Drivers of Soil Fungal Communities in Boreal Forests

Feedbacks on Soil Fertility and Decomposition

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Cover: Experimental plot where roots have been severed
(Photo: Erica Sterkenburg), soil core from which DNA was extracted and
fungal communities assessed (Photo: Anders Dahlberg)

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Drivers of Soil Fungal Communities in Boreal Forests – Feedbacks on Soil Fertility and Decomposition

Abstract

Boreal forests harbour diverse fungal communities with decisive roles in decomposition and plant nutrition. Difficulties in studying soil fungi have limited knowledge about how fungal communities are shaped. The objective of this thesis was to study factors influencing soil fungal communities, aiming for increased understanding of their effect on environmental processes.

Using next generation sequencing, responses of fungal communities to their physical-chemical environment, and responses of ectomycorrhizal (ECM) fungi to logging, were investigated. In a trenching experiment, this technology, combined with measurements of decomposition and vertical nitrogen distribution, enabled evaluation of direct and indirect involvement of ECM fungi in humus decomposition.

Fungal community composition was found to be significantly related to soil fertility, with ascomycetes dominating in less fertile forests, whereas basidiomycetes increased under more fertile conditions. ECM fungi were found to more or less disappear with complete clear-cutting and reestablishment of ECM diversity took several decades. However, a clear positive relationship between the amount of retention trees and ECM fungal species richness and abundance was found. By excluding ECM fungi, nitrogen limitation of saprotrophic fungi was released, increasing litter decomposition rates. However, this effect was overshadowed by an almost complete loss of oxidative enzyme activities in deeper humus layers, associated with removal of ECM fungi by trenching.

Our results indicate ECM fungi to be the principal decomposers of boreal forest humus layers. This, together with the predictability of soil fungal communities, reinforces the importance and ability of integrating rhizosphere microorganisms, in particular ECM fungi, in forest ecosystem models.

Keywords: Mycorrhiza, Decomposition, Gadgil effect, Forestry, Tree-retention, Ecosystem fertility, High-throughput sequencing, Ergosterol

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Dedication

Till Pappa

Contents

List of Publications

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

I Wallander, H., Johansson, U., Sterkenburg, E., Brandström Durling, M., Lindahl, BD. (2010). Production of ectomycorrhizal mycelium peaks during canopy closure in Norway spruce forests. *New Phytologist* 187, 1124-1134.

II Sterkenburg, E., Bahr, A., Brandström Durling, M., Clemmensen, KE. Lindahl, BD. (2015). Changes in fungal communities along a boreal forest soil fertility gradient. *New Phytologist* 207, 1145-1158.

III Sterkenburg, E., Clemmensen, KE., Lindahl, BD., Dahlberg, A. The significance of tree retention for ectomycorrhizal fungi in managed Scots pine forests (manuscript).

IV Sterkenburg, E., Clemmensen, KE., Ekblad, A., Finlay, RD., Lindahl, BD. Ectomycorrhizal fungi drive long-term humus decomposition but restrain short-term litter decomposition (manuscript).

The contribution of Erica Sterkenburg to the papers included in this thesis was as follows:

I Performed the laboratory DNA work.

II Planned the study together with supervisors. Collected samples and performed the laboratory work. Analysed the data and performed statistical analyses. Wrote the manuscript together with supervisors and input from co-authors. Responsible for correspondence with the journal.

III Collected samples. Analysed the data and performed statistical analyses. Wrote the manuscript together with supervisors.

IV Planned the study together with supervisors and responsible for setting up the experiment. Collected samples and performed the laboratory work. Analysed the data and performed statistical analyses. Wrote the manuscript with input from supervisors.

Abbreviations

C	Carbon
CCA	Canonical correspondence analysis
DCA	Detrended correspondence analysis
ECM	Ectomycorrhiza
F1	Defined as top 2/3 of F-layer
F2	Defined as bottom 1/3 of F-layer
H1	Defined as top 2/3 of H-layer
H2	Defined as bottom 1/3 of H-layer
ITS	Internal transcribed spacer of rDNA
Lm	Defined as moss litter layer
Ln	Defined as needle litter layer
MnP	Manganese peroxidase
N	Nitrogen
NH_4^+	Ammonium
OM	Organic matter
PCA	Principal component analysis
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
SH	Species hypothesis
SMRT	Single molecule real time (sequencing)

1 Background

1.1 Boreal forests

The northern hemisphere is to a major part covered by boreal forests (Bonan & Shugart, 1998). These forests act as a global sink of atmospheric carbon (C), with the main part sequestered below ground (Pan *et al.,* 2011). The soil is commonly acidic and poor in mineral nutrients, whereof nitrogen (N) generally limits primary production (Vitousek & Howarth, 1991). The dominant vegetation is coniferous trees, often with an understory vegetation of ericaceous plants, which produce recalcitrant litter with a high content of lignin and phenolic compounds (Aerts, 1995). Decomposition of litter in the boreal forests is negatively affected by its high content of recalcitrant compounds and low N levels. Together with climatic factors, this leads to an accumulation of organic matter in the soil (Swift *et al*., 1979). Further, the low pH constitutes a harsh environment for many soil animals (e.g. Haimi & Einbork 1992) with the consequence that the soil does not get mixed by e.g. earthworm, resulting in stratification of soil organic matter at different stages of decomposition.

Within the boreal forest biome there are ecological gradients, where for example, nutrient availability, hydrology and soil acidity are the principal and usually co-varying environmental determinants of plant communities (Lahti & Väisänen, 1987). Already in 1926, Cajander defined a forest site type classification based on the plant community of boreal forests in Finland, which ranges from heath-like *Pinus* forests to herb rich *Picea* forests (Cajander, 1926). This classification has been used for estimating the potential productivity of different sites. Since forest types differ quite dramatically between the different ends of this gradient, the "boreal forest" is not a uniform habitat, but rather a mosaic of habitats with similar features.

1.2 Soil fungi and boreal forests

In the boreal forests, soil fungi play a critical role in the cycling of nutrients and C, as symbionts of woody plants and as decomposers of organic matter. Symbiotic mycorrhizal fungi are mediators of plant nutrient uptake, providing their host plants with soil-derived N and other nutrients, receiving recently photosynthesised C in return. Saprotrophic fungi, on the other hand, are the principal decomposers of organic matter and acquire C via degradation of plant litter, thereby recycling C to the atmosphere (Smith & Read, 2008). The two main fungal phyla are Ascomycetes and Basidiomycetes, both including a wide range of species with important roles in biogeochemical transformation and ecological interactions.

