Succession of Wood-inhabiting Fungal Communities

Diversity and Species Interactions During the Decomposition of Norway Spruce

Elisabet Ottosson
Faculty of Natural Resources and Agricultural Sciences,
Department of Forest Mycology and Plant Pathology,
Uppsala

Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2013

Acta Universitatis agriculturae Sueciae
2013:17

Cover: Polyporus pinicola, aquarelle by E.P. Fries (1834-1858)
Belongs to the collections of the Museum of Evolution, Uppsala University

ISSN, 1652-6880
ISBN, 978-91-576-7774-7

Print: SLU Service/Repro, Uppsala 2013

Succession of Wood-inhabiting Fungal Communities – Diversity and Species Interactions During the Decomposition of Norway Spruce

Abstract

Dead wood constitutes an important substrate for biodiversity in boreal forests. As the wood decays, fungal communities develop and species associations are formed. Species interactions are thought to affect community development, but the mycelial dynamics within fungal communities are poorly understood.

In this thesis the diversity and temporal dynamics within fungal communities in Norway spruce logs are studied. In particular, patterns of diversity and mechanisms during community assembly are investigated. 454 sequencing is applied to study the less well-known fungal diversity and fine-scale mycelial distribution patterns in decaying logs. The influence of priority effects during community assembly is studied using time-series data from re-inventoried logs. The importance of wood-modification by a primary species and competition is examined in species interaction laboratory experiments.

454 sequencing revealed species-rich fungal communities with diverse ecological roles. Wood-decaying basidiomycetes was found to be the most abundant ecological group, and saprotrophic, mycorrhizal and parasitic fungi were regularly detected. Mycobiont partners of lichens were isolated from interior parts of logs. Fine-scale distribution within logs revealed that resource utilization reflects the life histories of fungal taxa. More decayed samples hosted a higher number of taxa, particularly ascomycetes, whereas wood-decaying basidiomycetes were found in less decayed wood. Priority effects in terms of different mortality factors of trees and the presence of primary decay species were found to affect the subsequent community composition. A species-specific response to primary decay and antagonistic interactions significantly affected decay rate and growth. It is concluded that priority effects are more important in early stages of community development while species more frequent in middle stages of decomposition relies more upon competitive abilities.

Keywords: Boreal forest, wood-decaying fungi, priority effects, dead wood, life-history traits, *Picea abies*, species interactions, environmental sequencing.

Author's address: Elisabet Ottosson, SLU, Department of Forest Mycology and Plant Pathology, Almas allé 5, 750 07 Uppsala, Sweden.
E-mail: elisabet.ottosson@slu.se

"I have always looked upon decay as being just as wonderful and rich an expression of life as growth."

Henry Miller

Till Pappa

Contents

List of Publications **7**

1 Wood-inhabiting fungi in forest ecosystems **9**

2 Fungal communities in dead wood **11**
2.1 Fungal decomposition in the boreal forest 11
2.2 Wood-inhabiting fungal communities 11
2.2.1 Wood-decaying fungi 12
2.2.2 Other fungal ecological roles in wood 14
2.3 Assembly of fungal communities in dead wood 15
2.3.1 Fungal life-histories 16
2.3.2 Fungal community development in decaying Norway spruce 17
2.4 Fungal-fungal interactions 18

3 Aims **21**

4 Materials and methods **23**
4.1 Study areas 23
4.2 Studies of fungal communities 23
4.2.1 The fruit body life-stage (Paper I and III) 23
4.2.2 The mycelial life-stage (Papers I and II) 25
4.2.3 Interspecific interactions (Paper IV) 27

5 Results and discussion **29**
5.1 Diverse ecological roles in fungal communities (Papers I and II) 29
5.2 Spatial patterns and life-history traits (Paper II) 32
5.3 The role of species interactions during community assembly 36
5.3.1 Priority effects and species associations (Papers I, III and IV) 36
5.3.2 Fungal competition and facilitation in decaying Norway spruce (Paper IV) 39
5.3.3 Ecological consequences of interspecific interactions 41

6 Synthesis and future directions **43**

References **47**

Acknowledgements **60**

List of Publications

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

I Ottosson, E., Kubartová, A., Jönsson, M., Edman, M., Lindhe, A., Dahlberg, A. and Stenlid, J. (2013). Deep sequencing of decomposing wood reveals the diverse ecological roles within fungal communities in logs. (*Submitted*)

II Kubartová, A., Ottosson, E., Dahlberg, A. and Stenlid, J. (2012). Patterns of fungal communities among and within decaying logs, revealed by 454 sequencing. *Molecular Ecology* 21(18), 4514–4532.

III Ottosson, E., Nordén, J., Dahlberg, A., Edman, M., Jönsson, M, Larsson, KH., Olsson, J., Penttilä, R., Stenlid, J. and Ovaskainen, O. (2013). Species associations during the assembly of wood-inhabiting fungal communities. (*Submitted*)

IV Ottosson, E., Dahlberg, A., Ovaskainen, O. and Stenlid, J. The initial colonizer affects the succession of wood-decaying fungi on Norway spruce. (*Manuscript*)

The contribution of Elisabet Ottosson to the papers included in this thesis was as follows:

I Participated in the research design and field sampling. identification of operational taxonomic units (OTUs), analysis of data and writing of paper together with supervisors and co-authors.

II Participated in the research design and field sampling. OTU identification and writing of paper together with supervisors and co-authors.

III Field sampling and species identifications together with Jenni Nordén and Karl-Henrik Larsson. Compilation and preparation of data sets. Data analysis and writing of paper with supervisors and co-authors.

IV Experimental design, collection and preparation of fungal strains. All laboratory work. Analysis of data and writing of paper together with supervisors.

1 Wood-inhabiting fungi in forest ecosystems

The interplay between trees and wood-inhabiting fungi is a key process in forest ecosystems. As trees grow, woody biomass accumulates; when trees die, fungi recycle the carbon and minerals that were fixed during the growth of the trees (Schwarze, 2007). The evolution of woody plants led to the formation of the world's first forests. Fungi have depended upon plants as their main source of energy throughout their evolutionary history, during which time some fungi developed parasitic abilities in order to access the energy captured in the living tree. Fungi enter the woody tissue of plants either through wounds or by infection of the roots. Even though the tree can resist fungal attack for some time, it will eventually drop its branches and ultimately die. This process can significantly contribute to the small-scale disturbance dynamics in a forest stand (Fig. 1a-c) (Edman et al., 2007; Hawkins and Henkel, 2011; Lännenpää et al., 2008). For fungi that are not able to overcome the defense mechanisms of the living tree, a recently fallen log presents an open resource with large carbon sources that are up for grabs. By being present as microscopic spores in the air or as filaments in the soil, fungi will never be far away from a recently fallen tree. Equipped with powerful enzymes, fungi penetrate the wood with their hyphae and modify the wood structure. Not only does the decay release the nutrients locked up in the wood; the decomposition process also opens up different niches, promoting the establishment of a range of other dead wood-dependent organisms, including other fungi, insects and hole-nesting birds (Stokland et al., 2012). As the main agents of wood decay, fungi can be considered as ecosystem engineers (Lonsdale et al., 2008).

Wood-inhabiting fungal communities are typically species-rich, and include multiple decomposer species in the same wood substrate. Throughout the decomposition of a fallen tree, fungal species interact with each other as community composition develops over time. The resident fungi must either defend an occupied domain or replace the mycelia of primary established species. This thesis aims to explore the diversity of wood-inhabiting fungi and investigate their interactions during the decomposition of coarse woody debris of Norway spruce (*Picea abies* (L.) Karst.).

2 Fungal communities in dead wood

2.1 Fungal decomposition in the boreal forest

The boreal forest is the main forest biome in the northern hemisphere and also the dominant forest type of the Scandinavian peninsula, Finland and Russian Karelia, i.e. Fennoscandia (Essen et al., 1997). Boreal forests are characterized by a cold climate with long winters and nutrient-poor podsolic soil. In Fennoscandia, the tree-layer is dominated by two coniferous tree species; Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.). These trees produce litter with a high lignin content that is also rich in polyphenolic compounds (Aerts, 1995). The combination of a cold climate, nutrient-poor soils and highly recalcitrant litter leads to a relatively slow decomposition rate of organic material in boreal forests (Coüteaux et al., 1995).

Plant litter consists of soluble compounds, cellulose, hemicelluloses and lignin, and can be classified in terms of how the chemical components are made available for degradation by microorganisms (Coüteaux et al., 1995). Fine woody debris and non-woody plant residues, such as needles, leaves and cones, comprise the major part of the litter input to the boreal forest floor (Berg et al., 2001; Dahlberg et al., 2011). Compared with these types of litter, coarse woody debris such as fallen stems of dead trees, contains only small amounts of readiliy available nutrients (Laiho and Prescott, 2004). The lignin fractions of conifers is to a large extent made up of coniferyl subunits, which make softwood more resistant to fungal degradation compared with hardwood (Blanchette, 1995). As a consequence it may take between 60 and 100 years or more for a fallen Norway spruce to be degraded in boreal forests (Edman et al., 2007; Holeksa et al., 2008).

2.2 Wood-inhabiting fungal communities

A community is defined as a group of species occurring together in a restricted space and time (Morin, 2011). This thesis investigates the diversity and dynamics of fungal communities that live and interact in dead logs of

Norway spruce. Degradation of wood in forest ecosystems is to a large extent a fungal affair. This is because of the intrusive mycelial habits of fungi as well as their ability to enzymatically degrade recalcitrant organic matter. Their filamentous growth enables fungi to penetrate complex substrates and the production of aggregated hyphae allows fungi to pass through unfavorable habitats to interconnect occupied nutrient sources. By connecting several resource patches, fungi may translocate energy from a nutrient-rich substrate to other parts of its mycelium, e.g. to support the decomposition of a new substrate such as wood (Tlalka et al., 2008). Even though wood-decaying fungi constitute an important part of fungal communities in dead wood, the fungal kingdom includes a wide diversity of species with different ecological roles and many of them also thrive in dead wood. In this thesis, the definition of wood-inhabiting fungal communities includes all different fungi that thrive in wood.

2.2.1 Wood-decaying fungi

Usually, when we think about wood-inhabiting fungi, species within the Ascomycota and Basidiomycota come to mind because these large phyla include species with the ability to degrade wood. Wood-decaying fungi target the cellulose and hemicellulose fractions, which comprise the major carbohydrate resources in wood. In the plant cell wall, the celluloses are coated with lignin, which is a more complex aromatic polymer than cellulose. To get access to the celluloses the fungi need to bypass the more recalcitrant lignin. Wood-decaying fungi have evolved different ways of solving this problem.

White-rot fungi degrade lignin by the production of oxidative lignolytic enzymes. The ability to decompose lignin appears to be mainly restricted to basidiomycete fungi within the Agaricomycotina (Floudas et al., 2012), even though some ascomycete fungi also have the ability to modify lignin (i.e. species within the Xylariales) (Worrall et al., 1997). The production of enzymes such as peroxidases enables the fungi to get access to the cellulose, but they do not use lignin as a source of energy (Baldrian, 2008). White-rot fungal species vary in regard to which enzyme systems they utilize to degrade the lignin and celluloses in the wood. Some white-rot fungi degrade cellulose during subsequent steps of the decomposition process (e.g. *Phellinus nigrolimitatus*) (Fig. 1i) whereas others degrade both lignin and celluloses at the same time (e.g. *Trichaptum abietinum* (Fig. 1g) and *Xylaria spp.*). Other combinations of enzymes allow some fungi to combine stepwise and simultaneous decay mechanisms (e.g. *Heterobasidion spa.*) (Baldrian, 2008; Hatakka and Hammel, 2010).

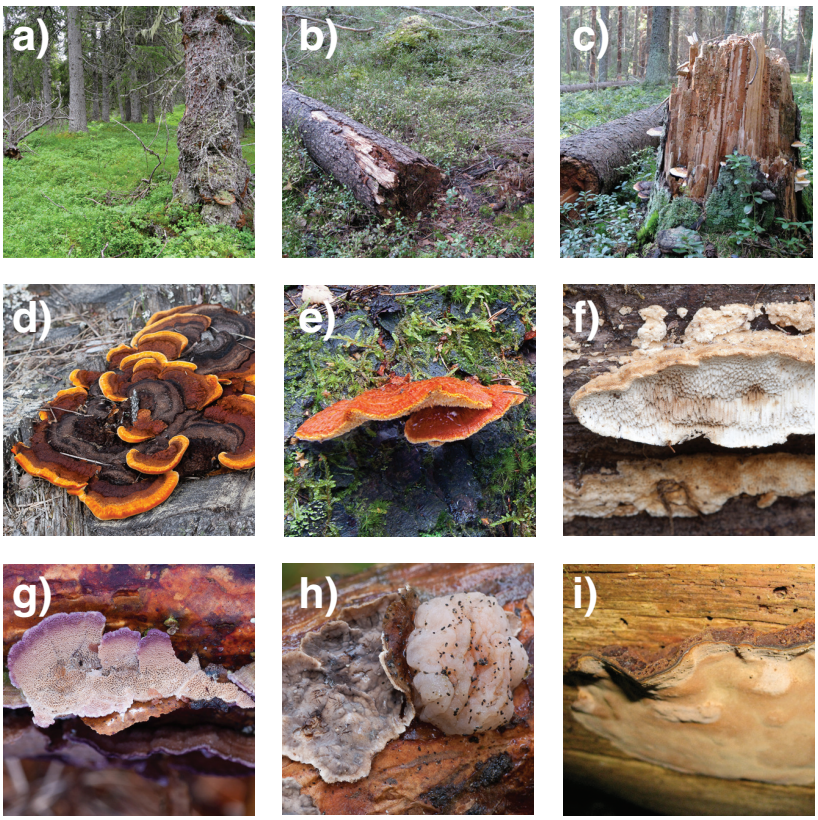

Figure 1: Norway spruce and some of the fungal species featured in this thesis. a) Norway spruce with cankers of *Phellinus chrysoloma* at Gardfjället. b) Butt rotted trunk of Norway spruce. c) Norway spruce with fruit bodies of *Fomitopsis pinicola* in Rörstrand. d) *Gloeophyllum sepiarium* (Photo: Michael Krikorev). e) *Pycnoporellus fulgens* (Photo: M. Krikorev). f) *Antrodia serialis* (Photo: M. Krikorev). g) *Trichaptum abietinum* (Photo: M. Krikorev). h) *Stereum sanguinolentum* parasitized by *Tremella encephala* (Photo: M. Krikorev). i) *Phellinus nigrolimitatus*.

