012

Hoffman, M.T., Arnold, A.E., 2008. Geographic locality and host identity shape fungal endophyte communities in cupressaceous trees. Mycol. Res. 112, 331–44. doi:10.1016/j.mycres.2007.10.014

Högberg, P., Näsholm, T., Franklin, O., Högberg, M.N., 2017. Tamm Review: On the nature of the nitrogen limitation to plant growth in Fennoscandian boreal forests. For. Ecol. Manage. 403, 161–185. doi:10.1016/j.foreco.2017.04.045

Hongsanan, S., Sánchez-Ramírez, S., Crous, P., Ariyawansa, H., Zhao, R., Hyde, K., 2016. The evolution of fungal epiphytes. Mycosphere 7, 1690–1712. doi:10.5943/mycosphere/7/11/6

Huang, Y.-L., Devan, M.M.N., U'Ren, J.M., Furr, S.H., Arnold, A.E., 2016. Pervasive effects of wildfire on foliar endophyte communities in montane forest trees. Microb. Ecol. 71, 452–468. doi:10.1007/s00248-015-0664-x

Hubbard, M., Germida, J.J., Vujanovic, V., 2014. Fungal endophytes enhance wheat heat and drought tolerance in terms of grain yield and second-generation seed viability. J. Appl. Microbiol. 116, 109–122. doi:10.1111/jam.12311

Hubbell, S.P., 2001. The unified neutral theory of biodiversity and biogeography. Princeton University Press.

Ibrahim, M., Sieber, T.N., Schlegel, M., 2017. Communities of fungal endophytes in leaves of *Fraxinus ornus* are highly diverse. Fungal Ecol. 29, 10–19. doi:10.1016/j.funeco.2017.05.001

Ibrahim, M.H., Jaafar, H.Z.E., Rahmat, A., Rahman, Z.A., 2011. Effects of nitrogen fertilization on synthesis of primary and secondary metabolites in three varieties of Kacip Fatimah (*Labisia pumila* Blume). Int. J. Mol. Sci. 12, 5238–54. doi:10.3390/ijms12085238

Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82, 666–677. doi:10.1111/j.1574-6941.2012.01437.x

Ishida, T.A., Nordin, A., 2010. No evidence that nitrogen enrichment affect fungal communities of *Vaccinium* roots in two contrasting boreal forest types. Soil Biol. Biochem. 42, 234–243. doi:10.1016/j.soilbio.2009.10.021

Jacobs, D.F., 2007. Toward development of silvical strategies for forest restoration of American chestnut (*Castanea dentata*) using blight-resistant hybrids. Biol. Conserv. 137, 497–506. doi:10.1016/j.biocon.2007.03.013

Jiang, S., Liu, Y., Luo, J., Qin, M., Johnson, N.C., Öpik, M., Vasar, M., Chai, Y., Zhou, X., Mao, L., Du, G., An, L., Feng, H., 2018. Dynamics of arbuscular mycorrhizal fungal community structure and functioning along a nitrogen enrichment gradient in an alpine meadow ecosystem. New Phytol. doi:10.1111/nph.15112

Jonsson, U., Jung, T., Sonesson, K., Rosengren, U., 2005. Relationships between health of *Quercus robur*, occurrence of *Phytophthora* species and site conditions in southern Sweden. Plant Pathol. 54, 502–511. doi:10.1111/j.1365-3059.2005.01228.x

Jumpponen, A., Jones, K.L., 2010. Seasonally dynamic fungal communities in the *Quercus macrocarpa* phyllosphere differ between urban and nonurban environments. New Phytol. 186, 496–513. doi:10.1111/j.1469-8137.2010.03197.x

Karban, R., Baldwin, I.T., 1997. Induced responses to herbivory. The University of Chicago Press.

Karlsson, I., Friberg, H., Steinberg, C., Persson, P., 2014. Fungicide effects on fungal community composition in the wheat phyllosphere. PLoS One 9, e111786. doi:10.1371/journal.pone.0111786

Keefover-Ring, K., Ahnlund, M., Abreu, I.N., Jansson, S., Moritz, T., Albrectsen, B.R., 2014. No evidence of geographical structure of salicinoid chemotypes within *Populus tremula*. PLoS One 9, e107189. doi:10.1371/journal.pone.0107189

Kehr, R.D., 1992. *Pezicula* canker of *Quercus rubra* L ., caused by *Pezicula cinnamomea* (DC.) Sacc. Eur. J. For. Pathol. 22, 29–40.