1.2.1 Mycorrhizal fungi

Ectomycorrhizal (ECM) fungal communities in boreal forests are highly diverse (Dahlberg, 2001) and almost all fine roots of boreal forest trees are colonized by ECM fungi. A major fraction of the microbial C in boreal forest soil is accounted for by ECM fungi (Högberg *et al*., 2002), and this functional group, together with ericoid mycorrhizal fungi, contributes significantly to long-term C sequestration (Clemmensen *et al*., 2013). The life span of individual ECM fungal genotypes varies, some only live for one growing seasons, while other become very old, potentially exceeding their host tree in age if there is a continuity of living trees at the site (Douhan *et al.* 2011). Some ECM fungal species appear to consist of multiple small, genetically distinct mycelia within a few decimetres, while other species seem to consist of few but large genotypes that may extend 10-50 m (Douhan *et al.* 2011). Large ECM fungal mycelial genotypes may associate with several trees and, as other groups of clonal organisms, potentially fragment into several physically distinct and independent but genetically identical mycelia that may re-form larger units (Beiler *et al.* 2010). ECM fungal individuals may be looked upon both as macroorganisms and microorganisms as they may vary in size from a few hyphae to mycelial networks of several tens of meters (Bahram *et al.,* 2015).

ECM fungi basically consist of three different structures. Around the tip of the host root there is a mantle built up by fungal tissue. The fungus penetrates the root with a net of hyphae called the Hartig's net. In order to get in contact with resources in the surrounding soil, the fungus have a system of emanating hyphae, the extramatrical mycelium. The extrametrical mycelium grows either as simple scattered hyphae from the mantle into the soil or it can be united into undifferentiated rhizomorphs. The rhizomorphs can either have a small reach or be highly organized into root like organs with vessel like hyphae for

efficient water and nutrient transport from distances of decimetres. Depending on the shape and structure of the emanating hyphae, ECM fungi have been described in terms of exploration types (Agerer, 2001). *The contact exploration type* is characterized by a smooth mantle and only a few emanating hyphae. Emanating hyphae, where present, are often in close contact with e.g. dead leaves. The *short-distance exploration type* has a voluminous envelope of emanating hyphae, but rhizomorphs are not found. *Medium-distance exploration type* form rhizomorphs and can be divided into three subtypes; *Fringe subtype* (form fans of emanating hyphae and rhizomorphs which ramify and interconnect repeatedly), *Mat subtype* (individual mycorrhizae have rather limited range of exploration and their rhizomorphs are undifferentiated) and *Smooth subtype* (rhizomorphs are internally undifferentiated, with a central core of thick hyphae. ECM fungal mantles appear rather smooth with almost no emanating hyphae). *Long distance exploration type* is characterized by rather smooth ECM fungal tips with few but highly differentiated rhizomorphs (Agerer, 2001).

Traditionally, ECM fungi have been viewed as a passive extension of root systems, mobilizing inorganic compounds from the soil solution. However, there is now increasing evidence that ECM fungi may act as decomposers in order to obtain nutrients (Lindahl & Tunlid, 2015). Many ECM fungi possess manganese-peroxidase encoding genes (Bödeker *et al.,* 2009) and a significant co-localization of high peroxidase activity and DNA from *Cortinarius* species has been found (Bödeker *et al.,* 2014). These enzymes are believed to play a major role in the degradation of recalcitrant soil organic matter (Sinsabaugh, 2010).

An established view of the N cycle is that saprotrophic fungi decompose soil organic matter and release access N into the soil, where ECM fungi can take up mineralized N and allocate it to their host. By being able to actively decompose and take up organically bound N, ECM fungi could short cut traditional pathways of N cycling (Lindahl *et al.,* 2002; Read and Perez-Moreno, 2003; Orwin *et al.,* 2011).

Most ECM fungi are basidiomycetes whereas ascomycetes dominate among ericaceous mycorrhizal fungi. Ericoid mycorrhiza is formed between fungi and members of the plant family *Ericaceae.* While basidiomycetes are relatively better competitors for space (Boddy, 2000), with many species possessing the ability to produce a potent repertoire of degrading enzymes (Floudas *et al*., 2012; Bödeker *et al.,* 2014), the ericoid mycorrhizal symbiosis represents an important adaptation to acidic and nutrient poor soils (Cairney and Meharg, 2003).

1.2.2 Saprotrophic fungi

Litter decomposition in boreal forests is to 95% carried out by microorganisms, e.g. bacteria and fungi (Persson *et al.,* 1980). Due to the acidic and nutrient poor conditions, together with the recalcitrant litter associated with boreal forest soil, fungi generally dominate the microbial biomass and respiration (Högberg *et al.,* 2007; Ekblad & Nordgren, 2002). Because of their production of ligninolytic enzymes essential for degradation of recalcitrant plant material, basidiomycetous litter fungi are considered especially important (Osono & Takeda, 2002). In addition to ligninolytic basidiomycetes, needle litter is frequently colonised by ascomycetes (mainly Leotiomycetes; Lindahl *et al.,* 2007). These ascomycetes generally have much lower decomposition capacity than basidiomycetes (Boberg *et al.,* 2011), but may be hypothesised to be more tolerant with respect to N deficiency and other stress factors.

Yeasts and moulds are short-lived saprotrophic fungi, with rapid growth and primarily asexual reproduction. Species mostly belong to the orders Eurotiales, Hypocreales, Morteriellales, Mucorales, Saccharomycetales, Tremellales and Sporidiales.

1.3 Community ecology

The projects in this thesis concern different factors shaping mycorrhizal and saprotrophic fungal communities in boreal forest soil. In ecology, a community is an assemblage of two or more species, occupying the same geographical area in a particular time. Organisms possess distinct traits, of which some influence their response to the environment and thereby influence how well they succeed in a particular community. Such traits are referred to as *response traits* (Lavorel and Garnier, 2002). For fungi, *preference* of N source (e.g. inorganic NH_4^+ or organically bound N) is an example of a response trait (Koide *et al*., 2013) that likely will influence the community composition under nutrient limited conditions. Another example is *tolerance* to low pH, probably an important trait for survival in the acidic boreal forest soil.

In turn, some traits of an organism affect environmental processes at different scales, these are referred to as *effect traits* (Lavorel and Garnier, 2002). An example of an effect trait is the ability of some ECM fungi to produce degrading enzymes to access organic N (Bödeker *et al*., 2009; 2014), which may affect the decomposition rate of the organic matter pool, and thereby nutrient and C cycles at ecosystem scale. A second example is mycelial persistence after death, which may affect the decomposability and thereby size of the organic matter pool. Depending on the context, some traits may act as both response and effect trait at the same time (Koide *et al.,* 2013).

Depending on their traits, species may be categorized according to different life strategies, favoured by different environmental conditions (Grime, 1977). Grime (1977) proposed plant evolution to be associated with the emergence of three primary life strategies; a theory which has been further adapted to fungi by Cooke and Rayner (1984). Below I outline these strategies, but also include hypotheses regarding which functional groups of fungi that will predominate within respective strategy.

The *stress-tolerant strategy* (S-strategy) predominates under continuously stressful but undisturbed conditions, such as low resource availability or harsh physical-chemical environment. Organisms associated with this strategy are slow growing and hardy. As in evergreen plants, long-lived mycelial structures may enable more economic resource utilization. To retain mycelium for a long time, fungi have to protect themselves against fungivores and harsh abiotic conditions, e.g. by impregnation of cell walls with melanin and hydrophobic compounds (Ekblad *et al*., 2013; Koide *et al*., 2013; Fernandez & Koide, 2014).