The brown-rot mechanism is only utilized by species within the Basidiomycota. Approximately 7% of all wood-decaying fungal species are known to cause brown-rot, and the majority of these are closely associated with conifers (Renvall, 1995; Hatakka and Hammel, 2010). Unlike white-rot fungi, brown-rotting species do not produce lignolytic enzymes in order to degrade lignin. Instead the brown-rot fungi synthesize low-molecular weight compounds that can pass through the lignified part of the inner plant

cell wall. After this, a Fenton reaction is initiated, resulting in the production of hydroxyl radicals, which in turn break up hemicelluloses and cellulose. These fragments are then assimilated by the fungus while the lignin is left behind (Eastwood et al., 2011).

A third wood-rot mechanism, soft-rot, is mainly utilized by ascomycete fungi and primarily targets the cellulose fractions in the wood, although lignin is sometimes modified (Hatakka and Hammel, 2010). Unlike the white- and brown-rot fungi that degrade the wood from inside the cell lumen, soft-rot fungi grow inside the cell walls (Schwarze et al., 2000). Soft-rot fungi are the main decay fungi of wood with high water content (Stokland et al., 2012).

2.2.2 Other fungal ecological roles in wood

Most saprophytic fungi are able to degrade cellulose although they may not be enzymatically equipped to degrade lignin (Blanchette, 1991). There are also fungi that may rely on the pre-modification of wood by a primary wood-decay species (Niemelä et al., 1995; Holmer et al., 1997; Fukasawa et al., 2011). In addition, a wide range of fungi rely upon readily available carbon compounds that are present in the wood. For instance, some species are able to assimilate sugars in the sap of wounded or recently dead trees, whereas others may utilize by-products from wood degradation or nutrients leaching during fungal-fungal interactions at more advanced stages of decomposition (Rayner and Boddy, 1988). Many of these fungi are ascomycetes; however, fungi growing as unicellular yeasts are also included in this group. Both ascocmycetes and basidiomycetes have yeast-growth forms. Although some yeasts seem to be specialized to degrade small aromatic compounds in soils (Botha, 2011), their role in decaying wood is uncertain.

Fungi that rely upon energy derived from other organisms are regularly encountered in wood (Papers I & II). Many ectomycorrhizal species use the wood primarily as support for their fruiting bodies (Tedersoo et al., 2003), whereas other species have certain enzymatic pre-requisites to degrade wood (Bödeker et al., 2009); however, their role in wood decomposition is uncertain and needs further attention. Some of the fungi found in wood parasitize the mycelia of other fungi. For example, some species within the Heterobasidiomycetes are biotrophic parasites of different species within the Corticales (Fig. 1h) (Zugmaier et al., 1994; Pippola and Kotiranta, 2008). A final example of fungi that have an alternative nutritional mode in wood is the predatory fungi. Fungal species with nematode-trapping devises are found both within the Ascomycota and Basidiomycota. Many of these species are also frequent in decaying wood (e.g. *Peniophorella praeter-*

missa) (Hallenberg et al., 2007). Given that these species also synthesize cellulose-degrading enzymes it has been suggested that the predation of nematodes provides a nutritional supplement and that these fungi are primarily saprotrophic or wood-decay fungi (Tzean and Liou, 1993; Barron, 2003).

2.3 Assembly of fungal communities in dead wood

Community assembly has been defined as the "construction and maintenance of local communities through sequential arrival of potential colonists from an external species pool" (Fukami, 2010a). After a disturbance, species arrive and colonize a new competition-free habitat. In this context, community assembly can be seen as the trajectory along which a community develops over time as colonizing species fail or succeed to establish in the community. Distinctive patterns in species composition and in the structure of different communities can be shaped by differences in environmental factors between habitat patches as well as biotic interactions within patches. During community development, a species may not establish in a patch because the abiotic conditions do not match its ecological requirements. Furthermore, biotic interactions within a community such as resource competition may act as a biological filter during community assembly. As more time elapses after a disturbance, species interactions will become more important as established species increase their abundance in the habitat patch (Fukami, 2010a). The presence of early established species might then affect the later arriving community. This phenomenon has been referred to as a priority effect (Young et al., 2001; Fukami, 2010a).

The importance of priority effects has mainly been studied in plant and animal communities, but recently also in fungal communities (Kennedy et al. 2009; Peay et al. 2012; Dickie et al. 2012; see also Papers III & IV). Few studies also addressed the question of what importance the primary species may have in wood-inhabiting fungal communities. In a laboratory study, Heilmann-Claussen and Boddy (2005) demonstrated that the identity of a primary established species highly influenced the ability of secondary species to colonize decayed beech wood. Similarly, Fukami (2010b), Lindner (2011) and Dickie (2012) demonstrated that the pre-decay of a primary decay species had significant effects on the subsequent community composition as well as decomposition rate. The composition of fungal communities may depend on the sequence in which species establish in a habitat patch (Kennedy et al., 2009; Peay et al., 2012; Dickie et al., 2012). One mechanism may be pre-emptive competition where the first species to establish in a habitat patch may get a head start in the exploitation of resources and will have occupied a larger domain in comparison to successive species (Vetro-

vsky et al., 2011). Priority effects in wood-inhabiting fungal communities also involve alternation of the chemical environment in the wood by the production of toxins (Heilmann-Clausen and Boddy, 2005; Woodward and Boddy, 2008). The pre-decomposition of wood by a primary species may inhibit but also facilitate the establishment of later-arriving species. This was one mechanism suggested by Holmer and colleagues (Holmer et al., 1997) who investigated the ability of late-successional species to replace pioneer species in a laboratory setting. They found that species succeeding a particular primary species under natural conditions were more successful in replacing that particular species in competitive interactions, compared with interactions involving primary species that the fungi did not regularly encounter in nature. However, the importance of species interactions in fungal community dynamics are not well understood and more detailed studies of specific species-associations are needed.

2.3.1 Fungal life-histories

Dead wood is a dynamic substrate, unevenly distributed in time and space. As the woody substrate is decomposed the physical and chemical microenvironment continually changes. To cope with these changing conditions, wood-inhabiting fungi have developed different ways in which they disperse, establish, compete and endure biotic and abiotic stress. According to Cooke and Rayner (1984), wood-inhabiting fungi can be assigned to have ruderal (R-selected), stress-tolerant (S-selected) or competitive (C-selected) life-histories, although many fungi may exert a combination of two or three categories in different sequences.

Ruderal species typically have efficient dispersal abilities, fast reproduction and rapid growth (Boddy and Heilmann-Clausen, 2008). These fungi can efficiently establish in an open, competition-free substrate, a process that is often referred to as primary resource capture (Rayner and Boddy, 1988). As the community develop, all colonization occurs from secondary resource capture, i.e. all new territory is gained from already established thalli (Boddy, 2001; Stenlid et al., 2008). Only species with better combative abilities, that are able to either defend their occupied space in the wood or overcome and take over an already occupied domain, will then persist in the community. These may be species that have captured large domains in the wood, for instance due to their ability to enzymatically degrade the wood or due to their arrival in the early stages of succession. Alternatively, late-stage colonizing species might have better competitive abilities (Holmer and Stenlid, 1997; Holmer et al., 1997). Replacement of primary species by such late-stage colonizer may be species-specific (Holmer et al., 1997) or late-stage

colonizers may have better competitive abilities in general. Throughout the whole decomposition process, fungal species experience changes in abiotic stress, hence different stress-tolerant species will increase and decrease in response to these factors.

2.3.2 Fungal community development in decaying Norway spruce

The way a tree dies may have consequences for the identity and composition of species in fungal communities during the decomposition of the fallen tree (Stokland et al. 2012; Paper II). A tree that has fallen in a windstorm has a different wood structure to that of a tree that has died and been standing as a snag for years. The community development of wood-inhabiting fungi may start before a tree dies because certain fungi (generally referred to as heart-rot fungi) can infect healthy and standing trees (Boddy, 2001; Arhipova et al., 2011). The combination of different mortality factors and the presence of heart-rot fungi may initiate different succession trajectories during the subsequent colonization of the dead tree.

Healthy trees are protected from fungal infection by the bark and the high water content in the sapwood. The conditions in the heartwood are more favourable for fungal growth, even though this woody tissue contain high levels of toxic extracts produced by the tree. A few species such as *Phellinus chrysoloma* and *Heterobasidion spp.* are able to overcome these stress factors and may enter the heartwood through wounds or by pathogenesis through the roots. An alternative route for establishment may be vectoring by insects (Persson et al., 2009, 2011). This may also be a way for saprotrophic species that are not pathogens to establish early and live as latent invaders in the tree. Indeed, a number of known saprotrophic species (e.g. *Fomitopsis pinicola*, and *Resinicium bicolor*) have been detected in healthy stems of Norway spruce (Vasaitis, 2013). When the tree eventually dies, species that were present in the heartwood may continue living as saprotrophs in the community.

A recently dead tree is immediately colonized by a wide range of fungi. While fungi and insects rapidly consume the inner bark, the outer bark cover can remain for a long time (Stokland et al., 2012). Among the fungi that commonly occur in these early decay stages we find *Phlebiopsis gigantea* and *Stereum sanguinolentum* (Persson et al., 2011). When the tree falls to the forest floor, these species continue to decay the log but now species that could not cope with the conditions in the standing trunk and species that primarily spread through the soil also colonize the wood. In these early to intermediate stages of decay communities become dominated by efficient wood-decaying species such as *Fomitopsis spp.*, *Hyphodontia spp.*, *Hy-*

phoderma spp. and *Antrodia spp.* (Renvall, 1995; Rajala et al., 2011) (Papers I and II).

As decomposition progresses, the fungi themselves increasingly influence their physical environment and the relative fitness of each species changes over time. Decomposition of cellulose and lignin changes the composition of nutrients. Fungal decay alters the physical strength of the wood, which leads to an increased moisture content when the log slowly sinks closer to the ground. At first, the nitrogen level in the log is low, but increases as more fungi colonize the log (Laiho and Prescott, 2004), as fungi translocate nitrogen from other parts of their mycelia and transport it into the log (Tlalka et al., 2008). When the carbon resources in the wood are depleted, much of the nutrients remain bound to recalcitrant compounds or are available in other sources, such as fungal hyphae and bacteria (Boddy and Heilmann-Clausen, 2008; Stenlid et al., 2008). In particular, brown-rot residues have high lignin content and may persist in the humus layer for a long time (Berg and McClaugherty, 2008).

2.4 Fungal-fungal interactions

In the initial stages of the community development, an established fungus can grow throughout the wood without encountering other fungal species. But as its mycelium expands and new species arrive, it will eventually encounter other fungi and they will interact (Boddy, 2000). Species interactions can be mutualistic, where both species benefit from each other. In parasitic or predatory interactions, one of the interacting species benefits at the expense of the other species. When neither species benefits from the presence of its antagonist, the interaction is of a competitive nature (Morin, 2011).

Competitive interactions are often described as being either direct or indirect. Interference competition involves direct contact between two species whereas exploitation competition occurs indirectly when the uptake of a common resource by one species limits the access for its competitors. In communities of wood-inhabiting fungi, competition for space inside the wood is the most common interaction (Boddy, 2000). Interactions involve direct interference at the hyphal or mycelial level or they may occur at a distance through the diffusion of volatile compounds that are induced during the recognition of a competitor (Hynes et al., 2007; Evans et al., 2008). When fungi grow in the proximity of each other they increase their production of lignolytic enzymes (Iakovlev and Stenlid, 2000; Hiscox et al., 2010) and other toxic secondary metabolites (Woodward and Boddy, 2008). The interactions may result in deadlock where both fungi retain their territory,

or replacement, either partial, mutual or complete (Boddy, 2000). In mycology, much research has focused on direct competition (Woodward and Boddy, 2008). However, fungal modification of the physicochemical properties of the wood may not only indirectly inhibit but also facilitate the nutrient acquisition of other species. This was suggested in a laboratory study where the production of secondary metabolites during decomposition not only inhibited but also stimulated the growth of interacting species (Heilmann-Clausen and Boddy, 2005).

A number of field studies of inventorying Norway spruce have found non-random species occurrence patterns in wood-inhabiting fungal communities (Renvall, 1995), even when the possibility that some species occur together merely because of similar habitat requirements were accounted for (Ovaskainen et al. 2010; Paper III). In a laboratory study, Holmer and Stenlid (Holmer and Stenlid, 1997) found that species in these communities differ in their competitive abilities, and that species occurring at later stages of decay had better combative abilities than early-successional species. Also, species that often form mycelial aggregates such as rhizomorphs or cords and then can allocate resources from external parts of their mycelia tend to have a competitive advantage in antagonistic species interactions (Stenlid et al., 2008). However, the tendency to form hyphal aggregates is not known for many species.

For the majority of species, the mechanisms behind the co-occurrence pattern observed in the field are not well understood. One reason for this could be that conclusions about species interactions are often made from one-time observation studies. Species interactions do not always result in species assemblages that differ from randomly structured communities. Therefore, it is important to observe temporal changes in community structure such as species immigration and persistence by repeated studies (Fukami, 2010a)(Paper III). In order to understand the nature of the interactions, field-studies should also be complemented by laboratory studies where the interactions can be studied directly. A better understanding of the interspecific dynamics in these communities is important because interactions may have significant influence on the structure and diversity of wood-inhabiting fungal communities, which in turn affects the decomposition of woody debris (Fukami et al., 2010b; Lindner et al., 2011; Dickie et al., 2012; van der Wal et al., 2012).