Klepzig, K.D., Kruger, E.L., Smalley, E.B., Raffa, K.F., 1995. Effects of biotic and abiotic stress on induced accumulation of terpenes and phenolics in red pines inoculated with bark beetle-vectored fungus. J. Chem. Ecol. 21, 601–626. doi:10.1007/BF02033704

Koskella, B., Meaden, S., Crowther, W.J., Leimu, R., Metcalf, C.J.E., 2017. A signature of tree health? Shifts in the microbiome and the ecological drivers of horse chestnut bleeding canker disease. New Phytol. 215, 737–746. doi:10.1111/nph.14560

Kwaśna, H., Szewczyk, W., Behnke-Borowczyk, J., 2016. Fungal root endophytes of *Quercus robur* subjected to flooding. For. Pathol. 46, 35–46. doi:10.1111/efp.12212

Laforest-Lapointe, I., Messier, C., Kembel, S.W., 2016. Tree phyllosphere bacterial communities: exploring the magnitude of intra- and inter-individual variation among host species. PeerJ 4, e2367. doi:10.7717/peerj.2367

Lamit, L.J., Lau, M.K., Sthultz, C.M., Wooley, S.C., Whitham, T.G., Gehring, C.A., 2014. Tree genotype and genetically based growth traits structure twig endophyte communities. Am. J. Bot. 101, 467–478. doi:10.3732/ajb.1400034

Lattanzio, V., Lattanzio, V.M.T., Cardinali, A., 2006. Role of polyphenols in the resistance mechanisms of plants against fungal pathogens and insects, in: Imperato, F. (ed.), Phytochemistry: Advances in Research. pp. 23–67.

Lebeis, S.L., 2015. Greater than the sum of their parts: characterizing plant microbiomes at the community-level. Curr. Opin. Plant Biol. 24, 82–86. doi:10.1016/j.pbi.2015.02.004

Lee, A.C.K., Maheswaran, R., 2011. The health benefits of urban green spaces: A review of the evidence. J. Public Health (Bangkok). 33, 212–222. doi:10.1093/pubmed/fdq068

Leff, J.W., Del Tredici, P., Friedman, W.E., Fierer, N., 2015. Spatial structuring of bacterial communities within individual *Ginkgo biloba* trees. Environ. Microbiol. 17, 2352–2361. doi:10.1111/1462-2920.12695

Legendre, P., Legendre, L., 1998. Numerical ecology. Elsevier.

Lehtonen, P., Helander, M., Saikkonen, K., 2005. Are endophyte-mediated effects on herbivores conditional on soil nutrients? Oecologia 142, 38–45. doi:10.1007/s00442-004-1701-5

Linaldeddu, B.T., Sirca, C., Spano, D., Franceschini, A., 2011. Variation of endophytic cork oak-associated fungal communities in relation to plant health and water stress. For. Pathol. 41, 193–201. doi:10.1111/j.1439-0329.2010.00652.x

Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, A., Garcia-Gonzalo, J., Seidl, R., Delzon, S., Corona, P., Kolström, M., Lexer, M.J., Marchetti, M., 2010. Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. For. Ecol. Manage. 259, 698–709. doi:10.1016/j.foreco.2009.09.023

Lindow, S.E., Leveau, J.H.J., 2002. Phyllosphere microbiology. Curr. Opin. Biotechnol. 13, 238–243. doi:10.1016/S0958-1669(02)00313-0

Livesley, S.J., McPherson, G.M., Calfapietra, C., 2016. The urban forest and ecosystem services: impacts on urban water, heat, and pollution cycles at the tree, street, and city scale. J. Environ. Qual. 45, 119. doi:10.2134/jeq2015.11.0567

Lockwood, J.L., Cassey, P., Blackburn, T., 2005. The role of propagule pressure in explaining species invasions. Trends Ecol. Evol. 20, 223–228. doi:10.1016/j.tree.2005.02.004

Lourenço, A., Rencoret, J., Chemetova, C., Gominho, J., Gutiérrez, A., del Río, J.C., Pereira, H., 2016. Lignin composition and structure differs between xylem, phloem and phellem in *Quercus suber* L. Front. Plant Sci. 7, 1612. doi:10.3389/fpls.2016.01612