The *competitive strategy* (C-strategy) predominates under high resource availability and relatively undisturbed conditions. For fungi, such conditions would involve high and continuous availability of C via mycorrhizal symbiosis or decomposable organic matter. Comparing two of the major phylogenetic groups of fungi (ascomycetes and basidiomycetes), there is a difference in ability to succeed in environments where C-strategic attributes are favoured. Basidiomycetes are relatively better competitors than ascomycetes (Boddy, 2000) and many also have the ability to produce a potent repertoire of degrading enzymes in order to access resources (Floudas *et al*., 2012; Bödeker *et al.,* 2014). By combining high combative strength with efficient substrate utilization and sometimes ECM symbiosis, basidiomycetes may maximize the efficiency by which they exploit available resources and convert them to biomass.

Finally, the *ruderal strategy* (R-strategy) is favoured in severely disturbed but potentially productive habitats with high resource availabilities. At high fertility, tree and understory species composition in boreal forests shifts to a higher contribution of deciduous species that shed litter seasonally (e.g. *Vaccinium myrtillus* and herbs), resulting in flushes of easily available resources into the soil. Furthermore, fertile soils with higher pH usually constitute a better environment for many soil animals (e.g. Haimi & Einbork 1992), which disturb the soil by mixing and grazing, preventing establishment of large and long-lived mycelial networks (Butenschoen *et al*., 2007; Crowther *et al*., 2013). In such disturbed and fluctuating environments, short-lived fungi

with rapid growth and asexual reproduction, such as yeasts and moulds, should be favoured together with bacteria.

The *fundamental niche* of an organism is shaped by the set of response traits involved in responses to the physical-chemical environment. This niche constitutes the full range of environmental conditions where an organism has the ability to survive. Factors that define the boundaries for this niche can be pH, water, N availability, presence of symbiotic partner etc. However, even though the conditions potentially permit establishment and growth, the distribution of the species may be limited due to competition from other, better adapted, species. This restricted fundamental niche is called the *realized niche* and defines the habitat where an organism actually lives.

Thus, in order to defend or expand the realized niche and accompanying resources, an organism needs to compete with other organisms. There are basically two different kinds of competition. *Interference competition* occurs when one individual directly affects another. It may appear as physical attack, threat behaviour, chemical poisoning or territoriality. *Exploitation competition* on the other hand, occurs when the effects are indirect, and only through reduction of the common available pool of resources (Keddy, 2001). While interference competition foremost is associated with the C-strategy, exploitation competition is connected to the R-strategy.

1.4 Community ecology and soil fungi

As mentioned, the projects in this thesis concern different factors shaping the mycorrhizal and saprotrophic fungal communities in boreal forest soil – both how environmental factors influence the fundamental niche of fungi, but also how fungi constrain the realized niches of each other. Some traits enable fungi to survive under stressful conditions, while others make them strong competitors for e.g. space and resources. Yet other traits will, in turn, affect the environment, such as mycelial persistence after death (organic matter input) or ability to produce enzymes that decompose organic matter (organic matter loss). Below, I will shortly address some factors shaping fungal communities that have been investigated in this thesis and in an additional section, how fungi may change environmental processes via effect traits.

1.4.1 Environmental gradients and fungi

If the conditions for plant growth changes e.g. along global or regional gradients, it is reasonable to believe that fungal communities will be affected. On a global scale, Read (1991) proposed that there is a gradient from the tundra with plants associating with ericoid mycorrhiza, via coniferous forests

with ECM fungal associating plants and finally to broad leave forests and grasslands that are dominated by plants associating with arbuscular mycorrhiza.

On a regional scale, within the boreal forests, there are gradients of shifting nutrient availability, pH, hydrology etc. (e.g. Lahti & Väisänen, 1987). Moving from one end of an environmental gradient to the other, the habitat can change quite dramatically.

The relative dominance of mycorrhizal fungi and saprotrophs has been hypothesised to change along a gradient in soil fertility, due to changed plant allocation of photosynthesis products between roots and leaves (Högberg *et al*., 2003). Potentially, at low soil fertility, mycorrhizal fungi may exchange root-derived C for soil nutrients, and the plant should preferentially allocate any surplus of C belowground. Mycorrhizal fungi have, thus, been proposed to out-compete saprotrophic fungi from the humus layers in less fertile boreal forests, restricting the saprotrophic niche to the uppermost litter layer (Lindahl *et al*., 2007; Clemmensen *et al*., 2015). With improved nutrient availability, trees shift production above ground (Janssens *et al*., 2010), resulting in lower relative C allocation to roots (Högberg *et al*., 2010) with expected negative effects for mycorrhizal fungi due to C limitation. In line with this hypothesis, nitrogen deposition (Nilsson *et al*., 2007; Kjøller *et al*., 2012) and N fertilization (Nilsson and Wallander, 2003; Högberg *et al*., 2006; Högberg *et al*., 2011) usually results in repressed mycorrhizal mycelial production and biomass.

Within the ECM fungal community, it is likely that C-strategic species dominates under nutrient poor conditions, as C allocation to roots is high. Under these conditions, species producing rhizomorphs that may allocate N from scarce patchy recourses, and species producing organic matter degrading enzymes should be favoured. As N levels are increased, there will be less C allocation to roots, favouring fungi with high C-use efficiency. Here, short and contact exploration types should have a competitive advantage.

By studying a N deposition gradient in Alaska, Lilleskov *et al.,* (2002) found a decline in belowground ECM species richness when moving from sites with lower N levels to sites with higher levels of N (closer to an anthropogenic point source). They also found a shift in species composition, where some ECM taxa disappeared completely at sites with higher N levels. Changes in species composition have also been observed along a short (90 m) natural gradient of N availability in Sweden (Toljander *et al.,* 2006). Further, by studying a N gradient in Europe, where the sites had been exposed to different levels of atmospheric N deposition, Taylor *et al.,* (2000) found decreased abundance of ECM species with high capacity to use complex organic N

sources. As N levels increases, there will be higher levels of inorganic N in the soil (e.g. NH_4^+) and having traits associated with organic matter decomposition (e.g. production of manganese peroxidases) will be of less competitive importance. A later review by Lilleskov *et al.,* (2011) presented evidence that ECM species with hydrophobic mycelium of the medium distance fringe exploration type (*Tricholoma, Cortinarius, Piloderma)* were especially sensitive to high inorganic N levels. By contrast, species with hydrophilic mycelium of the contact, short-distance and medium-distance exploration types (e.g. *Russula, Lactarius, Laccaria, Tylospora, Thelephora, Tomentella*) had mixed or positive responses to N deposition. High levels of inorganic N being toxic to certain species may induce shifts in fungal communities, probably together with competition from species adapted to perform well under nutrient rich conditions. Along an ecological gradient of for example N availability or pH, a species will have a tolerance span, the fundamental niche, where it can survive. Somewhere along the gradient, within the fundamental niche, there will be an optimum, where the species has the best possibility of competition and survival.