3 Aims

This thesis investigates the diversity and dynamics of wood-inhabiting fungal communities in Norway spruce. Wood-inhabiting fungi depend upon a patchy distributed habitat that continuously changes over time. Given that the dead wood is constantly subjected to decay, the fungal community present in each decaying log gradually alter their own habitat. In turn, the composition of fungal communities is affected by species interactions such as competition for space in wood. Ultimately, species interactions will indirectly affect the decomposition processes, such as decay rate and substrate qualities and, hence, also the dynamics of saproxylic biodiversity, but the mycelial dynamics within fungal communities are poorly understood.

The specific aim of this thesis is to study the diversity and the impact of species interactions on community development during the succession of fungal species. These questions are approached by investigating long-term trends in fungal communities as well as the fine-scale patterns of fungi within Norway spruce logs. Furthermore, detailed species interaction experiments are performed in laboratory microcosms.

Specifically, the objectives of this work are:

I To investigate the taxon identities, phylogenetic diversity and ecological roles of wood-inhabiting fungal communities in decaying Norway spruce (Papers I and II).

II To explore the spatial patterns of fungi with different ecology and life-histories in wood (Paper II).

III To examine if historical factors such as priority effects (Paper III) or different tree mortality factors (Paper I) affect the fungal community development.

IV To investigate the relative importance of substrate modification and competitive abilities on fungal succession (Paper IV).

4 Materials and methods

4.1 Study areas

The field data sets that part of this thesis is based upon came from studies conducted in the hemi-boreal and boreal zone (Ahti et al., 1968) in Sweden and Finland (Fig. 2). The study forests represent mature to old-growth forests in which the tree layers were dominated by Norway spruce, *Picea abies*. For papers I and II, field surveys were conducted in areas 1 and 2, and in addition, previously collected data were used in paper I (Table 1). In paper III, fruit body inventories in area 3 and 6 were combined with earlier collected data as well as data previously collected at site 2, 4 and 5 (Table 1).

4.2 Studies of fungal communities

4.2.1 The fruit body life-stage (Paper I and III)

Our understanding of wood-inhabiting fungal communities relies to a large extent on the observation of fruiting bodies that emerge upon the surface of decaying wood. The production of a fruit body represents an important fitness aspect because it reflects the reproductive outcome of fungi (Stenlid et al., 2008). Many studies have characterized the ecological requirements of wood-inhabiting fungi. Hence, habitat preferences in terms of tree-host, log dimension and wood decay stage are relatively well known for several species (Berglund et al., 2011a; Nordén et al., 2013; Stokland and Meyke, 2008; Niemelä, 2005; Renvall, 1995; Kruys and Jonsson, 1999). In addition, fruit body occurrence suggest that many fungal species are specialised in their habitat requirements (Nordén et al., 2013). Furthermore, specialist species are more sensitive to habitat fragmentation than generalist species that utilize different types of wood in both fragmented and connected forest landscapes (Nordén et al., 2013).

Assessment of the total species richness in fruit body inventories is hampered by the fact that the reproductive effort and the detectability of fruiting bodies varies between fungal species both in terms of the durability of the fruiting bodies and the seasonal variation of fruit body production. For

instance, many fungal species have annual fruit bodies, i.e. they produce short-lived fruit bodies that are only visible for weeks or months (Halme et al., 2009). Therefore, to detect as many species as possible, multiple surveys of each substrate should be conducted at a time in the season when most fungi produce fruit bodies (Halme and Kotiaho, 2012). In papers I and III we investigated fungal diversity based upon fruit body occurrences. In paper I, we used data from repeated monitoring during several years of fungal fruiting on individual logs to compare the previous and present fruit body occurrences with the species present as mycelia. In paper III, we looked at the succession of fungal assemblages inferred from fruiting body occurrences. Our conclusions concern the species found as fruit bodies and consisted of 1739 repeatedly inventoried logs, a sample number that so far has not been matched by any study using molecular methods.

Figure 2: Locations of the study forests included in this thesis. The associated studies are shown in parenthesis and are listed in Table 1. The position of Västerbotten is an average of seven pairs of forest stands (for details see Olsson et al. 2011). In study III, asterisks indicate where extra inventories were performed for the present study.

Table 1: Study sites, forest type and the associated papers in which field studies were performed or the associated studies from which data was compiled.

Study site	Forest type	Paper	References
1. Fagerön nature reserve, Sweden (Site F)	Mixed un-managed stand	I–II	Lindhe et al. 2004
2. Gardfjället, Brattiken nature reserve, Sweden (Site G)	Old-growth coniferous stand	I–III	Edman and Jonsson 2001, Berglund et al. 2005, Edman et al. 2007, Jönsson et al. 2008
3. Svartberget experimental forest, Sweden	Three old-growth and three managed coniferous stands	III	Edman et al. 2004b
4. Västerbotten, Sweden.	Seven pairs of old-growth and managed coniferous stands	III	Olsson et al. 2011
5. Patvinsuo national park, Finland	Old-growth coniferous stand	III	Penttilä 2004
6. Rörstrand nature reserve, Finland	Old-growth coniferous stand	III	Ovaskainen et al. 2010

4.2.2 The mycelial life-stage (Papers I and II)

While well-planned fruit body inventories will provide high-quality data on the fruiting species, many of the fungi that are present in the wood remain undetected in such inventories. In addition to the species recorded in the fruit body inventories that vary a lot in their production of fruit bodies, other species may not have retained sufficient resources to produce a fruit body or it exists in the log as an unmated homokaryotic mycelium (Stenlid et al., 2008). Furthermore, some species may not produce fruit bodies at all, or if they do these are so inconspicuous that they are rarely detected in a visual inspection of the log.

Mycelial isolation from natural substrates such as wood also targets the mycelial life-stage (Stenlid et al., 2008). However, as the conditions vary considerably in wood compared with nutrient media, the method itself is biased towards those species that are able to cope better with the artificial conditions than the other species present in the wood. Culture-independent molecular methods circumvent this problem by extracting DNA directly from wood samples and then amplifying it in PCR reactions (Johannesson and Stenlid, 1999). As for most fungal community studies, the ribosomal internal transcribed spacer (ITS) region has been targeted as a primer for species identification (White et al., 1990; Schoch et al., 2012). The amplified regions are sequenced and then taxonomic identification is carried out by

comparing the derived sequences with known sequences in databases such as GenBank and UNITE (Abarenkov et al., 2010). In many studies, molecular methods such as DGGE, T-RFLP and TGGE have been used to study wood-inhabiting fungal communities (e.g. Vainio and Hantula 2000; Allmér et al. 2006; Kulhanková et al. 2006; Rajala et al. 2010).

Studies of the mycelial life-stage in wood today focuses on high-throughput sequencing of directly extracted DNA. In this thesis, 454 sequencing was used to study wood-inhabiting fungal communities in Norway spruce (Papers I and II). Compared with the traditional Sanger sequencing methods where one sequence run results in 96 sequences, next-generation sequencing (NGS) methods such as 454 sequencing give approximately 1 000 000 sequences per run (Rothberg and Leamon, 2008). The fast developing 454 sequencing methodology enables the study of low abundant taxa and is now a widely applied method for studying diversity in microbial ecology (Rothberg and Leamon, 2008). It has been successfully used to study fungal diversity in a range of different environments, including indoor dust (Amend et al., 2010) agricultural (Rousk et al., 2010) and forest soils (Buée et al., 2009; Wallander et al., 2010; Hartmann et al., 2012) as well as the human oral cavity (Ghannoum et al., 2010).

Sampling from complex natural environments generates a huge amount of sequence data that is difficult to connect with the known sequences in current libraries (Delmont et al., 2012). Thus, a large proportion of the operational taxonomic units (OTUs) found in 454 sequencing studies remain unidentified or are not identified to species level. Whether or not an unidentified fungal taxon represents a previously unknown species is difficult to establish since about 70% of the fungal taxa that are already known to science are not represented by a sequence in GenBank (Brock et al., 2009). Given that global fungal diversity has been estimated to range between 1.5 million and 5.1 million species and only about 100 000 fungal species have been described to date, it will take centuries or millennia to describe all fungi (Hibbett et al., 2011) and it is unlikely that all species will be fully covered in sequence databases. As the use of NGS methods in diversity studies increases, so too is the number of unidentified OTUs. This calls for an accelerated rate of species description and the deposit of fully identified sequences in reference libraries such as GenBank. Possible ways to facilitate and speed up species description and naming well-supported OTUs by modernizing nomenclatural practices are under discussion (Hibbett et al., 2011).

4.2.3 Interspecific interactions (Paper IV)

Fungal interactions take place at the mycelial level. Although molecular methods have improved our chances of studying this life stage, detailed observations in axenic conditions are indispensible. Species interaction studies between wood-inhabiting fungi have a long history in fungal ecology, partly because of the potential of using antagonist species as biocontrol agents against forests pathogens (Woodward and Boddy, 2008). In addition to species interactions, the ability of fungi to assimilate different carbon resources (Boberg et al., 2011; Fukasawa et al., 2011) and produce enzymes (Iakovlev and Stenlid, 2000; Hynes et al., 2007; Hiscox et al., 2010) are other examples of important mechanisms that are difficult to study under natural conditions. Furthermore, laboratory studies allow detailed observations of differences in fitness, for instance, the growth of endangered species (Crockatt et al., 2008).

Laboratory studies have revealed that microclimate factors, such as water and gaseous content and also to a lesser extent temperature all affect interaction outcome in agar culture (Boddy, 2000). In interactions on wood, the size of the occupied resource and its stage of decay also result in different competitive outcomes (Holmer and Stenlid, 1993; Boddy, 2000). Even so, in vitro systems using natural substrates such as wood instead of nutrient media are likely to be more similar in terms of nutrient availability and spatial arrangement (Holmer and Stenlid, 1993) although the difficulty of mimicking natural conditions remains (Woodward and Boddy, 2008).

Another difficulty involved in studies of interspecific interactions is the complexity involved when including more than two species in an experiment. Furthermore, many studies of interactions include only one strain per species which may be misleading because competitive ability not only differs between species but also within species (Mgbeahuruike et al., 2011). Even under apparently identical conditions the interaction between two strains can result in different outcomes (Boddy2000), thus adding even more species often produces results that are difficult to interpret (Woodward and Boddy, 2008). Hence, it is challenging to predict the outcome of species interactions of three or more species based upon interactions between two species. However, paired interaction experiments with many combinations of species and isolates do yield valuable information about the degree of ease or difficulty with which a species may replace or defend its territory (Holmer et al. 1997; Holmer and Stenlid 1997; Boddy 2000; Paper IV). These patterns provide a wealth of data that should increase our understanding of how species interactions may influence non-random occurrence patterns found in natural conditions.

5 Results and discussion

5.1 Diverse ecological roles in fungal communities (Papers I and II)

454 sequencing of Norway spruce logs revealed species-rich fungal communities, spanning large parts of the fungal kingdom and representing several different ecological roles (Fig. 3). In particular, ascomycetes within the Helotiales and Chaetothyriales were common at both sites. Among the basidiomycetes, most OTUs clustered within the Agaricomycotina. Many OTUs belonged to the Hymenochaetales or Polyporales but OTUs within Tremellomycetes, Sebacinales and Russulales were also frequently detected. Within these groups we found wood-decaying fungi, litter-decaying saprotrophs, mycorrhizal fungi as well as endophytes and parasitic species.

The detection of lichen mycobionts inside wood in our study gives new insight into the lifecycle of lichens. It has been proposed that some lichen genera may have a free-living saprotrophic life-stage in wood (Wedin et al., 2004); however this topic has received little attention. In our study we frequently encountered mycobionts of species within *Cladonia*, *Trapeliopsis* and *Absconditella*. We found that the lichen *Parmeliopsis ambigua* was a frequent species in the inner part of logs, both at Fagerön (site F) and at Gardfjället site G.

A few OTUs were widespread and recorded on many logs; however the majority of OTUs were rare and only recorded in single or a few logs. Although we took at most 16 samples per log, which represents a tiny fraction of the total wood volume, from these samples we were able to detect up to 398 OTUs per log at site F and up to 274 OTUs per log at site G.

There can be many different reasons why we find such species-rich fungal communities in the wood. A decaying log presents a large number of different niches (Stokland et al., 2012). As a log decomposes its different parts will become more heterogeneous because the decomposer community creates changing patches with qualitative differences within a single log (Pyle and Brown, 1999). In addition, environmental factors, such as sun exposure, surrounding vegetation and soil properties will affect the wood conditions

(Progar et al., 2000). Species-specific interactions are also a contributing factor to the number of niches in wood because some species are favoured by the presence and decay of certain primary species. Niche partitioning might thus be one reason that we find so many species in dead wood. Supporting this view is the fact that fungi differ in their resource use (Hanson et al., 2008; McGuire et al., 2010), as well as their enzymatic abilities. Since the production of enzymes is costly, targeting different specific resources in wood might be an efficient way to obtain a niche in the fungal community. Furthermore, interaction experiments and studies of wood anatomy in decaying logs show that most fungal species have a compartmentalized growth and block the growth of other species (Boddy 2000; Holmer et al. 1997; paper IV). This may *de facto* reduce the competitive exclusion and provide an important clue to the high number of species coexisting in decaying wood.

If niche differentiation is a major factor determining the species coexistence in wood, a species that occupy a rare distinct niche in the community will persist even though it is not an efficient competitor. However, even though niche partitioning may be important, species coexistence also depends upon the differences in the competitive abilities among the interacting species. If there are large differences in competitive ability competitive exclusion will occur, even if the niche overlap is very small (Chesson, 2000). Hence species coexistence will ultimately depend upon the balance between these differences in competitive ability and niches between species in a community (Chesson, 2000). If species are equal or near equal in their competitive abilities and ecological niche the coexistence of species in a community will depend more upon random processes (Hubbell, 2005; Adler et al., 2007). In such cases, biological legacies, in the sense of what species are locally present and thus, have a high probability of becoming established at a certain habitat may be more important than niche differentiation. Regarding the species detected in papers I and II, those belonging to different ecological groups are likely to differ more in their resource use, and hence, depend more upon the availability of niches in the wood. However, within ecological groups, species are likely to be more similar in their resource use and, hence competitive abilities and random processes are more likely to influence species coexistence. As the decomposition of a log gives rise to both physical and chemical changes in the wood, the relative fitness between species changes over time. Hence, the conditions for coexistence of fungal species are dynamic, and given that so many different factors contribute to the decomposition of logs, fungal succession of species should not be looked upon as a deterministic pathway of species colonizations and extinctions, but rather a diverse multidimensional array of pathways (Boddy, 2001).