Luquez, V., Hall, D., Albrectsen, B.R., Karlsson, J., Ingvarsson, P., Jansson, S., 2008. Natural phenological variation in aspen (*Populus tremula*): the SwAsp collection. Tree Genet. Genomes 4, 279–292. doi:10.1007/s11295-007-0108-y

Martín, J.A., Macaya-Sanz, D., Witzell, J., Blumenstein, K., Gil, L., 2015. Strong in vitro antagonism by elm xylem endophytes is not accompanied by temporally stable in planta protection against a vascular pathogen under field conditions. Eur. J. Plant Pathol. 142, 185–196. doi:10.1007/s10658-015-0602-2

Martín, J.A., Witzell, J., Blumenstein, K., Rozpedowska, E., Helander, M., Sieber, T.N., Gil, L., 2013. Resistance to Dutch elm disease reduces presence of xylem endophytic fungi in elms (*Ulmus* spp.). PLoS One 8, e56987. doi:10.1371/journal.pone.0056987

McKinney, L. V., Thomsen, I.M., Kjaer, E.D., Nielsen, L.R., 2012. Genetic resistance to *Hymenoscyphus pseudoalbidus* limits fungal growth and symptom occurrence in *Fraxinus excelsior*. For. Pathol. 42, 69–74. doi:10.1111/j.1439-0329.2011.00725.x

McPherson, B.A., Mori, S.R., Opiyo, S.O., Conrad, A.O., Wood, D.L., Bonello, P., 2014. Association between resistance to an introduced invasive pathogen and phenolic compounds that may serve as biomarkers in native oaks. For. Ecol. Manage. 312, 154–160. doi:10.1016/j.foreco.2013.10.009

Meier, A.R., Hunter, M.D., 2018. Arbuscular mycorrhizal fungi mediate herbivore-induction of plant defenses differently above and belowground. Oikos. doi:10.1111/oik.05402

Mejía, L.C., Rojas, E.I., Maynard, Z., Bael, S. Van, Arnold, A.E., Hebbar, P., Samuels, G.J., Robbins, N., Herre, E.A., 2008. Endophytic fungi as biocontrol agents of Theobroma cacao pathogens. Biol. Control 46, 4–14. doi:10.1016/j.biocontrol.2008.01.012

Miller, J.D., Sumarah, M.W., Adams, G.W., 2008. Effect of a rugulosin-producing endophyte in *Picea glauca* on *Choristoneura fumiferana*. J. Chem. Ecol. 34, 362–368. doi:10.1007/s10886-007-9412-0

Mitchell, R.J., Beaton, J.K., Bellamy, P.E., Broome, A., Chetcuti, J., Eaton, S., Ellis, C.J., Gimona, A., Harmer, R., Hester, A.J., Hewison, R.L., Hodgetts, N.G., Iason, G.R., Kerr, G., Littlewood, N.A., Newey, S., Potts, J.M., Pozsgai, G., Ray, D., Sim, D.A., Stockan, J.A., Taylor, A.F.S., Woodward, S., 2014. Ash dieback in the UK: a review of the ecological and

conservation implications and potential management options. Biol. Conserv. 175, 95–109. doi:10.1016/j.biocon.2014.04.019

Morrison, E.W., Frey, S.D., Sadowsky, J.J., van Diepen, L.T.A., Thomas, W.K., Pringle, A., 2016. Chronic nitrogen additions fundamentally restructure the soil fungal community in a temperate forest. Fungal Ecol. 23, 48–57. doi:10.1016/j.funeco.2016.05.011

Mousa, W.K., Raizada, M.N., 2013. The diversity of anti-microbial secondary metabolites produced by fungal endophytes: an interdisciplinary perspective. Front. Microbiol. 4, 1–18. doi:10.3389/fmicb.2013.00065

Mueller, G., Bills, G., 2004. Introduction. In: Mueller GM, Bills GF, Foster MS (eds) Biodiversity of fungi: inventory and monitoring methods. Elsevier Academic Press, San Diego.

Mueller, G.M., Schmit, J.P., 2007. Fungal biodiversity: what do we know? What can we predict? Biodivers. Conserv. 16, 1–5. doi:10.1007/s10531-006-9117-7

Müller, E., Stierlin, H.R., 1990. Kronenbilder Couronnes d'arbres Le chiome degli alberi Tree crown photos, 2nd ed. Birmensdorf: Eidgenössische Forschnungstalt für Wald, Schnee und Landschaft.