1.4.2 Spatial separation of functional groups of fungi

In boreal forest soils, saprotrophic and ECM fungal communities are spatially separated. Saprotrophs are mainly restricted to litter components close to the surface, whereas ECM fungi dominate in well-decomposed litter and humus, older than 4-5 years (Lindahl *et al.,* 2007). Lindahl *et al.,* (2010) proposed this spatial separation of the two functional groups to be maintained by antagonistic interactions, where the two groups constrain the realized niche of each other via interference competition. Antagonistic interactions between saprotrophic and ECM fungi have been demonstrated in laboratory microcosms (Shaw *et al*., 1995; Lindahl *et al*., 1999, 2001, 2002; Leake *et al*., 2001). Already in 1971, Gadgil and Gadgil proposed that saprotrophic and mycorrhizal fungi compete for resources. They based their theory on an observation that decomposition rates of litter components increased when the roots (and thus C flow to mycorrhizal fungi) were severed – thereafter referred to as the 'Gadgil effect' (Gadgil and Gadgil, 1971). They proposed that, as mycorrhizal fungi decrease, nutrient limited saprotrophic fungi can expand, capture more nutrients and thereby decompose litter more efficiently.

However, ECM fungi may also compete with saprotrophic fungi via efficient exploitation competition, reducing the common pool of resources. After injecting ^{15}N into forest soil, Näsholm *et al.* (2013) found high levels of ^{15}N in mycorrhizal mycelium but little ^{15}N in tree canopies. Additions of N fertilizer to the soil before labelling, shifted allocation of ^{15}N from mycelium to

tree foliage, indicating a high capacity of ECM mycelium to compete with plants and other organisms for scarce resources. Many ECM fungi also possess genes encoding for manganese peroxidases (Bödeker *et al.,* 2009) which are enzymes believed to play a major role in the degradation of recalcitrant soil organic matter (Sinsabaugh, 2010). Thus, ECM fungi may not only have a high capacity to compete for resources, but also ability to compete for resources in the same substrates as saprotrophic fungi.

1.4.3 Soil fungi and forest management

Forest management generally focuses on the production of wood with clear-cutting as the main harvesting regime (MillenniumEcosystemAssessment 2005). Industrialized forestry has resulted in simplified forest structures and even-aged stands with short rotation times, which dramatically change the environment of the organisms living there. After clear-cutting the habitat may no longer cover the fundamental niche of a species. This will consequently have adverse impacts on biodiversity and ecosystem functions (Bengtsson *et al.* 2000; Puettmann *et al.,* 2009; Butchart *et al.,* 2010). In Finland and Scandinavia, where the status and trends of animal, fungal and plant species has been evaluated since 1980s, large scale clear-cutting is the main threat to 75% of the about 5000 red-listed forest species included in the recent versions of the national Red Lists of Finland, Norway and Sweden, of these 200 species are ECM fungi (Rassi P, 2010; ArtDatabanken 2015; Henriksen & Hilmo 2015).

Thus, clear-cutting has profound effects on the abundance and composition of ECM soil fungal communities (Hartmann *et al.,* 2012), as the quantity of trees, or rather the amount of carbohydrates allocated to tree roots regulates C and energy supply to ECM fungal communities.

After clear-cutting, there will also be changes in environmental factors such as increased pH, higher temperature and moisture. As harvest residues, dying roots and mycelia starts to degrade there will be a flush of nutrients released in the soil. Since ECM fungal species respond to changed environmental conditions differently this could cause a shift in the ECM community composition (Jones *et al.,* 2003).

During a rotation period of a forest stand, from planting to logging, the allocation of C to fine roots has been shown to vary (King *et al*., 2007). It appears that most C is allocated below ground at canopy closure, when tree nutrient demand is high (Simard *et al*., 2002). The amount of C to ectomycorrhiza may follow a similar pattern during this period, as the growth of fine roots is positively correlated with that of ECM mycelium (Majdi *et al*.,

2008). As a forest ages, soil chemistry (e.g. pH) and N availability varies and the organic material usually becomes more recalcitrant (Deacon and Flemming, 1992; Jumpponen *et al.,* 1999). This, together with changes in C delivery by the host, will result in a changing environment, probably with different fungal traits and life-form strategies being favoured at different ages of the forest.

1.4.4 Fungi affecting environmental processes

Depending on their response traits, the fungal community in the boreal forest soil is shaped by different environmental properties. In turn, the fungal community will affect the environment and environmental processes via different effect traits of its members (Koide *et al.,* 2013). Soil organic matter dynamics is an important example of an environmental process that is affected, or even largely regulated, by the effect traits of the fungal community (e.g. production of enzymes, C use efficiency, melanisation of mycelium, competitive interactions).

In a global-scale comparison of ecosystems dominated by different types of mycorrhizal symbioses, Averill *et al*. (2014) found that ecosystems dominated by ecto- and ericoid mycorrhiza had 70% more C per unit N compared with those dominated by arbuscular mycorrhiza. They ascribed the greater C storage to competitive suppression of free-living decomposers (with high decomposer capacity) by ecto- and ericoid mycorrhizal fungi (with presumed low decomposer capacity), whereby mycorrhizal fungi indirectly would protect soil bound C from decomposition. Competitive interaction between ECM fungi and saprotrophs was first proposed by Gadgil & Gadgil (1971). They showed that severing of tree roots growing into experimental plots significantly increased litter decomposition rates and attributed this phenomenon - the 'Gadgil effect' - to competition between ECM and saprotrophic fungi for limiting nutrients. Thus, being effective competitors for space and resources is an example of an effect trait that, combined with variation in decomposer capacity, would indirectly affect C sequestration.

In contrast to the 'Gadgil effect', ECM fungi may also act directly to stimulate degradation of organic matter. Most nutrients in soils are immobilised in organic macromolecules that require depolymerization before uptake, and most ECM fungi produce a variety of extracellular enzymes involved in the degradation of organic substrates (Abuzinadah *et al.,* 1986; Read & Perez-Moreno, 2003; Lindahl *et al.,* 2005). Degradation of recalcitrant organic matter, such as lignin and humus compounds, requires potent oxidative enzymes, and extracellular manganese peroxidases are believed to play a major role in the degradation of recalcitrant soil organic matter (Sinsabaugh, 2010).

These enzymes have previously primarily been studied in saprotrophic wood decomposers, but many ECM taxa also possess Mn-peroxidase encoding genes (Bödeker *et al.,* 2009). Further, Bödeker *et al*. (2014) found significant co-localization of high peroxidase activity and DNA from *Cortinarius* species, supporting the idea that some ECM fungi may play an important role in decomposition of complex organic matter. The ability to produce oxidative enzymes is thus another example of an effect trait, which may act to directly counteract C sequestration.

The 'Gadgil effect' and ECM fungal decomposition may occur simultaneously with opposing effects on decomposition, and their relative importance is likely to depend on ecosystem properties.

1.5 Methods to study fungi and their activity

A major obstacle when studying soil fungal communities is the fact that they live under ground. Occasionally some, but not all, fungi produce fruiting bodies that make it possible to detect and identify them without too much effort. Another complicating issue is that fungi have to be identified to species level in order to determine their ecological function, since species within the same family and order may have very different ecologies (Matheny *et al.* 2006). During evolution of fungi, switches between a saprotrophic life strategy and mycorrhizal symbiosis have occurred frequently. The mycorrhizal life-strategy is therefore distributed across the fungal phylogenetic tree, and many of the litter saprotrophs of today might have evolved from mycorrhizal ancestors (Hibbet *et al*., 2000). Thus, in order to separate and quantify functional groups, the entire fungal community has to be analysed down to the level of genera and species.