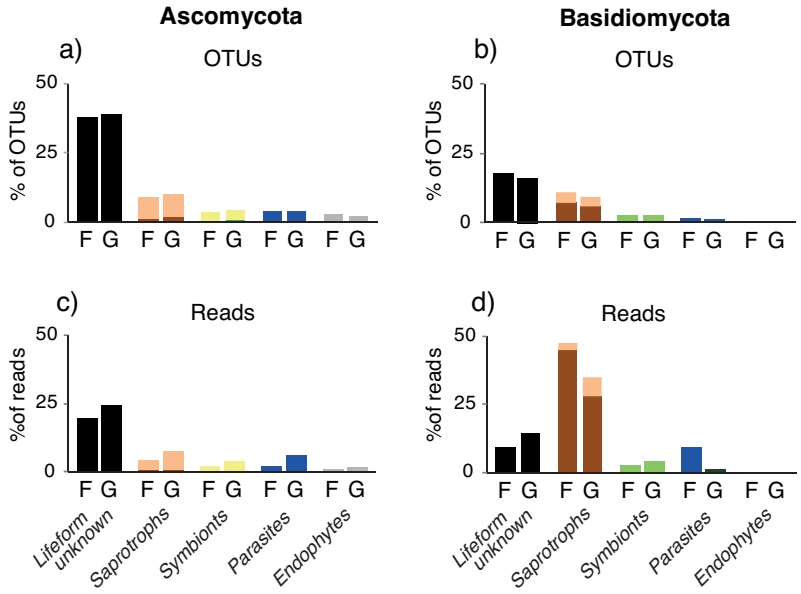

Figure 3: Assumed ecological roles used by the OTUs detected in the logs at Fagerön (F) and Gardfjället (G). The percentage comparison is based on the total number of OTUs, n=973 at F and n=1406 at G, (panels a and b) and the total number of sequence reads, n=90114 at F and n=141223 at G, (panels c and d). These parameters are separately shown for Ascomycota (panels a and c), and for Basidiomycota (panels b and d). Symbionts refers to mycorrhizal fungi and lichens. Black = ecological role unknown, Brown = wood-decayer, Pink = other saprotrophs, Green = mycorrhizal, Yellow = lichen, Red = parasite and Grey = endophyte. Glomeromycota, Mucoromycotina and fungi with unidentified affiliation are not shown (fraction of OTUs 10.1% and 11.5% at F and G respectively; corresponding fraction of reads 2.6% and 2.1% at F and G respectively).

Another mechanism that allows for coexistence of competitive species is if there is a trade-off between dispersal and competitive abilities. By having better dispersal abilities, a competitively inferior species can utilize similar resources to that of a competitively superior one because it may colonize patches where the better competitor has not yet reached (Tilman, 1994). These types of trade-offs are often referred to as r- and K-strategies, and this concept has been further developed for wood-decaying fungi (Cooke and Rayner 1984, outlined in Chapter 2 and exemplified below).

5.2 Spatial patterns and life-history traits (Paper II)

In paper II, 454 sequencing enabled the investigation of fine-scale distribution of fungal communities in Norway spruce logs. In addition, we took advantage of this high-throughput method to explore the spatial patterns of fungal groups that have not been as well studied as wood decaying species. One reason for this is that earlier methods have been restricted in their ability to detect OTUs with low levels of abundance (Peay et al., 2008). In paper II we hypothesized that the spatial patterns of fungal species and ecological groups would differ in response to factors such as the position inside a log and decay stage.

We expected that the number of sequences for each OTU would correspond to the number of logs in which an OTU was found. However, many OTUs significantly diverged from this pattern, meaning that some OTUs occurred in more samples than was expected based on their number of sequences, and other species occurred in fewer number of logs than was expected based on their number of sequences (Fig. 4). In general, ascomycete taxa were found in more samples than expected. Many of these OTUs were often species that are known as ruderal or R-selected species (Boddy and Heilmann-Clausen, 2008). Among the OTUs that showed the opposite trend (i.e. they occurred in a fewer logs than was predicted by their sequence abundance), most species were wood-decaying fungi. These patterns reflect the different utilization of the wood resource by the different taxonomic groups. Saprotrophic and wood-decaying basidiomycetes invest much energy to produce wood-degrading enzymes whereas the ascomycetes in general rely upon a more opportunistic strategy. This opportunistic life-strategy may allow them to allocate more resources for reproduction, increasing their chances of being present at the time when resources become available. Since a species competitive ability is related to the size of its occupied domain in wood (Holmer and Stenlid, 1993), the low abundance of these opportunistic species indicates that as they have a restricted space in the wood they may be quickly replaced by more abundant species. These species are not particularly well known as wood-inhabiting fungi and the detection of them in decaying wood generates more information on their distribution and putative ecology.

Long-lived polypores such as *Heterobasidion parviporum* at site F and *Phellinus nigrolimitatus* at site G were encountered in the majority of the studied logs where they typically dominated the inner parts. This reflects a competitive life-strategy (C-selected) where one species may dominate a large part of the inner wood column in a log. *P. nigrolimitatus* has been primarily considered as a late-stage fruiter (Jönsson et al., 2008). The data

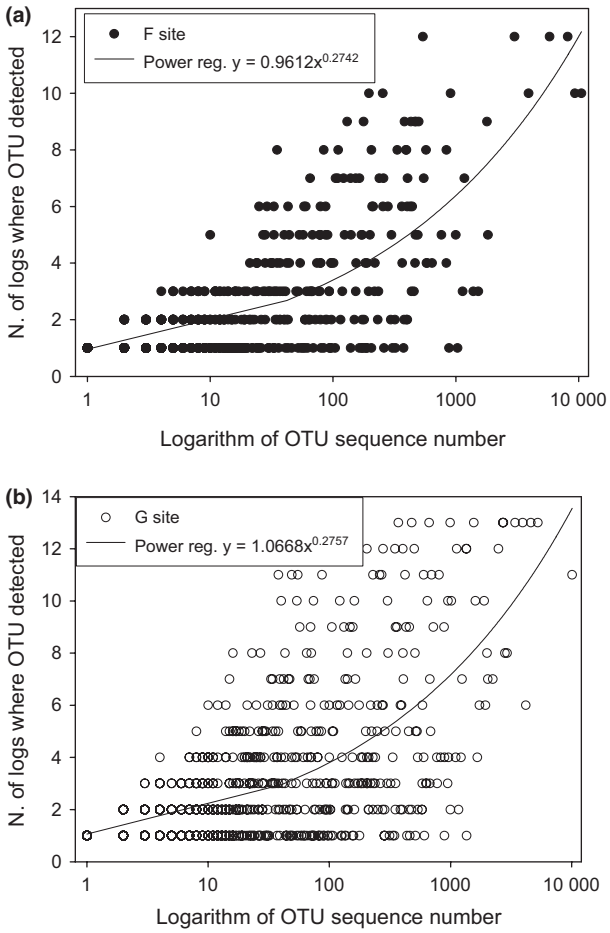

Figure 4: Number of logs in which the OTU was detected at sites F (a) and G (b) plotted against the number of sequences for each OTU (i.e. abundance, on x-axis with logarithmic scale). The majority of OTUs were not highly abundant and were detected in one or few logs only. By contrast, few OTUs had high sequence numbers and were present in the majority of logs. Residual values to the power regression line were counted for each OTU. OTUs with the highest/lowest residuals are listed in Table 2, in Paper II and the rest are listed in Table S2 (Supporting information, Paper II).

in our study reveals that this species may invest in fruit-body production by the middle stages of decay but more importantly that it may continue

living as mycelia for a long time after the initial fruiting. At site G, *P. nigrolimitatus* was found fruiting at the same log at three separate inventories in 1997, 2003 and 2008. Hence, the species may live in the same log for at least eleven years and probably longer given that it was detected as a fruit body in 1997. This suggests that once a species is dominant as mycelia in a log it may control those resources for a long time, possibly throughout the lifetime of the log.

At both sites, mycelium of *H. parviporum* at was detected in more logs than it had been detected as a fruit body; however, the investment in fruit body production was less obvious for this species compared with *P. nigrolimitatus* (Fig. 5). *H. parviporum* is able to infect living trees, and it is often considered to be a stress-tolerant species (S-selected). As the tree dies the defence mechanisms and the hostile environment of the wood disappear. As a result, there will more competition for space as more species colonize the wood. Thus, *H. parviporum* has been selected to allocate resources for fruiting in the earlier stages when competition is less severe, although, it may continue to live inside the fallen trunk as a saprotroph.

We expected different fungal ecological groups to be distributed differently inside logs. For instance, mycorrhizal species are thought to colonize logs from the forest floor (Tedersoo et al., 2003), accordingly we expected to find mycorrhizal species in more decayed wood samples taken from lower parts of logs. Contrary to our expectation, mycorrhizal species occurrence was independent of log-decay stage (Fig. 6). We did recover mycorrhizal species from the lower parts of log, but they were also frequent in samples taken from the upper parts. This finding suggests that some of the mycorrhizal fungi found in our study may also colonize wood by spore dispersal.

Many wood-decaying fungi, such as *Hyphodontia alutaria*, *Ascocoryne cylichnium*, *H. parviporum* and *F. pinicola* were more abundant in less-decayed samples. These are all efficient wood-decomposers and some may also be found in living trees. Other species such as the mycoparasite *Hyphodiscus hymenophilus* and the saprotrophs *Botryobasidium botryosum*, *Tubulicrinis borealis* and *Atheliopsis subinconspicua* were more frequent in more decayed samples. This reflects a species turnover towards a community that depends upon a pre-modified wood environment as well as the presence of senescing mycelia and fruit bodies of primary species that may act as a food source for mycoparasitic species. At site G, we also detected a significant increase of OTU richness in more decayed samples. Other wood-decay species such as *Resinicum bicolor* exhibited no correlation with decay stage. This species forms well-developed cords, which are aggregated hyphae that can form an extensive and long-lived hyphal system that connects different resource

patches occupied by the fungus (Boddy, 1993). This feature enables *R. bicolor* to establish in already occupied wood during different decay stages of a log (Holmer and Stenlid, 1996).

One interesting aspect is the low consistency between fruit body production and presence as mycelia. We found a trend that species producing

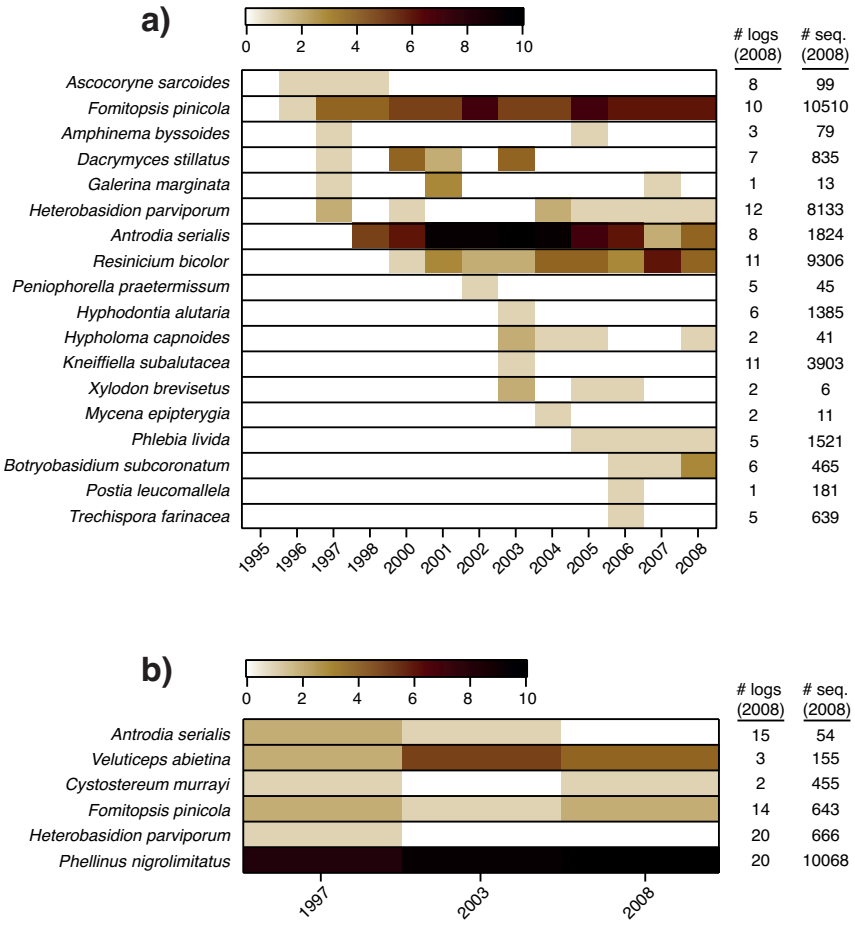

Figure 5: Species recorded both as fruiting bodies and DNA for a) experimentally cut logs at site F (n = 12) between 1995 and 2008 (no inventory was performed in 1999) and for b) naturally fallen logs (n = 26) between 1997 and 2008 at site G. The figure bar shows the number of fruiting bodies present on logs each year: the darker the colour the greater the number of occurrences.