Müller, M.M., Hallaksela, A.-M., 1998. Diversity of Norway spruce needle endophytes in various mixed and pure Norway spruce stands. Mycol. Res. 102, 1183–1189. doi:10.1017/S0953756298006285

Newcombe, G., 2011. Endophytes in forest management: four challenges, in: Pirttilä, A.M., Frank, A.C. (eds.), Endophytes of forest trees: biology and applications. Springer, Dordrecht, pp. 251–262. doi:10.1007/978-94-007-1599-8_16

Newcombe, G., Dugan, F.M., 2010. Fungal pathogens of plants in the homogocene, in: Gherbawy, Y., Voigt, K. (eds.), Molecular identification of fungi. Springer Berlin Heidelberg.

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248. doi:10.1016/j.funeco.2015.06.006

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Simpson, G., Solymos, P., Stevens, M., Wagner, H., 2016. vegan: Community Ecology Package. R package version 2.4-0.

Osono, T., 2008. Endophytic and epiphytic phyllosphere fungi of *Camellia japonica*: seasonal and leaf age-dependent variations. Mycologia 100, 387–391. doi:10.3852/07-110R1

Pansanit, A., Pripdeevech, P., 2018. Antibacterial secondary metabolites from an endophytic fungus, *Arthrinium* sp. MFLUCC16-1053 isolated from *Zingiber cassumunar*. Mycology 1–9. doi:10.1080/21501203.2018.1481154

Parfitt, D., Hunt, J., Dockrell, D., Rogers, H.J., Boddy, L., 2010. Do all trees carry the seeds of their own destruction? PCR reveals numerous wood decay fungi latently present in sapwood of a wide range of angiosperm trees. Fungal Ecol. 3, 338–346. doi:10.1016/j.funeco.2010.02.001

Paungfoo-Lonhienne, C., Yeoh, Y.K., Kasinadhuni, N.R.P., Lonhienne, T.G.A., Robinson, N., Hugenholtz, P., Ragan, M.A., Schmidt, S., 2015. Nitrogen fertilizer dose alters fungal communities in sugarcane soil and rhizosphere. Sci. Rep. 5, 8678. doi:10.1038/srep08678

Pautasso, M., Aas, G., Queloz, V., Holdenrieder, O., 2013. European ash (*Fraxinus excelsior*) dieback – A conservation biology challenge. Biol. Conserv. 158, 37–49. doi:10.1016/j.biocon.2012.08.026

Pawłowska, J., Wilk, M., Śliwińska-Wyrzychowska, A., Mętrak, M., Wrzosek, M., 2014. The diversity of endophytic fungi in the above-ground tissue of two *Lycopodium* species in Poland. Symbiosis 63, 87–97. doi:10.1007/s13199-014-0291-1

Peay, K.G., Schubert, M.G., Nguyen, N.H., Bruns, T.D., 2012. Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. Mol. Ecol. 21, 4122–4136. doi:10.1111/j.1365-294X.2012.05666.x

Peñuelas, J., Rico, L., Ogaya, R., Jump, A.S., Terradas, J., 2012. Summer season and long-term drought increase the richness of bacteria and fungi in the foliar phyllosphere of *Quercus ilex* in a mixed Mediterranean forest. Plant Biol. 14, 565–575. doi:10.1111/j.1438-8677.2011.00532.x

Pérez-Izquierdo, L., Zabal-Aguirre, M., Flores-Rentería, D., González-Martínez, S.C., Buée, M., Rincón, A., 2017. Functional outcomes of fungal community shifts driven by tree genotype and spatial-temporal factors in Mediterranean pine forests. Environ. Microbiol. 13, 385–392. doi:10.1111/1462-2920.13690

Peršoh, D., 2015. Plant-associated fungal communities in the light of meta'omics. Fungal Divers. 75, 1–25. doi:10.1007/s13225-015-0334-9

Petrini, O., Fisher, P.J., 1990. Occurrence of fungal endophytes in twigs of *Salix fragilis* and *Quercus robur*. Mycol. Res. 94, 1077–1080. doi:10.1016/S0953-7562(09)81336-1