By studying fruit bodies or mycorrhizal root tips, it has been possible to analyse a fungal community to species level with identification based on morphological traits, such as sporocarp structure, shape and colour, but also microscopic features, such as hyphal structure. DNA based methods, such as restriction fragment length polymorphism (RFLP) fingerprinting or sequencing of the ITS region have been employed to increase accuracy (Horton & Bruns, 2001). The limitation herewith is that such methods do not allow analysis of the whole fungal communities, but only the fruiting or symbiotic components.

1.5.1 High-throughput sequencing of fungal community markers

Methodological advances in molecular biology, for example high-throughput sequencing of molecular markers (Lindahl *et al*., 2013; Nguyen *et al.,* 2015), enable at least semi-quantitative descriptions of fungal communities with

unprecedented capacity and resolution. Herewith, the entire fungal community in, for example, a soil sample can be identified. High-throughput sequencing is basically a method for DNA sequencing, generating large amounts of data. To begin with, a traditional PCR reaction is performed, using fungal specific primers. Molecular identification of fungi largely relies on amplification of the internal transcribed spacer (ITS) regions of the ribosome encoding genes. Due to high evolutionary rates of this region, flanked by highly conserved regions with suitable target sites for universal primers (Begerow *et al.* 2010), the ITS region can be used to identify fungal species.

High-throughput sequencing uses multiplex assays where multiple samples can be analysed simultaneously. Accordingly, each sample needs to be marked in order to be traceable. This is done by the use of sample specific identification tags attached to the primer.

During this thesis work, high-throughput sequencing has evolved from an experimental stage to more of a standard procedure.

Paper I

Two years before the first articles using high-throughput sequencing in fungal research was published (Buee *et al*., 2009; Jumpponen *et al*., 2009; Öpik *et al*., 2009), the DNA work for the first paper in this thesis (Paper I) was performed. In that point of time, we used primers with both the identification tag and adaptors required for the used sequencing technique (Roche 454 sequencing) attached. This resulted in long primers (44-46 base pairs) that formed substantial quantities of primer dimers. There was also a problem with the long primers not attaching to the template, which was solved by performing two PCR reactions: a first with primers without adaptors and tag, and a second with the adaptors and tag.

The high proportion of primer dimers did, accordingly, lead to a low yield of usable sequences, where only about 5% of the total information could be used. This was solved in the following projects by using primers with only identification tag attached and instead ligating the adaptors just before performing the sequencing.

Papers II and III

The DNA work and sequencing for the second paper (Paper II) of this thesis was actually performed twice. First with the primer combination ITS1F-ITS4, (Gardes & Bruns, 1993; White *et al*., 1990) then redone with the newly developed primers gITS7 and fITS9 (Ihrmark *et al.,* 2012) in combination with

the ITS4 primer. These primers are now widely used among international fungal ecologists.

As mentioned, the ITS region can be used to identify fungal species, and it consists of the ITS1 and ITS2 regions separated by the conserved 5.8S gene. While the ITS1F-ITS4 primer combination covers all of ITS1, 5.8S and ITS2, the fITS9 or gITS7 in combination with ITS4 primer only covers the ITS2 region and a part of 5.8S. By using the primer combinations gITS7-ITS4 and fITS9-ITS4 the PCR fragments become shorter (250-350 base pairs long) which leads to high amplification efficiency reducing the number of required PCR cycles. Thereby the distortion of community composition during PCR is minimized (Polz & Cavanaugh, 1998; Kanagawa, 2003). Further, the ITS1 region of some species has insertions, resulting in long PCR fragments of theses particular species (Johansson *et al.,* 2010). When using the ITS1F-ITS4 primer combination, this length variation between species may lead to PCR amplification biases against long amplicon fragments in mixed communities (Ihrmark *et al.,* 2012). This bias was largely avoided in Papers II and III by using the new primer combinations, amplifying only the ITS2 region.

Paper IV

During high-throughput sequencing, some DNA fragments may switch identification tags (Carlsen *et al.,* 2012), which will create false positives in downstream analyses. Usage of the new gITS7 and fITS9 primers, resulting in shorter PCR fragments, enables sequencing of the whole fragments. Thereby, tags in both ends of the PCR fragment can be sequenced and tracked. In the fourth article of this thesis, a new set of gITS7-ITS4 primer pairs were used. These primers were elongated with identification tags on both the forward and reverse primer, in order to be able to clear the dataset from false positives.

In the first three projects, 454 pyrosequencing was used, but because of the rapid evolution of these technologies, 454 pyrosequencing was no longer in use when the fourth project was to be sequenced. Instead there is now a so-called "third generation" sequencing technology and our fourth project was sequenced using single molecule real time (SMRT) sequencing from Pacific Biosciences. These two technologies are the least prone to sequence length biases. Two other "third generation" technologies are LifeTech's IonTorrent and Illumina, which both suffer from sequence length bias such there is preferential sequencing of shorter amplicons.

Our sample preparation protocol is optimised to maintain the relative abundances of fungal taxa, which is of decisive importance when investigating fungal ecological niches.

1.5.2 Methods to assess fungal biomass and activity

When using DNA markers to study fungal communities, a species may be represented by the same relative abundance of sequences in two different samples. However, even if the relative abundance of a species is the same in these samples, the biomass of the species might actually be larger in on of the samples due to differences in total fungal biomass. Extraction of the fungal specific sterol ergosterol, which is found in the cell membranes of all fungi, can give an indication of the fungal biomass in a sample. This biomass estimate might then be taken into consideration when analysing sequencing output.

Another way to study the fungal community is to estimate its activity by measuring organic matter decomposing enzymes.

In order to decompose organic matter, fungi use extra-cellular enzymes. There are two big groups of decomposing enzymes, which catalyse either hydrolytic or oxidative reactions.

Plant litter consist of several groups of compounds. The principal, dominant C rich components are: soluble organic compounds, hemicellulose, cellulose and lignin. When needle litter fall to the forest floor, soluble organic compounds are generally easily degraded or lost from the litter through dissolution or leaching within the first year of decomposition (Berg *et al.,* 1982).

Cellulose and hemicellulose are polysaccharides that are degraded into monomers or oligomers, primarily by hydrolytic enzymes, which are subsequently taken up and enter fungal metabolism (Baldrian & Valaskova, 2008). Lignin, on the other hand, is a complex aromatic polymer. A substantial part of the cellulose in plant material is protected by lignin. Thus, by degrading lignin, the cellulose within becomes available for degradation and utilization (Jennings and Lysek, 1996). Degradation of recalcitrant organic matter, for example lignin, requires potent oxidative enzymes such as laccases and peroxidases, whereof the basidiomycete specific manganese peroxidases are believed to play a major role (Sinsabaugh, 2010). Oxidative enzymes may also be important for accessing the large pool of N sequestered in complex organic forms in the humus layer.

The activity of different enzymes shifts in accordance with the decomposition stage of the organic matter. At the soil surface, with recently shed litter, hydrolytic enzymes are most active. As litter decomposes, the organic matter becomes more and more depleted in cellulose and hemicellulose, and after about a year, ligninolytic, oxidative enzymes are most active (Snajdr *et al.,* 2011).