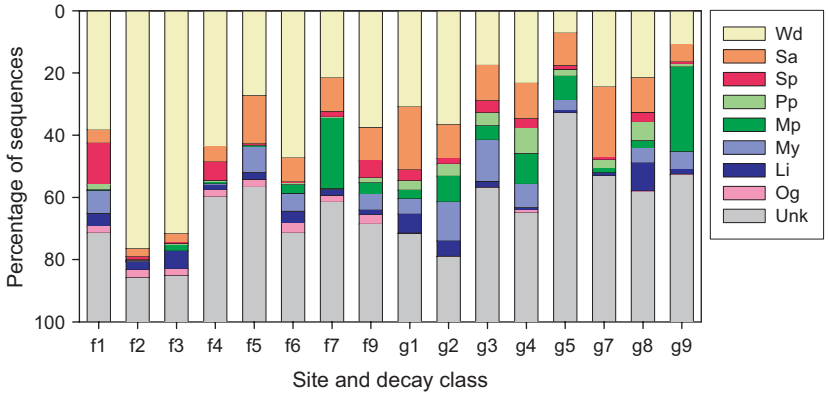

Figure 6: Ecological composition of fungal communities in different decay classes (1-9), Wd = wood decay fungi; Sa = saprophytic fungi; Sp = Fungi a combined saprophytic and parasitic strategy; Pp = plant parasites; Mp = mycoparasites; My = mycorrhizal fungi; Li = lichenized fungi; Og = other ecological roles; Unk = no data about ecology available. f = site F; g = site G.

long-lived fruit-bodies are more abundant in less decayed wood samples, which would typically hold more available energy. Species that invest fewer resources in reproduction in terms of the size and longevity of fruit bodies are typically found in more-decayed samples where there is less energy available. These results support the idea of an energy-driven control of fruit body production for some species. However, fruit body production, particularly regarding species with short-lived and less sturdy fruit bodies, may also be triggered by other factors, such as shifts in temperature and humidity as well as interspecific interactions (Moore et al., 2008).

5.3 The role of species interactions during community assembly

5.3.1 Priority effects and species associations (Papers I, III and IV)

Based on extensive surveys, Renvall (1995) proposed the idea that the presence of certain primary fungal species determines the composition of succeeding communities. The effects of primary species on the succeeding community can be referred to as priority effects because the predecessor species creates conditions that will have positive or negative effects on the colonization of a secondary species (Fukami et al., 2010b). Many studies have reported distinct patterns of fungal community composition for Nor-

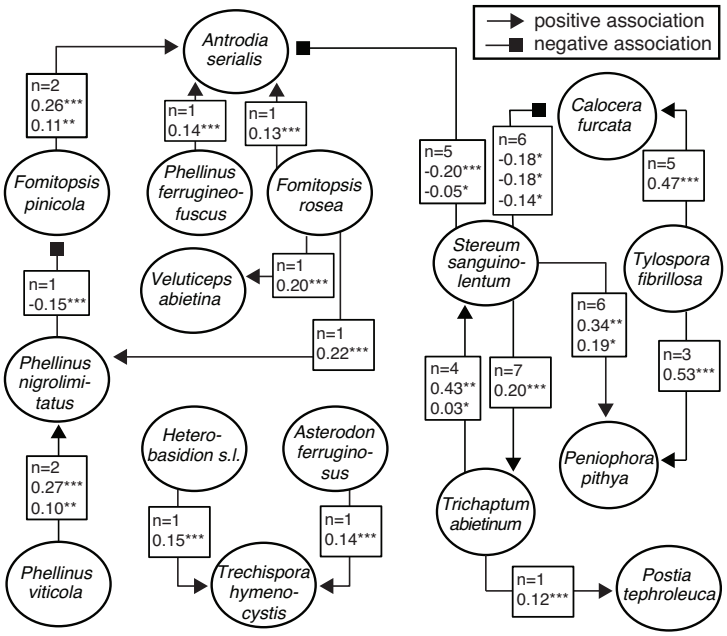

Figure 7: A summary of the priority effects (influence of already established species to the establishment probability of subsequent colonizers) found in Paper III. Species associations that were significant at the p=0.000085 level (Bonferroni corrected) in one site and associations that were significant at the p=0.05 level (without Bonferroni correction) in at least two sites are included. The numbers within the boxes give the number of sites (n) in which the association was tested for, and the effect sizes and their significance levels coded as * (0.01<p<0.05), ** (0.001<p<0.01) and *** (p<0.001). The direction of the influence is denoted by a symbol, either an arrowhead (positive influence) or a square (negative influence).

way spruce during different stages of decay (e.g. Renvall 1995; Niemelä et al. 1995; Lindblad 1998; Rajala et al. 2011, 2012). These studies are based on chronosequence studies, i.e. where wood-inhabiting fungi that are fruiting at logs at different stages of decay are compared to infer successional patterns rather than following how the fungal community develops over time on specific logs. Therefore, the previous species assemblages are not known in these studies and thus, potential priority effects are difficult to assess (but see Ylisirniö et al. 2009).

In paper III we hypothesized that the presence of a specific primary decay species would affect the colonization of a secondary established species.

We also expected that species having particular life-history traits would have an especially strong influence on the succeeding species, or alternatively, be sensitive to the primary species. Therefore, we expected that the different types of decay produced by primary species would influence the occurrence of succeeding species, and that species that are abundant as mycelia would have a strong influence on the succeeding species. Furthermore, we hypothesized that specialist species would be more influenced by priority effects than generalist species with a wider niche breadth. In order to explore these hypotheses, we analyzed data from fruit body inventories on repeated surveys of individual Norway spruce logs (paper III).

We found a strong positive association between *Trichaptum abietinum* and *Stereum sanguinolentum*, and that the occurrence of both species affects the probability of detecting ten and seven secondary species, respectively (Paper III, Fig. 7). From his study on successional pathways, Renvall (1995) found that these two species were associated with uprooted logs, which on average hosted a low number of fruiting species. Based upon species occurrence as mycelia, we also found that uprooted logs with fruit bodies of *T. abietinum* differed in their associated species compositions compared to logs that originated from stem breakages (Paper I). A similar association was reported by Pouska et al. (2011) who found that *T. abietinum* and *S. sanguinolentum* were fruiting on logs that had been killed by bark beetles. Also, Ylisirniö et al (2009) found that uprooting and the presence of fruiting *T. abietinum* influenced the probability of detecting certain species on the same logs. Interestingly, the pre-decay experiment in paper IV implies that many species have difficulties in degrading wood pre-modified by *S. sanguinolentum* compared with undecayed wood. However, we show that *T. abietinum* was one of the few species which could grow on the wood pre-decayed by *S. sanguinolentum*. The growth of *T. abietinum* on *S. sanguinolentum* was similar to its growth on the undecided control wood. In the competition experiment, *T. abietinum* overgrew *S. sanguinolentum*, but was not able to completely replace it. These results suggest that *S. sanguinolentum* can be considered to be a R-selected species, inhibiting the colonization of other species, whereas *T. abietinum* is more combative (C-selected) and is associated with *S. sanguinolentum* one reason being that it is able to degrade and take over the wood occupied by *S. sanguinolentum* as well as many other species (Fig. 8L). Strid (2012) also reported an association with bark beetle galleries and *S. sanguinolentum* that suggests that early arrival in the community is facilitated by bark beetles (Strid, 2012).

The brown-rot species *Antrodia serialis* was one of the species that was negatively associated with *S. sanguinolentum* (Paper III). In turn, *A. seri-*

alis formed positive associations with two other brown-rot species, *Fomitopsis pinicola* and *Fomitopsis rosea*. These positive associations between the brown-rot species were also found in an analysis of co-occurrence data from single-time observations of fruit bodies (Ovaskainen et al., 2010), suggesting that the coexistence of these species might be explained by niche partitioning by excluding each other from their occupied domain, and, thus, being C-selected species. This observation was supported in the pre-decay experiment in paper IV, were the decay rate of *A. serialis* and *F. rosea* increased on brown-rotted wood. Furthermore *F. rosea* overgrew, but did not completely replace *A. serialis*, and these species were also able to coexist with *F. pinicola* in the interaction experiments.

In earlier laboratory studies, the tree pathogen species *Heterobasidion parviporum* has been shown to have weak competitive abilities (Holmer and Stenlid, 1993). It is obvious that *H. parviporum* occurs more frequently in forests as mycelia compared with the occurrence levels detected by fruit body inventories (Papers I and II). This species, together with *T. abietinum* and *S. sanguinolentum* was associated with uprooted logs (Paper I) but they were negatively associated in the analysis based upon fruit bodies (Paper III). The finding of both *H. parviporum* and *F. pinicola* as mycelia in middle to late stage decay classes supports the view that once a primary species is established in a fallen trunk it may persist in the community for a long time (Vetrovsky et al., 2011). Thus, they can be considered as stress-selected (S) species that move towards competitive-selected (C) life-histories as the tree decays. Other early-stage decayers, like *S. sanguinolentum* and *Gloeophyllum sepiarium* are more prominent in the early decay stages (Strid, 2012; Nordén et al., 2013) and, thus, we only detected *S. sanguinolentum* from DNA in three samples and did not detect *G. sepiarium* in our studies.

5.3.2 Fungal competition and facilitation in decaying Norway spruce (Paper IV)

In paper IV, we focused on species that are found in early and middle stages of wood decomposition. We observed that even though the late-stage colonizers were efficient competitors, some of the early-stage decomposers were able to capture space and deadlock with late-stage colonizers. Some species impair the decay of secondary species whereas others secondary species are facilitated by the pre-decay of certain primary species. This suggests that when established in a log, both pre-decay by primary species and competitive abilities are important mechanisms influencing the fungal species community. Physicochemical modification of the wood substrate appears to be of greater importance in the early stages of decay, whereas species that

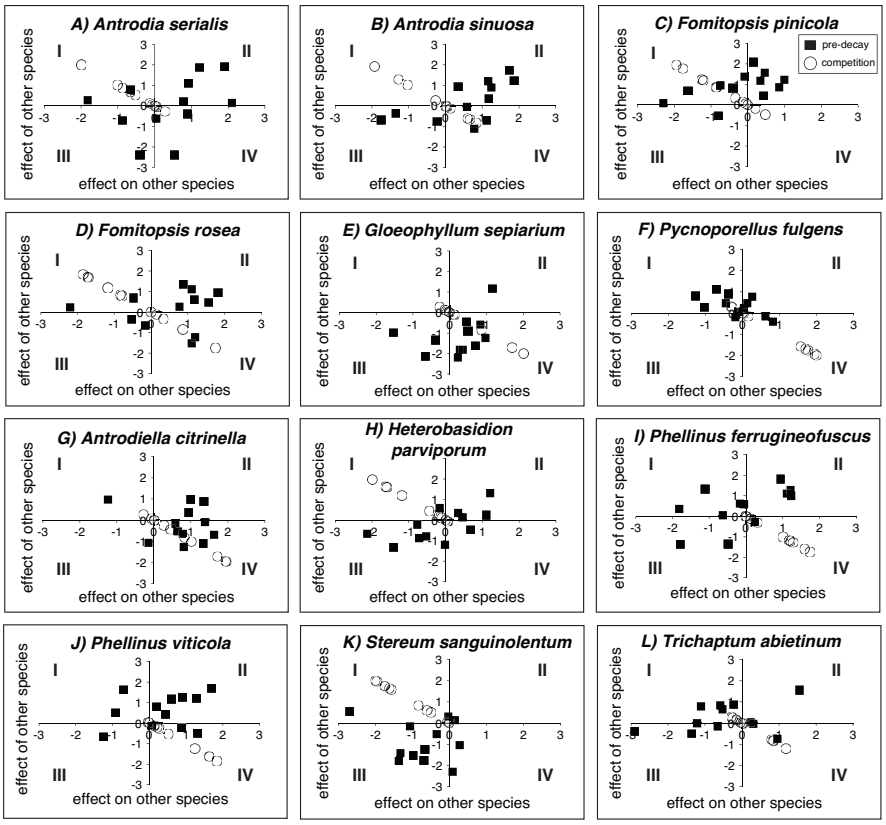

Figure 8: A-L Plots showing the results from the pre-decay and interaction experiments in paper IV. Closed squares show the influence in terms of species decay rate in the wood decay experiment compared with the non-decayed control (origo), and open circles show the species ability to capture space in the interaction experiment compared to dead-lock interactions (origo). Panel I in each figure shows the cases where the species A-L are promoting decay, but not promoted by the decay of the associated species, or the cases when species A-L gets overgrown by the antagonist species in the competition experiment. Panel II shows the cases where decay rate of associated species were increased by the pre-decay of species A-L. Panel III shows the cases where the decay rate of both associated species were impaired on wood decayed by the associated species. Panel IV shows the cases in which the pre-decay of the species A-L impaired the decay of other species but in which the species A-L was able to degrade those associated species, or the cases when species A-L overgrew its opponents in the competition experiment.

appear in the middle decay stages rely more upon their combative abilities (i.e. *Phellinus ferrugineofuscus* and *Phellinus viticola*) (Fig. 8I and J). It appears that a number of early successional species, e.g. *H. parviporum* and *F. pinicola*, which typically have relatively weak combative abilities (Holmer and Stenlid, 1993; Holmer et al., 1997; Holmer and Stenlid, 1997), are selected for and rely upon their early arrival in wood. These species may persist in the community for a long time even though they are not aggressive competitors. This view is further supported by the finding that species that had a dominant position in the wood as mycelia had a greater influence on secondary species in the analysis of different life-history traits in paper III. Many species had increased decay rates on wood pre-decayed by *F. pinicola*, suggesting that the early presence of this species causes positive priority effects, whereas the early establishment of *G. sepiarium* may act as a negative priority effect on secondary species given that many were inhibited by the pre-decay of this species (Fig. 8E).