Porras-Alfaro, A., Bayman, P., 2011. Hidden fungi, emergent properties: endophytes and microbiomes. Annu. Rev. Phytopathol. 49, 291–315. doi:10.1146/annurev-phyto-080508-081831

Porter, J.K., Bacon, C.W., Cutler, H.G., Arrendale, R.F., Robbins, J.D., 1985. In vitro auxin production by *Balansia epichloë*. Phytochemistry 24, 1429–1431. doi:10.1016/S0031-9422(00)81037-7

Potter, C., Harwood, T., Knight, J., Tomlinson, I., 2011. Learning from history, predicting the future: the UK Dutch elm disease outbreak in relation to contemporary tree disease threats. Philos. Trans. R. Soc. B Biol. Sci. 366, 1966–1974. doi:10.1098/rstb.2010.0395

Power, M.W.P., Hopkins, A.J.M., Begtsson, S.B.K., Vasaitis, R., Cleary, M.R., 2017. European *Fraxinus* species introduced into New Zealand retain many of their native endophytic fungi. Balt. For. 23, 74–81.

Promputtha, I., Lumyong, S., Dhanasekaran, V., McKenzie, E.H.C., Hyde, K.D., Jeewon, R., 2007. A phylogenetic evaluation of whether endophytes become saprotrophs at host senescence. Microb. Ecol. 53, 579–590. doi:10.1007/s00248-006-9117-x

Pusztahelyi, T., Holb, I.J., Pócsi, I., 2015. Secondary metabolites in fungus-plant interactions. Front. Plant Sci. 6, 573. doi:10.3389/fpls.2015.00573

R Development Core Team (2016). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, n.d.

Ragazzi, A., Moricca, S., Capretti, P., Dellavalle, I., Turco, E., 2003. Differences in composition of endophytic mycobiota in twigs and leaves of healthy and declining *Quercus* species in Italy. For. Pathol. 33, 31–38. doi:10.1046/j.1439-0329.2003.3062003.x

Rai, M., Agarkar, G., 2014. Plant–fungal interactions: what triggers the fungi to switch among lifestyles? Crit. Rev. Microbiol. 7828, 1–11. doi:10.3109/1040841X.2014.958052

Rajala, T., Velmala, S.M., Vesala, R., Smolander, A., Pennanen, T., 2014. The community of needle endophytes reflects the current physiological state of Norway spruce. Fungal Biol. 118, 309–15. doi:10.1016/j.funbio.2014.01.002

Reynolds, D.R., Gilbert, G.S., 2005. Epifoliar fungi from Queensland, Australia. Aust. Syst. Bot. 18, 265. doi:10.1071/SB04030

Rodriguez, R.J., White, J.F., Arnold, A.E., Redman, R.S., 2009. Fungal endophytes: diversity and functional roles. New Phytol. 182, 314–30. doi:10.1111/j.1469-8137.2009.02773.x

Romeralo, C., Santamaría, O., Pando, V., Diez, J.J., 2015. Fungal endophytes reduce necrosis length produced by *Gremmeniella abietina* in *Pinus halepensis* seedlings. Biol. Control 80, 30–39. doi:10.1016/j.biocontrol.2014.09.010

Romeralo, C., Witzell, J., Diez, J.J., 2016. Aleppo pine provenances vary in susceptibility and secondary chemical response to *Gremmeniella abietina* infection. Plant Pathol. 65, 664–672. doi:10.1111/ppa.12452

Rudgers, J.A., Koslow, J.M., Clay, K., 2004. Endophytic fungi alter relationships between diversity and ecosystem properties. Ecol. Lett. 7, 42–51. doi:10.1046/j.1461-0248.2003.00543.x

Saikkonen, K., 2007. Forest structure and fungal endophytes. Fungal Biol. Rev. 21, 67–74.