Thus, analysing enzymatic activities in samples taken e.g. at different soil depths, can be linked to fungal communities and say something about their activities and effect on important processes.

Objectives

The projects in this thesis concern different factors that shape mycorrhizal and saprotrophic fungal communities in boreal forest soils, aiming at a better understanding of how soil fungi affect ecosystem processes, such as C and nutrient cycling. An additional aim was to identify logging impacts on ECM fungal community composition, providing information about how to preserve a diverse ECM fungal community after logging.

The specific objectives were to:

I Assess if a change occurs in ECM fungal community composition with increasing forest age and if there is peak in ECM fungal growth during tree canopy closure (Paper 1).

II Establish whether there are predictable patterns in how fungal communities in litter and humus layers respond to variation in soil fertility, focusing particularly on the balance between broad functional groups (Paper II).

III Find out to what extent retaining trees at clear-cutting can moderate the short-term negative impacts of logging, and to what degree ECM fungi thereby can be life-boated through the regeneration phase (Paper III).

IV Determine whether ECM fungi indirectly hamper decomposition or in contrary, acting as decomposers themselves, directly contributing to soil organic matter decomposition (Paper IV).

2 Project descriptions

2.1 Paper I: Norway spruce chronosequence

In Paper I was the ECM fungal community composition analysed and the production of ECM fungal biomass (as extraradical mycelium) estimated, over a Norway spruce chronosequence. The 40 Norway spruce sites were located in southwestern Sweden (56°42´N, 13°06´E) and ranged from 0-130 years in age. Sand-filled ingrowth mesh bags were used to estimate the active, extraradical ECM fungal community in the soil. The mesh bags were left at the site for an incubation period of 22 weeks. Accumulated fungal biomass at the end of the incubation was estimated by ergosterol analyses.

Development of the ECM fungal community in the chronosequence was analysed in a subsample from the mesh bags, using high-throughput 454 sequencing of internal transcribed spacer (ITS) amplicons (further described in section 1.5.1). Sequences were clustered into species hypotheses, which accordingly were taxonomically identified. The forests were organized into five age classes, and variations in mycelial production and fungal biomass between age-classes were analysed. A Shannon diversity index was calculated for each stand and relationship between stand age and ECM fungal diversity was tested by linear regression. Correlation between stand age and fungal community composition was established by canonical correspondence analysis (CCA) and evaluated for statistical significance by Monte Carlo permutation tests.

2.2 Paper II: Boreal forest soil fertility gradient

In Paper II, it was investigated how fungal community composition, in humus and litter, varies along a gradient in soil fertility. Twenty-five, old-growth forests, located in central Sweden, were selected to represent a natural gradient

in soil fertility. The relative composition of Norway spruce (*Picea abies* (L.) H. Karst.) and Scots pine (*Pinus sylvestris* L.) as well as understory plants shifted along the gradient. To reduce variation due to anthropogenic disturbance, the sites were located outside areas of significant N-deposition. The forest stands were old (>100 years) and had never been subjected to intensive forestry.

In September of 2008, ten soil cores were randomly collected from each forest and pooled within sites. In addition, 10 needles were picked from the forest floor surface adjacent to each soil core. Extractable ammonium (NH_4^+), C and N content, water content, soil pH and mineral content were analysed in each soil sample, while needles were analysed for C and N content only. Fungal biomass was estimated by ergosterol analysis and fungal community composition was analysed using high-throughput 454 sequencing of internal transcribed spacer (ITS) amplicons (further described in section 1.5.1). Sequences were clustered into species hypotheses, which accordingly were taxonomically identified and divided into functional groups. Relationship between environmental parameters and functional groups, as well as environmental parameters and ergosterol content, in both soil and litter were tested by linear regression. By using a detrended correspondence analysis (DCA), fungal community similarity was graphically demonstrated. Correlation between environmental variables and fungal community composition was established by CCA and evaluated for statistical significance by Monte Carlo permutation tests. With this method, we could establish whether there are predictable patterns in how fungal communities in litter and humus layers respond to variation in soil fertility.

2.3 Paper III: Tree retention

In Paper III was the effect of retention trees on the survival of ECM fungi at logging investigated. An experimental field study was established in an old-growth Scots pine forest in northern Sweden (66°98´N, 20°46´E).

The study consisted of four treatments with different proportions of trees retained: (1) unlogged control with all trees retained (100% of the trees retained); (2) 60% of the trees retained; (3) 30% of the trees retained and (4) all trees cut (0% of the trees retained). The plots were not planted after harvest, but naturally established seedlings were left at site for regeneration purposes. Treatments were replicated five times across five randomized blocks (figure 1). Each treatment plot was 50 m x 50 m, and retained trees were evenly distributed within each plot.

Nine soil-cores were collected in the centre of each plot and divided into O-, E- and B-horizons, which were separately pooled into three composite

samples per plot. Pre-treatment samples were collected in September 2009. Re-sampling was done one year after harvest (September 2011) and three years after harvest (September 2013).

Fungal community composition was analysed using high-throughput 454 sequencing of internal transcribed spacer (ITS) amplicons (further described in section 1.5.1). Sequences were clustered into species hypotheses, and the species hypotheses identified to be ECM were used for statistical analyses. Relationship between proportion of retained trees and ECM relative abundance, ECM species richness and frequency of samples with ECM fungi still present was tested using a linear mixed model. Beta diversity was calculated with alpha and gamma diversities considered at different spatial scales. Correlations between tree retention and ECM fungal community composition were established by canonical correspondence analysis (CCA) and evaluated for statistical significance by Monte Carlo permutation tests. Hereby the effect of retention trees on the ECM fungal community composition could be assessed.

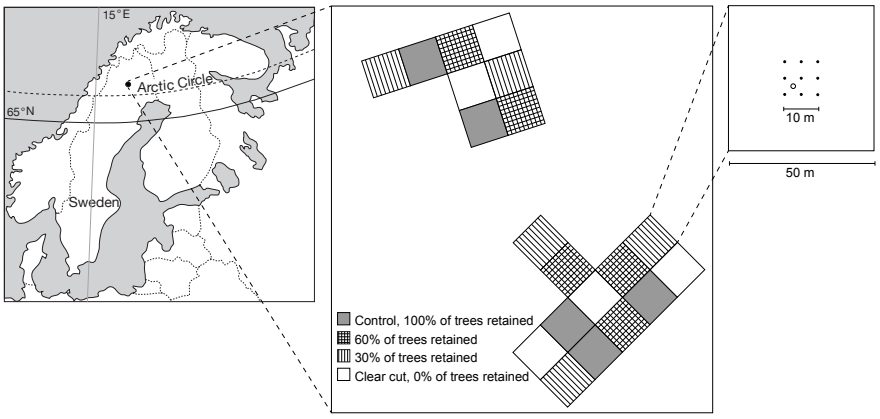

Figure 1. Location of the study area, experimental design and layout of soil-core sampling. The experimental design contained four retention levels: 100% (unlogged), 60%, 30% and 0% (clear-cut) retained trees, in five replicated blocks. Nine soil-cores were collected according to the figure, one year before harvest (2009), as well as one (2011) and three (2013) years after harvest. The cores were divided into O-, E- and B-horizons and pooled correspondingly into three composite samples per plot. In 2013, an additional 10 soil cores were collected from the O-horizon and kept separate; 9 at the same location as for the pooled samples. The circle indicates where the extra 10th core was sampled.