5.3.3 Ecological consequences of interspecific interactions

Species community structure and composition is considered to influence ecosystem functions (Loreau et al., 2001; Hooper et al., 2005) and it is generally acknowledged that decomposer organisms are key drivers of the carbon dynamics in ecosystems (Hättenschwiler et al., 2005; Gessner et al., 2010). The decomposition ability of a decay fungi is determined by the enzymatic attributes of the species and affected by substrate quality and abiotic factors. Hence, the composition and interspecific interactions do affect decomposition rates in fungal communities (van der Wal et al., 2012). However, to link fungal diversity with wood decomposition remains a challenging task, because results are often idiosyncratic and difficult to interpret. Studies on the effect of saprotrophic fungal diversity in terms of both species richness and community composition report both positive (Robinson et al., 1993; Tiunov and Scheu, 2005; Setälä and MacLean, 2004; Lebauer, 2010) and negative (Cox et al., 2001; Fukami et al., 2010b) effects on decomposition rates. Resource partitioning and facilitative interactions in decomposer communities have been put forward as mechanisms explaining a positive diversity effect (Gessner et al., 2010; Hättenschwiler et al., 2005). Furthermore, with an increased number of species, there is a greater chance that the community includes efficient decomposer species (Lebauer, 2010). However, competitive interactions within diverse communities can result in reduced decomposition rates because competition involves metabolic costs (Wells and Boddy, 2002; Gessner et al., 2010). In wood-inhabiting fungal communities, deadlock interactions that result in a compartmentalization of differ-

ent fungal individuals may somehow reduce the costs of competitive interference, as long as there is a competitive balance between two neighboring species under stable environmental conditions. However, as conditions in the wood are dynamic during decomposition, the relative fitness and combative abilities of fungal species constantly changes (Toljander et al., 2006).

Recently, Lindner et al (2011) demonstrated the significant effects of an initial decomposer species on fungal diversity and decomposition. Inoculation of a brown-rot species enhanced decomposition rates and resulted in a different community composition compared with the white-rot community, which was more similar to the non-treated logs. The results presented in this thesis also highlight the impact that brown-rot species have on community composition due to being fast decomposers, long-lived and abundant in Norway spruce wood as well as having an influence on other species in the fungal communities. Brown-rot residues are recalcitrant and long-lived in forest soils; hence, the decay activities of brown-rot fungi have a large input to the soil formation and nutrient dynamics in conifer-dominated forests (Eriksson et al., 1990). More studies are needed to elucidate the relationships and the long-term effects of the contribution made by white- and brown-rot fungi to nutrient turnover and carbon sequestration in forest ecosystems.

6 Synthesis and future directions

This thesis contributes with new data, which increases our understanding of the community development and mycelial life stage of wood-inhabiting fungi. The basis of our present understanding of the ecology of wood-inhabiting fungal communities is based upon observations of the fruiting stage in the fungal life cycle. In this thesis, we have studied fungal communities at different levels, from succession of fruit bodies at several sites, to the fine-scale distribution within logs and interactions between fungal individuals in laboratory microcosms. Put togehter, some interesting patterns emerge.

A main finding of this thesis is the demonstration of species-rich fungal communities in wood representing a wide range of ecological roles. A classification of fungal biodiversity based upon functionally relevant traits is a challenging task in fungal ecology since functional response is not always consistent in different situations (Hättenschwiler et al., 2011). For instance, typical wood decay fungi have been shown to form mycorrhizal-like relationships with conifer seedlings (Vasiliauskas et al., 2007), or species classified as endophytes may become saprotrophs under certain circumstances (Korkama-Rajala et al., 2008). Furthermore, much of the knowledge we have about fungal functions are derived from laboratory experiments, hence studies from field conditions are required in order to connect genetic identity and functional response (McGuire et al., 2010; Lindner et al., 2011).

When compiling the information of ecological data on wood-inhabiting species, it became clear that knowledge of fungal species' ecology is still rudimentary compared to other organisms such as plants or animals. This is particularly true for many ascomycete species. NGS of environmental samples have resulted in a dramatic increase of the number of OTUs of which many cannot be properly assigned to taxonomic identity (Hibbett et al., 2011). To get more out of sequence data from environmental samples, and to be able to communicate fungal diversity found in metagenomic studies, there is a need to develop fungal taxonomy further, both in terms of taxon description but equally important a classification system based upon environmental sequences needs to be established (Taylor, 2011).

In this thesis, high-throughput sequencing was used to study the fine-scale distribution of wood-inhabiting fungi inside wood for the first time. We demonstrated that differences in fungal life-histories are reflected in the way fungal communities are structured inside wood. Species that are known to produce perennial fruit bodies were often dominating the wood samples, in particular the ones that were in early to intermediate stages of decay, and hence contained more energy for fruit body production. The findings in

this thesis supports the view that early established and long-lived species will affect the succeeding fungal community in both direct and indirect ways.

Interspecific interactions within fungal communities can also have consequences on a stand or at a regional level. Modern forestry has created a new substrate for wood-inhabiting fungi, namely cut stumps. Studies suggest that even though many species produce fruit bodies on cut stumps and logs, some species are more common on this new type of substrate compared to natural fallen logs (Berglund et al., 2011a; Nordén et al., 2013). Since cut stumps and logs are nowadays very common on the landscape level, these fungi may experience a positive feed back, as their high abundance in the landscape will enable high production of spores and hence their potential to establish in new substrates (Norros et al., 2012). So far, it is not known how forest management affects the number of genets in different logs and stumps. However, it is likely that if certain species are more common on the landscape level; this will have direct effects on the competitive balance within fungal communities and also within individual logs. As the results of this thesis suggests, priority effects may affect community assembly in decaying wood, and species that have a dominant position inside logs are more prone to affect subsequent species colonization. Also, certain early successional species have good competitive abilities, and this will increase their fitness if they are also abundant in substrates that are common in the landscape.

Molecular tools provide a less biased measure to study fungal communities compared to fruit body inventories as well as the isolation and culturing of mycelia. Molecular methods also allow the opportunity to address questions about fungal life-stages inside logs. We found that individual logs may be suitable habitats for wood-inhabiting fungal species after forming a heterokaryotic mycelium and production of fruit bodies. Few studies have addressed the homokaryotic stage inside logs. Laboratory studies suggest that the homokaryotic mycelium may have better competitive abilities than the heterokaryotic mycelium (Crockatt et al., 2008). Species that are rare in the landscape may have a prolonged homokaryotic stage inside the wood since the probability of mating and forming fruit bodies is low (Crockatt et al., 2008). However, overall, the ecological importance of the homokaryotic mycelium life stage is largely unknown. Another aspect of the fungal life cycle in wood that has been difficult to study is the establishment phase. Recently, Strid (2012) demonstrated that some early successional species are facilitated by the activity of bark-beetles. Furthermore, a trade-off between resource allocation to produce many small spores or few larger spores has

been suggested (Nordén et al., 2013) but so far this relationship has not been confirmed, and it is not known how this is related to the establishment success in wood. Colonization patterns have suggested dispersal limitation at the stand and regional level for some rare wood-decaying fungi (Edman et al., 2004b; Jönsson et al., 2008). Establishment success may be related to the fitness of the spores. Genetic differentiation and low germination rates has been found in fragmented populations of wood-decaying species (Högberg and Stenlid, 1999; Franzén et al., 2007; Edman et al., 2004a) but in general the population genetics of wood-inhabiting fungi is not known and needs more attention.

The relationships between species associations and species interactions found in this thesis stress the importance of a high availability of different types of dead wood substrates, both at a stand and at a regional level for wood-inhabiting fungal diversity (Junninen and Komonen, 2011). One way to promote saproxylic biodiversity in fragmented forest landscapes is to restore habitats by actively creating habitats and substrates that are lacking in the landscape (Stokland et al., 2012). The finding that the way in which trees are broken is associated with different fungal communities a long time after tree death suggest that active restoration practices, such as the creation of different types of logs, could result in different compositions of fungal communities. However, we also noted that the root rot pathogen *Heterobasidion parviporum* was frequently detected in cut logs left as retention logs. Hence, more research is needed to establish which parts of fungal diversity that is promoted in restoration and retention practices, and also where these practices are most efficient. In order to promote saproxylic diversity, it has been suggested that priority effects could be utilized in ecological restoration by actively promoting the diversity of pioneer fungi to create a more diverse array of niches for the successor species (Berglund et al., 2011b). As many pioneer fungi are able to sustain high populations in a fragmented forest landscape (Nordén et al., 2013; Berglund et al., 2011a), an interesting alternative to the active promotion of pioneer species is the direct inoculation of rare specialist species. The success of such inoculations would also bring valuable insights regarding the relevance of priority effects in fungal communities.

References

K. Abarenkov, R. H. Nilsson, K. H. Larsson, I. J. Alexander, U. Eberhardt, S. Erland, K. Hoiland, R. Kjoller, E. Larsson, T. Pennanen, R. Sen, A. F. S. Taylor, L. Tedersoo, B. M. Ursing, T. Vralstad, K. Liimatainen, U. Peintner, and U. Koljalg. The UNITE database for molecular identification of fungi - recent updates and future perspectives. *New Phytologist*, 186(2):281–285, 2010.

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

R. Aerts. The advantages of being evergreen. *Trends in ecology & evolution*, 10(10):402–407, 1995.

T. Ahti, Hämet-Ahti, and J. L, Jalas. Vegetation zones and their sections in Northwestern Europe. *Annalaes Botanici Fennici*, 5:169–211, 1968.

J. Allmér, R. Vasiliauskas, K. Ihrmark, J. Stenlid, and A. Dahlberg. Wood-inhabiting fungal communities in woody debris of Norway spruce (*Picea abies*(L.) karst.), as reflected by sporocarps, mycelial isolations and T-RFLP identification. *FEMS Microbiology Ecology*, 55(1):57–67, 2006.

A. Amend, K. Seifert, R. Samson, and T. Bruns. Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics. *Proceedings of the National Academy of Sciences of the United States of America*, 107:13748–13753, 2010.

N. Arhipova, T. Gaitnieks, J. Donis, J. Stenlid, and R. Vasaitis. Butt rot incidence, causal fungi, and related yield loss in *Picea abies* stands of Latvia. *Canadian Journal of Forest Research*, 41(12):2337–2345, 2011.

P. Baldrian. *Ecology of Saprotrophic Basidiomycetes*, chapter Enzymes of Saprotrophic Basidiomycetes, pages 19–41. Elsevier, 2008.

G. Barron. Predatory fungi, wood decay, and the carbon cycle. *Biodiversity*, 4(1):3–9, 2003.

B. Berg and C. McClaugherty. *Plant Litter - Decomposition, Humus Formation, Carbon Sequestration*. Springer, Berling Heidelberg, 2008.

B. Berg, C. McClaugherty, A. V. D. Santo, and D. Johnson. Humus buildup in boreal forests: effects of litter fall and its N concentration. *Canadian Journal of Forest Research*, 31(6):988–998, 2001.

H. Berglund, M. Edman, and L. Ericson. Temporal variation of wood-fungi diversity in boreal old-growth forests: implications for monitoring. *Ecological Applications*, 15(3):970–982, 2005.

H. Berglund, J. Hottola, R. Penttilä, and J. Siitonen. Linking substrate and habitat requirements of wood-inhabiting fungi to their regional extinction vulnerability. *Ecography*, 34(5):864–875, 2011a.

H. Berglund, M. T. Jönsson, R. Penttilä, and I. Vanha-Majamaa. The effects of burning and dead-wood creation on the diversity of pioneer wood-inhabiting fungi in managed boreal spruce forests. *Forest Ecology and Management*, 261(7):1293–1305, 2011b.

R. A. Blanchette. Delignification by wood-decay fungi. *Annual Review of Phytopathology*, 29(1):381–403, 1991.

R. A. Blanchette. Degradation of the lignocellulose complex in wood. *Canadian Journal of Botany*, 73(1):999–1010, 1995.

J. B. Boberg, K. Ihrmark, and B. D. Lindahl. Decomposing capacity of fungi commonly detected in pinus sylvestris needle litter. *Fungal Ecology*, 4(1):110–114, 2011.

L. Boddy. Saprotrophic cord-forming fungi: Warfare strategies and other ecological aspects. *Mycological Research*, 97(6):641–655, 1993.

L. Boddy. Interspecific combative interactions between wood-decaying basidiomycetes. *FEMS Microbiology Ecology*, 31:185–194, 2000.

L. Boddy. Fungal community ecology and wood decomposition processes in angiosperms: from standing tree to complete decay of coarse woody debris. *Ecological Bulletins*, 49:43–56, 2001.

L. Boddy and J. Heilmann-Clausen. *Ecology of Saprotrophic Basidiomycetes*, chapter Basidiomycete community development in temperate angiosperm wood, pages 211–237. Elsevier, 2008.

I. T. M. Bödeker, C. M. R. Nygren, A. F. S. Taylor, Å. Olson, and B. D. Lindahl. ClassII peroxidase-encoding genes are present in a phylogenetically wide range of ectomycorrhizal fungi. *ISME Journal*, 3(12):1387–1395, 2009.

A. Botha. The importance and ecology of yeasts in soil. *Soil Biology and Biochemistry*, 43(1):1–8, 2011.

P. M. Brock, H. Döring, and M. I. Bidartondo. How to know unknown fungi: the role of a herbarium. *New Phytologist*, 181(3):719–724, 2009.

M. Buée, M. Reich, C. Murat, E. Morin, R. H. Nilsson, S. Uroz, and F. Martin. 454 pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytologist*, 184(2):499–56, 2009.

P. Chesson. Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, 31:343–366, 2000.

R. Cooke and A. D. M. Rayner. *Ecology of Saprotrophic fungi*. Longman, London and New York, 1984.

M.-M. Coüteaux, P. Bottner, and B. Berg. Litter decomposition, climate and liter quality. *Trends in Ecology & Evolution*, 10(2):63–66, 1995.

P. Cox, S. P. Wilkinson, and J. M. Anderson. Effects of fungal inocula on the decomposition of lignin and structural polysaccharides in pinus sylvestris litter. *Biology and Fertility of Soils*, 33(3):246–251, 2001.

M. Crockatt, G. Pierce, R. Camden, P. Newell, and L. Boddy. Homokaryons are more combative than heterokaryons of *Hericium* coralloides. *Fungal Ecology*, 1:40–48, 2008.