Saikkonen, K., Faeth, S.H., Helander, M., Sullivan, T.J., 1998. Fungal endophytes: a continuum of interactions with host plants. Annu. Rev. Ecol. Syst. 29, 319–343. doi:10.1146/annurev.ecolsys.29.1.319

Santamaría, J., Bayman, P., 2005. Fungal epiphytes and endophytes of coffee leaves (*Coffea arabica*). Microb. Ecol. 50, 1–8. doi:10.1007/s00248-004-0002-1

Santamaría, O., Diez, J.J., 2005. Fungi in leaves, twigs and stem bark of *Populus tremula* from northern Spain. For. Pathol. 35, 95–104. doi:10.1111/j.1439-0329.2004.00389.x

Sanz-Ros, A. V., Müller, M.M., San Martín, R., Diez, J.J., 2015. Fungal endophytic communities on twigs of fast and slow growing Scots pine (*Pinus sylvestris* L.) in northern Spain. Fungal Biol. 119, 870–883. doi:10.1016/j.funbio.2015.06.008

Scalbert, A., 1991. Antimicrobial properties of tannins. Phytochemistry 30, 3875–3883. doi:10.1016/0031-9422(91)83426-L

Scholtysik, A., Unterseher, M., Otto, P., Wirth, C., 2013. Spatio-temporal dynamics of endophyte diversity in the canopy of European ash (*Fraxinus excelsior*). Mycol. Prog. 12, 291–304. doi:10.1007/s11557-012-0835-9

Schulz, B., Boyle, C., 2005. The endophytic continuum. Mycol. Res. 109, 661–686. doi:10.1017/S095375620500273X

Schweitzer, J.A., Madritch, M.D., Bailey, J.K., LeRoy, C.J., Fischer, D.G., Rehill, B.J., Lindroth, R.L., Hagerman, A.E., Wooley, S.C., Hart, S.C., Whitham, T.G., 2008. From Genes to Ecosystems: The genetic basis of condensed tannins and their role in nutrient regulation in a *Populus* model system. Ecosystems 11, 1005–1020. doi:10.1007/s10021-008-9173-9

Sherwood, P., Bonello, P., 2013. Austrian pine phenolics are likely contributors to systemic induced resistance against *Diplodia pinea*. Tree Physiol. 33, 845–854. doi:10.1093/treephys/tpt063

Siddikee, M.A., Zereen, M.I., Li, C.-F., Dai, C.-C., 2016. Endophytic fungus *Phomopsis liquidambari* and different doses of N-fertilizer alter microbial community structure and function in rhizosphere of rice. Sci. Rep. 6, 32270. doi:10.1038/srep32270

Sieber, T.N., 2007. Endophytic fungi in forest trees: are they mutualists? Fungal Biol. Rev. 21, 75–89. doi:10.1016/j.fbr.2007.05.004

Sieber, T.N., 1989. Endophytic fungi in twigs of healthy and diseased Norway spruce and white fir. Mycol. Res. 92, 322–326. doi:10.1016/S0953-7562(89)80073-5

Stener, L.-G., 2013. Clonal differences in susceptibility to the dieback of *Fraxinus excelsior* in southern Sweden. Scand. J. For. Res. 28, 205–216. doi:10.1080/02827581.2012.735699

Stone, J.K., Bacon, C.W., White, J.F., 2000. An overview of endophytic microbes : endophytism defined, In: Bacon. ed.

Sun, X., Ding, Q., Hyde, K.D., Guo, L.D., 2012. Community structure and preference of endophytic fungi of three woody plants in a mixed forest. Fungal Ecol. 5, 624–632. doi:10.1016/j.funeco.2012.04.001

Tadych, M., Bergen, M.S., Johnson-Cicalese, J., Polashock, J.J., Vorsa, N., White, J.F., 2012. Endophytic and pathogenic fungi of developing cranberry ovaries from flower to mature fruit: diversity and succession. Fungal Divers. 54, 101–116. doi:10.1007/s13225-012-0160-2

Tanney, J.B., McMullin, D.R., Green, B.D., Miller, J.D., Seifert, K.A., 2016. Production of antifungal and antiinsectan metabolites by the *Picea* endophyte *Diaporthe maritima sp. nov*. Fungal Biol. 120, 1448–1457. doi:10.1016/j.funbio.2016.05.007

Tedersoo, L., Lindahl, B., 2016. Fungal identification biases in microbiome projects. Environ. Microbiol. Rep. 8, 774–779. doi:10.1111/1758-2229.12438

Thomma, B.P.H.J., Van Esse, H.P., Crous, P.W., De Wit, P.J.G.M., 2005. *Cladosporium fulvum* (syn. *Passalora fulva*), a highly specialized plant pathogen as a model for functional studies on plant pathogenic *Mycosphaerellaceae*. Mol. Plant Pathol. 6, 379–393. doi:10.1111/j.1364-3703.2005.00292.x