2.4 Paper IV: Root trenching

The aim of Paper IV was to find out whether ECM fungi hamper decomposition or in contrary, acting as decomposers themselves, directly contributing to soil organic matter decomposition. ECM fungi were excluded (trenching) from eight experimental plots in a mixed coniferous forest in central Sweden (59°57'07.9"N, 16°45'18.8"E), while eight plots were designated as controls.

Trenching was performed in 2009 by digging a ditch around a central plot (1x1m) using an excavator, leaving the plot as undisturbed as possible. A steel barrier was thereafter pressed down round the core and the adjacent area was refilled (figure 2).

Soil cores were collected one and four years after trenching respectively and carefully divided into six different horizons. Mesh bags were inserted horizontally between the F1 and moss layer and left for one or two growing seasons.

Community composition in the different soil layers was assessed by high-throughput SMRT-sequencing of PCR amplified ITS2 markers and ergosterol was used as a marker for fungal biomass (further described in section 1.5.1). Sequences were clustered into species hypotheses, which accordingly were taxonomically identified and divided into functional groups.

Organic matter decomposition was assessed by monitoring weight loss of litter in the litterbags and by measuring the activities of selected extracellular enzymes. In order to monitor the vertical N distribution, $^{15}N/^{14}N$-ratios were determined and δ^{15} N calculated.

The relationship between trenching and differences in ergosterol concentration, relative abundance of functional groups, enzyme activities and δ^{15} N in the different soil layers were analysed by generalized linear mixed models.

This approach enabled us to follow how different functional groups of fungi reacted to disrupted C-input via roots and disentangle the mechanisms underlying ECM influences on decomposition.

Figure 2. Trenching was performed by digging a ditch around a central plot using an excavator, leaving the plot as undisturbed as possible. A steel barrier was thereafter pressed down round the core and the adjacent area was refilled.

3 Results and discussion

3.1 Ecological niches (Papers II and IV)

Depending on the response traits of an organism, the boundaries of the fundamental niche is defined. However, the fundamental niche might be restricted due to competition from other, better adapted, species. This restricted fundamental niche is called the realized niche and defines the habitat where an organism actually lives. In this thesis, ecological niches of soil fungi are investigated - both on a larger, landscape scale, covering several forests (Paper II), but also on the small scale, within a few centimetres of soil (Paper IV).

3.1.1 Landscape scale

The fungal community composition in relation to a soil fertility index was investigated in Paper II. The fertility index was established by correlating environmental parameters of the humus layer (pH, C:N and NH_4^+, mineral content) and dominant tree and understory species of the 25 investigated forests. With increasing pH and NH_4^+, vegetation became more dominated by *Picea* and herbs, while *Pinus*, *V. vitis-idaea* and *Calluna* predominantly were found in forests with low NH_4^+. The C:N ratio in the needle litter followed this fertility index with significantly higher ratio in *Pinus* compared to *Picea* litter.

Along this gradient of soil fertility, the fungal community could, to a large extent, be explained by the combined influence of soil pH, C:N ratio and NH_4^+ content (figure 3).

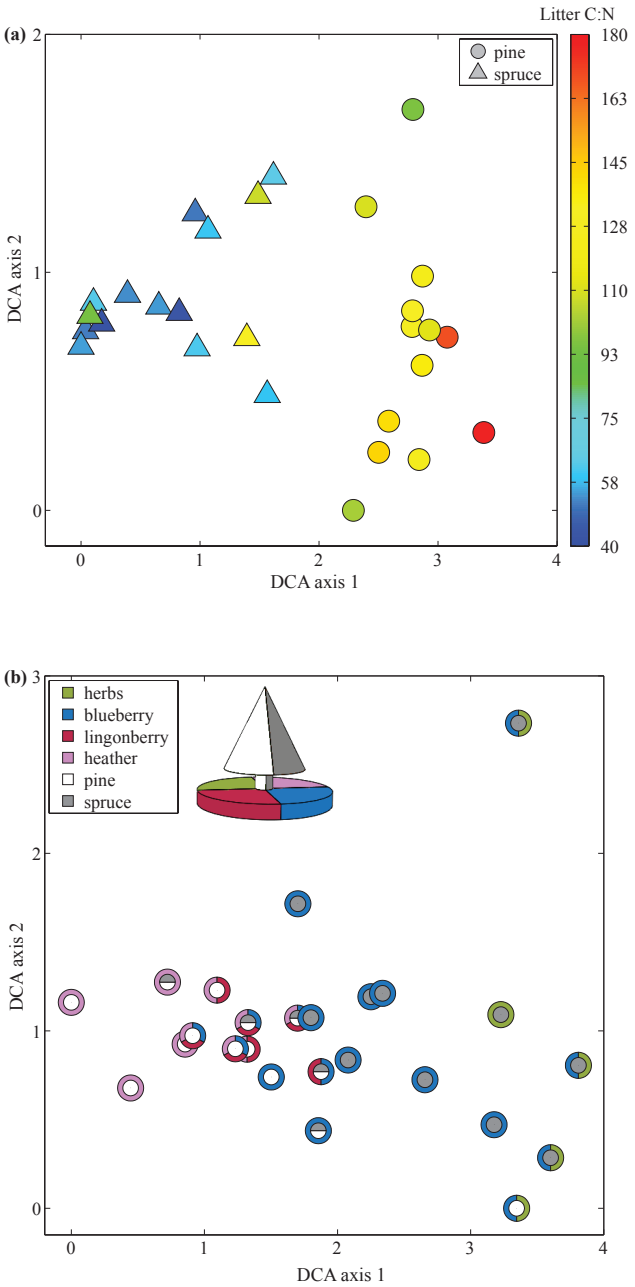

Figure 3. Sample plots of detrended correspondence analyses (DCA) of fungal communities in a) litter and b) humus layers of 25 Swedish old growth boreal forests. In a) circles represent fungal communities in *Pinus sylvestris* litter and triangles represent fungal communities in *Picea abies* litter. Symbols are color-coded according to a) litter C:N ratio b) understory vegetation (outer ring) and dominant tree species (inner circle).

Further, we found support for a trade-off between S-strategic ascomycetes and C-strategic basidiomycetes in the assembly of fungal communities (figure 4). In the *Pinus*-dominated forests with low soil fertility, both litter and soil were dominated by ascomycetes, largely assigned to species in Leotiomycetes, Chaetothyriales and Archaerhizomycetes (humus/soil only). Fungi in Leotiomycetes (Vrålstad et al., 2002) and Chaetothyriales (Zhao et al., 2010) commonly display S-strategic traits, such as melanised cell walls. Our result is in line with the observed preference of Leotiomycetes for higher latitudes and more acidic soils (Tedersoo et al., 2014) and the persistence of Leotiomycetes and Chaetothyriales in retrogressing ecosystems (Clemmensen *et al.,* 2015).