A. Dahlberg, G. Thor, J. Allmér, M. Jonsell, M. Jonsson, and T. Ranius. Modelled impact of Norway spruce logging residue extraction on biodiversity in sweden. *Canadian Journal of Forest Research*, 41:1220–1232, 2011.

T. O. Delmont, P. Simonet, and T. M. Vogel. Describing microbial communities and performing global comparisons in the 'omic era. *ISME Journal*, 6(9):1625–1628, 2012.

I. A. Dickie, T. Fukami, J. P. Wilkie, R. B. Allen, and P. K. Buchanan. Do assembly history effects attenuate from species to ecosystem properties? a field test with wood-inhabiting fungi. *Ecology Letters*, 15:133–141, 2012.

D. C. Eastwood, D. Floudas, M. Binder, A. Majcherczyk, P. Schneider, A. Aerts, F. O. Asiegbu, S. E. Baker, K. Barry, et al. The plant cell wall decomposing machinery underlies the functional diversity of forest fungi. *Science*, 333(6043):762–765, 2011.

M. Edman and B. G. Jonsson. Spatial pattern of downed logs and wood-decaying fungi in an old-growth picea abies forest. *Journal of Vegetation Science*, 12(5):609–620, 2001.

M. Edman, M. Gustafsson, J. Stenlid, and L. Ericson. Abundance and viability of fungal spores along a forestry gradient - responses to habitat loss and isolation? *Oikos*, 104(1):35–42, 2004a.

M. Edman, N. Kruys, and B. G. Jonsson. Local dispersal sources strongly affect colonization patterns of wood-decaying fungi on spruce logs. *Ecological Applications*, 14(3):893–901, 2004b.

M. Edman, M. T. Jönsson, and B. G. Jonsson. Fungi and wind strongly influence the temporal availability of logs in an old-growth spruce forest. *Ecological Applications*, 17(2):482–490, 2007.

K.-E. Eriksson, R. Blanchette, and P. Ander. *Microbial and enzymatic degradation of wood components.* Springer-Verlag, Berlin, 1990.

P.-A. Essen, B. Ehnström, L. Ericson, and S. K. Boreal forests. *Ecological Bulletins*, 46:16–47, 1997.

J. A. Evans, C. A. Eyre, H. J. Rogers, L. Boddy, and C. T. Müller. Changes in volatile production during interspecific interactions between four wood rotting fungi growing in artificial media. *Fungal Ecology*, 1 (2-3):57–68, 2008.

D. Floudas, M. Binder, R. Riley, K. Barry, R. A. Blanchette, B. Henrissat, A. T. Martinez, R. Otillar, J. W. Spatafora, J. S. Yadav, et al. The paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science*, 336(6089):1715–1719, 2012.

I. Franzén, R. Vasaitis, R. Penttilä, and J. Stenlid. Population genetics of the wood-decay fungus *Phlebia centrifuga* P. Karst. in fragmented and continuous habitats. *Molecular Ecology*, 16(16):3326–3333, 2007.

T. Fukami. *Community Ecology - Processes, Models and Applications*, chapter Community assembly dynamics in space, pages 45–54. Oxford University Press, Oxford, 2010a.

T. Fukami, I. A. Dickie, J. P. Wilkie, B. C. Paulus, D. Park, A. Roberts, P. K. Buchanan, and R. B. Allen. Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. *Ecology Letters*, 13(6):675–684, 2010b.

Y. Fukasawa, T. Osono, and T. Hiroshi. Wood decomposing abilities of diverse lignicolous fungi on nondecayed and decayed beech wood. *Mycologia*, 103(3):474–482, 2011.

M. O. Gessner, C. M. Swan, C. K. Dang, B. G. Mckie, R. D. Bardgett, D. H. Wall, and S. Hättenschwiler. Diversity meets decomposition. *Trends in Ecology & Evolution*, 25(6):372–380, 2010.

M. Ghannoum, R. Jurevic, P. Mukherjee, F. Cui, M. Sikaroodi, A. Naqvi, and P. Gillevet. Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. *PLoS Pathogens*, 6(1):e1000713, 2010.

N. Hallenberg, R. H. Nilsson, A. Antonelli, S.-H. Wu, N. Maekawa, and B. Nordén. The *Peniophorella praetermissa* species complex (Basidiomycota). *Mycological Research*, 111(12):1366–1376, 2007.

P. Halme and J. Kotiaho. The importance of timing and number of surveys in fungal biodiversity research. *Biodiversity and Conservation*, 21(1):205–219, 2012.

P. Halme, J. S. Kotiaho, A. L. Ylisirniö, J. Hottola, K. Junninen, J. Kouki, M. Lindgren, M. Mönkkönen, R. Penttilä, P. Renvall, J. Siitonen, and M. Similä. Perennial polypores as indicators of annual and red-listed polypores. *Ecological Indicators*, 9(2):256–266, 2009.

C. Hanson, S. Allison, M. Bradford, M. Wallenstein, and K. Treseder. Fungal taxa target different carbon sources in forest soil. *Ecosystems*, 11:1157–1167, 2008.

M. Hartmann, C. G. Howes, D. VanInsberghe, H. Yu, D. Bachar, R. Christen, H. R. Nilsson, S. J. Hallam, and W. W. Mohn. Significant and persistent impact of timber harvesting on soil microbial communities in northern coniferous forests. *The ISME Journal*, 6:2199–2218, 2012.

A. Hatakka and K. Hammel. *The Mycota : a comprehensive treatise on fungi as experimental systems for basic and applied research. X, Industrial applications*, chapter Fungal biodegradation of lignocelluloses, pages 319–340. Springer, Berlin, Heidelberg, second edition, 2010.

S. Hättenschwiler, A. Tiunic, and S. Sheu. Biodiversity and litter decomposition in terrestrial ecosystems. *Annu. Rev. Ecol. Evol. Syst.*, 36:191–218, 2005.

S. Hättenschwiler, N. Fromin, and S. Barantal. Functional diversity of terrestrial microbial decomposers and their substrates. *Comptes Rendus Biologies*, 334(5-6):393–402, 2011.

A. E. Hawkins and T. W. Henkel. Native forest pathogens facilitate persistence of Douglas-fir in old-growth forests of northwestern California. *Canadian Journal of Forest Research*, 41(6):1256–1266, 2011.

J. Heilmann-Clausen and L. Boddy. Inhibition and stimulation effects in communities of wood decay fungi: Exudates from colonized wood influence growth by other species. *Microbial Ecology*, 49(3):399–406, 2005.

D. S. Hibbett, A. Ohman, D. Glotzer, M. Nuhn, P. M. Kirk, and R. H. Nilsson. Progress in molecular and morphological taxon discovery in *Fungi* and options for formal classification of environmental sequences. *Fungal Biology Reviews*, 25:38–47, 2011.

J. Hiscox, P. Baldrian, H. J. Rogers, and L. Boddy. Changes in oxidative enzyme activity during interspecific mycelial interactions involving the white-rot fungus *Trametes versicolor*. *Fungal Genetics and Biology*, 47(6): 562–571, 2010.

N. Högberg and J. Stenlid. Population genetics of *Fomitopsis rosea* – a wood-decay fungus of the old-growth european taiga. *Molecular Ecolgy*, 8(5): 703–710, 1999.

J. Holeksa, T. Zielonka, and M. Zywiec. Modeling the decay of coarse woody debris in a subalpine Norway spruce forest of the West Carpathians, Poland. *Canadian Journal of Forest Research*, 38(3):415–428, 2008.

L. Holmer and J. Stenlid. The importance of inoculum size for the competitive ability of wood decomposing fungi. *Fems Microbiology Ecology*, 12 (3):169–176, 1993.

L. Holmer and J. Stenlid. Diffuse competition for heterogeneous substrate in soil among six species of wood-decomposing basidiomycetes. *Oecologia*, 106(4):531–538, 1996.

L. Holmer and J. Stenlid. Competitive hierarchies of wood decomposing basidiomycetes in artificial systems based on variable inoculum sizes. *Oikos*, 79(1):77–84, 1997.

L. Holmer, P. Renvall, and J. Stenlid. Selective replacement between species of wood-rotting basidiomycetes, a laboratory study. *Mycological Research*, 101:714–720, 1997.

D. U. Hooper, F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä,

A. J. Symstad, J. Vandermeer, and D. A. Wardle. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs*, 75(1):3–35, 2005.

S. H. Hubbell. Neutral theory in community ecology and the hypothesis of functional equivalence. *Functional Ecology*, 19:166–172, 2005.

J. Hynes, C. T. Müller, T. H. Jones, and L. Boddy. Changes in volatile production during the course of fungal mycelial interactions between *Hypholoma fasciculare* and *Resinicium bicolor*. *Journal of Chemical Ecology*, 33(1):43–57, 2007.

A. Iakovlev and J. Stenlid. Spatiotemporal patterns of laccase activity in interacting mycelia of wood-decaying basidiomycete fungi. *Microbial Ecology*, 39(3):236–245, 2000.

H. Johannesson and J. Stenlid. Molecular identification of wood-inhabiting fungi in an unmanaged *Picea abies* forest in sweden. *Forest Ecology and Management*, 115(2-3):203–211, 1999.

M. T. Jönsson, M. Edman, and B. G. Jonsson. Colonization and extinction patterns of wood-decaying fungi in a boreal old-growth *Picea abies* forest. *Journal of Ecology*, 96:1065–1075, 2008.

K. Junninen and A. Komonen. Conservation ecology of boreal polypores: A review. *Biological Conservation*, 144(1):11–20, 2011.

P. G. Kennedy, K. G. Peay, and T. D. Bruns. Root tip competition among ectomycorrhizal fungi: are priority effects a rule or an exception? *Ecology*, 90(8):2098–2107, 2009.

T. Korkama-Rajala, M. M. Müller, and T. Pennanen. Decomposition and fungi of needle litter from slow- and fast-growing Norway spruce (*Picea abies*) clones. *Microbial Ecology*, 56(1):76–89, 2008.

N. Kruys and B.-G. Jonsson. Fine woody debris is important for species richness on logs in managed boreal spruce forests of northern Sweden. *Canadian Journal of Forest Research*, 29(8):1295–1299, 1999.

A. Kulhanková, T. Béguiristain, J. Moukoumi, J. Berthelin, and J. Ranger. Spatial and temporal diversity of wood decomposer communities in different forest stands, determined by ITS rDNA targeted TGGE. *Annals of Forest Science*, 63:547–556, 2006.

R. Laiho and C. E. Prescott. Decay and nutrient dynamics of coarse woody debris in northern coniferous forests: a synthesis. *Canadian Journal of Forest Research*, 34(4):763–777, 2004.

A. Lännenpää, T. Aakala, H. Kauhanen, and T. Kuuluvainen. Tree mortality agents in pristine Norway spruce forests in northern Fennoscandia. *Silva Fennica*, 42(2):151–163, 2008.

S. Lebauer, David. Litter degradation rate and β-glucosidase activity increase with fungal diversity. *Canadian Journal of Forest Research*, 40:1076–1085, 2010.

I. Lindblad. Wood-inhabiting fungi on fallen logs of Norway spruce: relations to forest management and substrate quality. *Nordic Journal of Botany*, 18(2):243–255, 1998.

A. Lindhe, N. Åsenblad, and H.-G. Toresson. Cut logs and high stumps of spruce, birch, aspen and oak—nine years of saproxylic fungi succession. *Biological Conservation*, 119(4):443–454, 2004.

D. L. Lindner, R. Vasaitis, A. Kubartová, J. Allmér, H. Johannesson, M. T. Banik, and J. Stenlid. Initial fungal colonizer affects mass loss and fungal community development in *Picea abies* logs 6 yr after inoculation. *Fungal Ecology*, 4(6):359–460, 2011.

D. Lonsdale, M. Pautasso, and O. Holdenrieder. Wood-decaying fungi in the forest: conservation needs and management options. *European Journal of Forest Research*, 127(1):1–22, 2008.

M. Loreau, S. Naeem, P. Inchausti, J. Bengtsson, J. P. Grime, A. Hector, D. U. Hooper, M. A. Huston, D. Raffaelli, B. Schmid, D. Tilman, and D. A. Wardle. Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science*, 294(5543):804–808, 2001.

K. L. McGuire, E. Bent, J. Borneman, A. Majumder, S. D. Allison, and K. K. Treseder. Functional diversity in resource use by fungi. *Ecology*, 91 (8):2324–2332, 2010.

A. C. Mgbeahuruike, H. Sun, P. Fransson, R. Kasanen, G. Daniel, M. Karlsson, and F. O. Asiegbu. Screening of *Phlebiopsis gigantea* isolates for traits associated with biocontrol of the conifer pathogen *Heterobasidion annosum*. *Biological Control*, 57(2):118–129, 2011.

D. Moore, A. Gange, E. Gange, and L. Boddy. *Ecology of Saprotrophic Basidiomycetes*, chapter Fruit bodies: their production and development in relation to environment, pages 79–102. Elsevier, 2008.

P. J. Morin. *Community Ecology*. Wiley-Blackwell, New Bruswick, 2nd edition, 2011.

T. Niemelä. *Polypores, lignicolous fungi*. Helsinki University Press, Helsinki, 2005.

T. Niemelä, P. Renvall, and R. Penttila. Interactions of fungi at late stages of wood decomposition. *Annales Botanici Fennici*, 32(3):141–152, 1995.

J. Nordén, R. Penttila, J. Siitonen, E. Tomppo, and O. Ovaskainen. Specialist species of wood-inhabiting fungi struggle while generalists thrive in fragmented forests. *Journal of Ecology (In press)*, 2013.

V. Norros, R. Penttilä, M. Suominen, and O. Ovaskainen. Dispersal may limit the occurrence of specialist wood decay fungi already at small spatial scales. *Oikos*, 121(6):961–974, 2012.

J. Olsson, B. G. Jonsson, J. Hjältén, and L. Ericson. Addition of coarse woody debris - the early fungal succession on *Picea abies* logs in managed forests and reserves. *Biological Conservation*, 144(3):1100–1110, 2011.