Tilman, D., 2004. Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. Proc. Natl. Acad. Sci. 101, 10854–10861. doi:10.1073/pnas.0403458101

Toti, L., Chapela, I.H., Petrini, O., 1992. Morphometric evidence for host-specific strain formation in *Discula umbrinella*. Mycol. Res. 96, 420–424. doi:10.1016/S0953-7562(09)81085-X

Turnbaugh, P.J., Ley, R.E., Hamady, M., Fraser-Liggett, C.M., Knight, R., Gordon, J.I., 2007. The human microbiome project. Nature 449, 804–810. doi:10.1038/nature06244

Unterseher, M., 2011. Diversity of fungal endophytes in temperate forest trees, in: Pirttilä, A.M., Frank, A.C. (eds.), Endophytes of forest trees: biology and applications. Springer, Dordrecht, pp. 31–46. doi:10.1007/978-94-007-1599-8_2

Unterseher, M., Peršoh, D., Schnittler, M., 2013. Leaf-inhabiting endophytic fungi of European beech (*Fagus sylvatica* L.) co-occur in leaf litter but are rare on decaying wood of the same host. Fungal Divers. 60, 43–54. doi:10.1007/s13225-013-0222-0

Unterseher, M., Schnittler, M., 2009. Dilution-to-extinction cultivation of leaf-inhabiting endophytic fungi in beech (*Fagus sylvatica* L.) - different cultivation techniques influence fungal biodiversity assessment. Mycol. Res. 113, 645–54. doi:10.1016/j.mycres.2009.02.002

Vannette, R.L., Fukami, T., 2014. Historical contingency in species interactions: towards niche-based predictions. Ecol. Lett. 17, 115–124. doi:10.1111/ele.12204

Vaz, A.B.M., da Costa, A.G.F.C., Raad, L.V.V., Góes-Neto, A., 2014. Fungal endophytes associated with three south American *Myrtae* (*Myrtaceae*) exhibit preferences in the colonization at leaf level. Fungal Biol. 118, 277–286. doi:10.1016/j.funbio.2013.11.010

Vega, F.E., Posada, F., Catherine Aime, M., Pava-Ripoll, M., Infante, F., Rehner, S. a., 2008. Entomopathogenic fungal endophytes. Biol. Control 46, 72–82. doi:10.1016/j.biocontrol.2008.01.008

Veresoglou, S.D., Barto, E.K., Menexes, G., Rillig, M.C., 2013. Fertilization affects severity of disease caused by fungal plant pathogens. Plant Pathol. 62, 961–969. doi:10.1111/ppa.12014

Videira, S.I.R., Groenewald, J.Z., Braun, U., Shin, H.D., Crous, P.W., 2016. All that glitters is not *Ramularia*. Stud. Mycol. 83, 49–163. doi:10.1016/J.SIMYCO.2016.06.001

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7, 737–750. doi:10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2

Wang, Y., Guo, L., 2007. A comparative study of endophytic fungi in needles, bark, and xylem of *Pinus tabulaeformis*. Can. J. Bot. 85, 911–917. doi:10.1139/B07-084

Weidlich, E.W.A., von Gillhaussen, P., Delory, B.M., Blossfeld, S., Poorter, H., Temperton, V.M., 2017. The importance of being first: exploring priority and diversity effects in a grassland field experiment. Front. Plant Sci. 7, 2008. doi:10.3389/fpls.2016.02008

Wennekes, P.L., Rosindell, J., Etienne, R.S., 2012. The neutral-niche debate: a philosophical perspective. Acta Biotheor. 60, 257–271. doi:10.1007/s10441-012-9144-6

White, J.F., Torres, M.S., 2010. Is plant endophyte-mediated defensive mutualism the result of oxidative stress protection? Physiol. Plant. 138, 440–446. doi:10.1111/j.1399-3054.2009.01332.x

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomial RNA genes for phyologenetics, in: Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T.J. (Ed.), PCR protocols: a guide to methods and applications. Academic Press Inc., New York.©W°, pp. 315–322.