O. Ovaskainen, J. Hottola, and J. Siitonen. Modeling species co-occurrence by multivariate logistic regression generates new hypotheses on fungal interactions. *Ecology*, 91(9):2514–2521, 2010.

K. G. Peay, P. G. Kennedy, and T. D. Bruns. Fungal community ecology: A hybrid beast with a molecular master. *Bioscience*, 58(9):799–810, 2008.

K. G. Peay, M. Belisle, and T. Fukami. Phylogenetic relatedness predicts priority effects in nectar yeast communities. *Proceedings of the Royal Society B: Biological Sciences*, 279(1729):749–758, 2012.

R. Penttilä. *The impacts of forestry on polyporous fungi in boreal forests*. PhD thesis, 2004.

Y. Persson, R. Vasaitis, B. Långström, P. Öhrn, K. Ihrmark, and J. Stenlid. Fungi vectored by the bark beetle *Ips typographus* following hibernation under the bark of standing trees and in the forest litter. *Microbial Ecology*, 58(3):651–659, 2009.

Y. Persson, K. Ihrmark, and J. Stenlid. Do bark beetles facilitate the establishment of rot fungi in Norway spruce? *Fungal Ecology*, 4(4):262–269, 2011.

E. Pippola and H. Kotiranta. The genus *Tremella* (Basidiomycota, Tremellales) in Finland. *Annales Botanici Fennici*, 45401-434, 2008.

R. A. Progar, T. D. Schowalter, C. M. Freitag, and J. J. Morrell. Respiration from coarse woody debris as affected by moisture and saprotroph functional diversity in Western Oregon. *Oecologia*, 124(3):426–431, 2000.

C. Pyle and M. M. Brown. Heterogeneity of wood decay classes within hardwood logs. *Forest Ecology and Management*, 114(2-3):253–259, 1999.

T. Rajala, M. Peltoniemi, T. Pennanen, and R. Mäkipää. Relationship between wood-inhabiting fungi determined by molecular analysis (denaturing gradient gel electrophoresis) and quality of decaying logs. *Canadian Journal of Forest Research*, 40(12):2384–2397, 2010.

T. Rajala, M. Peltoniemi, J. Hantula, R. Mäkipää, and T. Pennanen. RNA reveals a succession of active fungi during the decay of Norway spruce logs. *Fungal Ecology*, 4(6):437–448, 2011.

T. Rajala, M. Peltoniemi, T. Pennanen, and R. Mäkipää. Fungal community dynamics in relation to substrate quality of decaying Norway spruce (*Picea abies* [L.] Karst.) logs in boreal forests. *FEMS Microbiology Ecology*, 81(2):494–505, 2012.

A. D. M. Rayner and L. Boddy. *Fungal decomposition of wood: its biology and ecology*. A Wiley-Interscience publication. John Wiley & Sons, Chichester, 1988.

P. Renvall. Community structure and dynamics of wood-rotting Basidiomycetes on decomposing conifer trunks in northern Finland. *Karstenia*, 35:1–51, 1995.

C. Robinson, J. Dighton, J. Frankland, and P. A. Coward. Nutrient and carbon dioxide release by interacting species of straw-decomposing fungi. *Plant and soil*, 151(1):139–142, 1993.

J. Rothberg and J. Leamon. The development and impact of 454 sequencing. *Nature Biotechnology*, 10:1117–1124, 2008.

J. Rousk, E. Baath, P. Brookes, C. Lauber, C. Lozupone, J. Caporaso, R. Knight, and N. Fierer. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME Journal*, 4:1340–1351, 2010.

C. L. Schoch, K. A. Seifert, S. Huhndorf, V. Robert, J. L. Spouge, C. A. Levesque, W. Chen, and C. Fungal Barcoding. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. *Proceedings of the National Academy of Sciences*, 109(16):6241–6246, 2012.

F. Schwarze. Wood decay under the microscope. *Fungal Biology Reviews*, 21(4):133–170, 2007.

F. Schwarze, J. Engels, and C. Mattheck. *Fungal Strategies of Wood Decay in Trees*. Springer, Berlin, Heidelberg, 2000.

H. Setälä and M. MacLean. Decomposition of organic substrates in relation to the species diversity of soil saprophytic fungi. *Oecologia*, 139:98–107, 2004.

J. Stenlid, R. Penttilä, and A. Dahlberg. *Ecology of Saprotrophic Basidiomycetes*, chapter Wood-decay Basiodiomycetes in boreal forests: distribution and community development, pages 239–262. Elsevier, 2008.

J. Stokland and E. Meyke. The saproxylic database: an emerging overview of the biological diversity in dead wood. *Revue d'Ecologie (Terre Vie)*, 63: 29–40, 2008.

J. Stokland, J. Siitonen, and B.-G. Jonsson. *Biodiversity in Dead Wood*. Ecology, Biodiversity and Conservation. Cambridge University Press, Cambridge, 2012.

Y. Strid. *Bark beetles facilitate the establishment of wood decay fungi*. PhD thesis, Swedish University of Agricultural Sciences, 2012.

J. W. Taylor. One fungus = one name: DNA and fungal nomenclature twenty years after PCR. *IMA Fungus*, 2:113–120, 2011.

L. Tedersoo, U. Köljalg, N. Hallenberg, and K.-H. Larsson. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. *New Phytologist*, 159(1): 153–165, 2003.

D. Tilman. Competition and biodiversity in spatially structured habitats. *Ecology*, 75(1):2–16, 1994.

A. V. Tiunov and S. Scheu. Facilitative interactions rather than resource partitioning drive diversity-functioning relationships in laboratory fungal communities. *Ecology Letters*, 8(6):618–625, 2005.

M. Tlalka, D. Bebber, P. Darrah, and S. Watkinson. *Ecology of Saprotrophic Basidiomycetes*, chapter Mycelial networks: nutrients uptake, translocation and role in ecosystems., pages 43–62. Elsevier, 2008.

Y. Toljander, B. Lindahl, L. Holmer, and N. Högberg. Environmental fluctuations facilitate species co-existence and increase decomposition in communities of wood decay fungi. *Oecologia*, 148(4):625–631, 2006.

S. Tzean and J. Liou. Nematophagous resupinate basidiomycetous fungi. *Phytopathology*, 83:1015–1020, 1993.

E. J. Vainio and J. Hantula. Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. *Mycological Research*, 104(8):927–936, 2000.

A. van der Wal, T. D. Geydan, T. W. Kuyper, and W. de Boer. A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition processes. *FEMS Microbiology Reviews*, pages 1–18, 2012.

R. Vasaitis. *Infectious Forest Diseases*, chapter Heart Rots, Sap Rots and Canker Rots. CAB International, 2013.

R. Vasiliauskas, A. Menkis, R. D. Finlay, and J. Stenlid. Wood-decay fungi in fine living roots of conifer seedlings. *New Phytologist*, 174(2):441–446, 2007.

T. Vetrovsky, J. Vorisková, J. Snajdr, J. Gabriel, and P. Baldrian. Ecology of coarse wood decomposition by the saprotrophic fungus *Fomes fomentarius*. *Biodegradation*, 22(4):709–718, 2011.

H. Wallander, U. Johansson, E. Sterkenburg, M. B. Durling, and B. D. Lindahl. Production of ectomycorrhizal mycelium peaks during canopy closure in Norway spruce forests. *New Phytologist*, 187(4):1124–1134, 2010.

M. Wedin, H. Döring, and G. Gilenstam. Saprotrophy and lichenization as options for the same fungal species on different substrata: environmental plasticity and fungal lifestyles in the *Stictis–Conotrema* complex. *New Phytologist*, 164(3):459–465, 2004.

J. M. Wells and L. Boddy. Interspecific carbon exchange and cost of interactions between basidiomycete mycelia in soil and wood. *Functional Ecology*, 16(2):153–161, 2002.

T. White, T. D. Bruns, S. Lee, and J. Taylor. *PCR Protocols: a Guide to Methods and Applications*, chapter Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics., pages 315–322. Academic Press, San Diego, 1990.

S. Woodward and L. Boddy. *Ecology of Saprotrophic Basidiomycetes*, chapter Interactions between saprotrophic fungi, pages 125–141. Elsevier, 2008.

J. J. Worrall, S. Anagnost, and R. Zabel. Comparison of wood decay among diverse lignicolous fungi. *Mycologia*, 89(2):199–219, 1997.

A.-L. Ylisirniö, H. Berglund, T. Aakala, T. Kuuluvainen, A.-M. Kuparinen, Y. Norokorpi, V. Hallikainen, K. Mikkola, and E. Huhta. Spatial distribution of dead wood and the occurrence of five saproxylic fungi in old-growth timberline spruce forests in northern Finland. *Scandinavian Journal of Forest Research*, 24(6):527–540, 2009.

T. Young, J. Chase, and R. Huddleston. Community succession and assembly: Comparing, contrasting and combining paradigms in the context of ecological restoration. *Ecological Restoration*, 19(1):5–18, 2001.

W. Zugmaier, R. Bauer, and F. Oberwinkler. Mycoparasitism of some *Tremella* species. *Mycologia*, 86(1):49–56, 1994.

Acknowledgements

Imagine you are working a long day in the forest, you start to sing a song you learnt when you were, say eight, and suddenly you realize you are drilling logs in the rain with the man who wrote the lyrics of this song you have been humming at for twenty years. Janne, you get a lot of great things done that become classics in many different fields. That is probably because you always see possibilities and interesting patterns no one else can spot, both in the big and the molecular. Thank you for opening the doors to Mykopaten for me, for introducing me to the part of the fungal life where all the action takes place, and for allowing me to follow different paths and giving me the opportunity to work as a scientist. Also thanks for teaching me Nydalspojkarnas ovanifrån. It has been handy to know that one too.

There will not be many opportunities to meet a person like you Anders. Well, it is probably a once in a lifetime experience. You are intelligent, fun, critical, tall and you also make excellent lentil-soup. You make everyone feel welcome and special, and you always have five minutes. I'm sorry that you had to read all those texts so many times, in such late hours. However I truly enjoyed the writing and brainstorming, and I also really much appreciate how its always "högt i tak" when talking to you. Thanks for your contagious enthusiasm, positive thinking and everlasting support.

Otso, during the hours of analyzing the time-series data I often wondered how it would sound if one should attach a cable from your keyboard to a piano or something else: How great music you would make with that genius mind and rapid fingers! Thank you for welcoming me at MRG, for the grand excursion with twenty modelers to Lammi and for explaining and running those analyses just one more time.

Polypores are beautiful things, as long as they stay put on the log. But when it comes to Corticoids its a different story. Thank you Karl-Henrik for unravelling this hidden world of star-formed spores, gloeocystidia and ampullate septa during my visits in Göteborg and Oslo. You have such an extraordinary sharp eye for the details that make all the difference. And you ask the right questions (the tricky ones).

It is great to be surrounded with inspiring colleagues that can help you out and remind you again why you are doing all this. Thanks for that Ariana. You were a great support in the lab, with data analysis and you carried the field equipment when I drilled the wasp nest at site F, and fed my cats! Thanks Jenni for all the work you put into this thesis, sharing your knowledge on polypores and forest, and letting me stay at Hylocomium splendens for such a long time, and most of all for writing those e-mails which always makes me happy.

During this thesis work I had the opportunity to meet with some of the best polypore persons! Thank you Mari, Mattias, Anders, Jörgen and Reijo for sharing your field-sites, data and expertise! A special thanks Mari for the extra trip to Skoterhotellet (I mean Gardfjället).

Mykopaten is a great place to work in thanks to the exciting research and the friendly and inspiring people (and the cupboard with the dusty dry old specimens inside). Thanks all old and new PhD-students, it's been great to be part of the gang! Good luck to all of you! Stina you have been a great long-time roomy, I have enjoyed our philosophical chats and your far-out sense of humor! Lunch walks, pep-talks, laughs and strong food were never far away - thanks for that Anna, Ylva, Srivatsha, Srisailam and Inga. Thanks Natalija for late night coffee breaks! Mykopaten would not be running without Karin, Erica, Katta, Maria and Rena. Thanks for all help! Petra, you have been my mentor in life, science and vampire TV-shows, and Björn L you took me through the 454 and decomposition jungle with chats and great courses. Thanks Mikael for being my SCATA-therapist and Rimvys and Audrius for advice and letting me use your isolates! Kalle, Fredrik, Kerstin, Cajsa, Johanna, Anna, Matt, Nick, Jonás, Malin, Åke, Nisse, Elna and Shahid; thanks for lunches, chats, parties, banjo-picking, and knitting patterns.

Through my fascination for fungi I have met many persons, which also helped me a great deal throughout this thesis work. Dmitry, I am amazed of your vast polypore, beetle and Fandorin knowledge. Thanks for the Tver-Moscow-trip, Pisa-trip and all the Polypore-trips to come. Thanks also to Pekka for help with isolates and good company, Bettan, Martin, Johan for cheering and corticoiding and Ismo for letting me stay at your house during many of my stops in Helsinki! Thanks Micke for lending me your great pics, and Sven-Gunnar for great help at Fytoteket, EBC. At last, thank you Björn N for all your support.

Uppsala var ju inte så tokigt, med tanke på alla fina vänner som fanns här. Tack Helena, Joel, Bodil, Vita, Anna, Jonas, Kranzen och Dans-Pelle för att ni har fått mig att tänka på annat. Tack fina vännerna Erica, Majsan, Åsa och Marie i Göteborg för att ni står ut med allt flyttande.

Jag vet inte hur jag ska tacka min familj. Ni har stöttat mig så otroligt mycket. Eva och KG, tack för att ni har överseende med trästockar i trädgården. Magnus, Tobbe, Runa och Erik. Jag hoppas att vi ses mer snart! Mamma, Pappa och Karin för att ni alltid finns där.

Meklaf, tack för alla koppar kaffe, din superba matlagning, uppmuntran, pepp, och all hjälp! Det hade aldrig gått utan dig. Jag lovar att bli en roligare fru nu.