Wilson, D., 1995. Fungal endophytes which invade insect galls: insect pathogens, benign saprophytes, or fungal inquilines? Oecologia 103, 255–260. doi:10.1007/BF00329088

Witzell, J., Martín, J.A., 2018. Endophytes and forest health, in: Pirttilä, A.M., Frank, C.A. (eds.), Endophytes of forest trees. Forestry Sciences 86, Springer International Publishing AG, Springer Nature, pp. 261–282. doi:10.1007/978-3-319-89833-9_12

Witzell, J., Martín, J.A., 2008. Phenolic metabolites in the resistance of northern forest trees to pathogens - past experiences and future prospects. Can. J. For. Res. 38, 2711–2727. doi:10.1139/X08-112

Yang, M.-Z., Ma, M.-D., Yuan, M.-Q., Huang, Z.-Y., Yang, W.-X., Zhang, H.-B., Huang, L.-H., Ren, A.-Y., Shan, H., 2016. Fungal endophytes as a metabolic fine-tuning regulator for wine grape. PLoS One 11, e0163186. doi:10.1371/journal.pone.0163186

Zabalgogeazcoa, I., 2008. Fungal endophytes and their interaction with plant pathogens. Spanish J. Agric. Res. 6, 138–146. doi:10.5424/sjar/200806S1-382

Zimmerman, N.B., Vitousek, P.M., 2012. Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. Proc. Natl. Acad. Sci. 109, 13022–13027. doi:10.1073/pnas.1209872109

Acknowledgements

My deepest gratitude goes to my main supervisor, associate professor Johanna Witzell, who patiently guided me through this project, challenging me with questions and pushing me to look at deeper and broader meanings. Thanks to you I started this journey and I am grateful for you being present when in need of guidance. I have learnt so much about research and myself along the way, thank you.

I would like to thank my co-supervisors associate professors Michelle Cleary and Juan Antonio Martín for their support and time during these years. Michelle, thank you for pushing me out of the comfort zone every time it was needed and to show me things from a different angle. I am grateful for the time you dedicated to me and the project, your most-needed English corrections, and the opportunity to travel with you to OSU. Juan, thank you for your time spent on skype to discuss about statistics and EstimateS. I would like to thank associate professor Benedicte Albrectsen, who, despite not being officially on the list, has guided me through some statistical analyses and provided a different point of view on the project.

Thanks to the people at the Southern Swedish Forest Research Centre, particularly the fellow PhD students who have contributed to create a nice environment. A special thanks goes to Violeta and Kent for your time helping with bureaucratic issues and anything that was needed in the lab.

Thanks to Diem and Carl, you are my R-rescuers.

Thanks to Vilis and Ola for the opportunity to work with the ALTERFOR team and their understanding of the situation in these months of overlapping work.

I like to thank the financers of the PhD project, FORMAS (2012-1358) and the Southern Swedish Forest Research Centre, and those who contributed covering travel and publication expenses, COST Action No. FP1103, Åforsk, and KSLA.

A special thank goes to the new friends found along the way, for your support and the fun shared in these years: Kathrin & Michael, Giulia & Johannes, Rebecka, Clem, Ida & Alex, Maria & Guillermo, Carl, Lars, Emma & Emil, and Chiara. And to the old friends that followed all along: Barbara, Francesca, Alessandra, Monica, and Francesca. A special mention goes to Marta, thanks for being my *super partes* advisor, providing insightful and objective advices at any time of the day and the night!

Finally, I want to thank my family from the deepest of my heart. This thesis is dedicated to Adelina, la mia nonnissima, because she thought me to see the positive in everything. Stefano, you are the most stubborn and uncompromising person I have ever met, but I know I can always count on you. I am grateful to have you as a brother. Mami and Papo, there are no words to thank you enough for the support of these years. Thanks to your passion for mountains, hikes, and nature, I have got curious to know how trees 'work', and here I am today. Thank you for letting me pursuing my way, even if that meant to have me far away for almost 10 years.

Marco, this journey started with the two of us living in different countries and it finishes with the two of us living in different countries. I don't know how many hours we have spent in busses, ferries, trains, and planes to see each other over a weekend, but they were all worth it. Luckily, we have our special place where to plant trees and compensate for our CO_2 footprint! You are the best *compagno*[2] I could have wished for this journey, the one who pushes me to do always my best, the one who makes me laugh the most, and who reminds me what is important in life. I am grateful I could share the bread along the way with you, especially if it's the one you have baked. It is time to bake fresh bread, a new journey is awaiting…

2. c*ompagno* comes from Medieval latin and means 'with bread' - 'cum'=with 'panis'=bread