Increasing N-availability and decreasing acidity should reduce the need for S-strategic traits. In the *Picea*-dominated forests of the gradient, with higher soil fertility, basidiomycetes increased relative to ascomycetes, both in litter and humus. Probably, by trading traits coping with a stressful environment (e.g. acidity tolerance and high N use efficiency), for traits associated with high combative strength (Boddy, 2000), basidiomycetes could proliferate in the forests with more fertile soil.

Among the root-associated fungi in the soil, the change in abundance of ascomycetes in relation to basidiomycetes also implied a shifting proportion of ericoid mycorrhizal fungi (mainly ascomycetes) and ECM symbionts (mainly basidiomycetes)(c.f. Read and Perez-Moreno, 2003). While some studies have observed higher production of ECM mycelium in more fertile forests (Kalliokoski *et al*., 2010) and positive responses of ECM fungi to N additions in unproductive tundra (Clemmensen *et al.,* 2006), the established view is that ECM fungi decreases with increasing N-availability (Nilsson *et al*., 2003; 2005; Toljander *et al.,* 2006). Low soil fertility would increase plant C allocation to roots and thereby drive a dominance of ECM fungi in the humus layer of poor forests. In forests with higher fertility levels, C allocation to mycorrhizal fungi would decline, with the consequence of decreased ECM fungal abundance (Högberg *et al*., 2003).

However, we found that the relative abundance of ECM fungi did not decline, even under the most nutrient rich conditions of our gradient, but remained at 50-70% of the amplicons. The lower abundance of ECM fungi that we found in the N-limited forests with low pH, might be a result of ECM fungi approaching the limit of their fundamental niche with respect to N-availability and soil acidity.

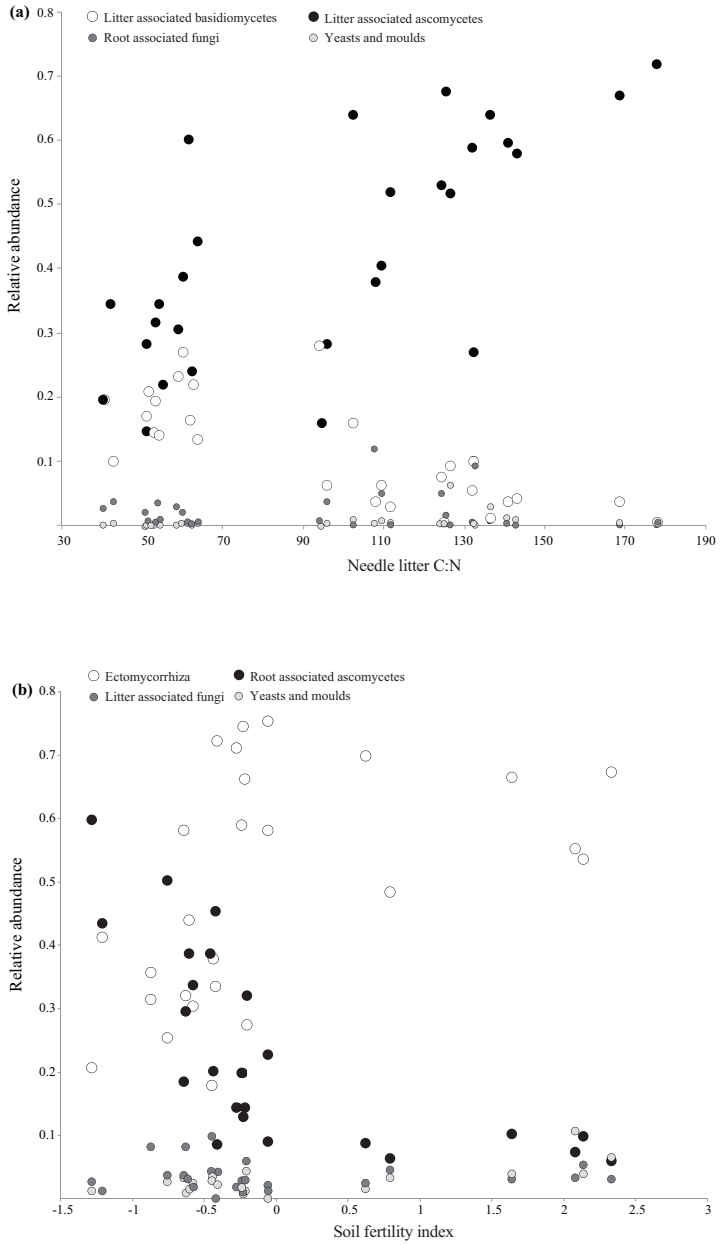

Figure 4. Relative abundance of functional groups in a) litter and b) humus from 25 old-growth boreal forests in relation to C:N ratio of the substrate and soil fertility index (ordination scores on the first axis of a PCA analysis of pH, C:N, NH_4^+ and vegetation), respectively. Data is based on 454-pyrosequencing of ITS2 amplicons.

Our gradient represented forests with N-levels and pH that are typical in Scandinavian forest without N-deposition and N-fertilization. If our gradient would have been expanded to also include areas of higher N-availability, such as forests within the N-deposition zone, we may approach the border of the ECM fungal niches where the high N-levels would decrease root C-allocation by trees, leading to lowered recourse availability for the ECM fungal community, weakening their position as efficient competitors. Accordingly, in one of the *Pinus* forests, mostly resembling a ”parkland” (not included in the study) with less acidic soil (pH 5.8) and higher inorganic N levels (86 µg NH_4^+-N g OM^{-1}), we observed lower fungal biomass and ECM fungal relative abundance than in any of the other investigated forests. This single observation may be an indication of a decrease in fungal biomass further along the fertility gradient, supporting a maximum in fungal (ECM) biomass around pH 4.5.

In accordance with Lilleskov *et al*. (2002), we found increased abundance of short exploration types of ECM fungi (e.g. *Tylospora* and *Inocybe*) with increasing fertility index, while long exploration types (e.g. *Cortinarius*) predominantly were found at lower index. Lilleskov *et al.* (2002) speculated that as N input increase, the ECM fungal community will shift from taxa specialised for N uptake under low N conditions, toward taxa specialised for high overall nutrient availability.

3.1.2 Micro scale

In Paper IV, competition between saprotrophic and ECM fungal communities was investigated, in other words, how these two functional groups restrict the realized niches of each other. We studied the vertical distribution of soil fungi by dividing soil cores into fine layers. The cores were collected in control plots and in plots where roots and associated ECM fungi were excluded. We also followed the activity of the litter saprotrophic fungal community by burying mesh bags filled with litter beneath the moss layer, monitoring litter decomposition rates.

Already in 1971, Gadgil & Gadgil found increased decomposition rates in the absence of ECM fungi. They prescribed the increased activity of the saprotrophic community to reduced competition for nutrients from ECM fungi. Consistent with their results, we also found increased decomposition rates in the litter layer after exclusion of ECM fungi.

Further, in litterbags and in needle litter retrieved from soil cores, levels of ^{15}N were higher in trenched plots. During transfer of N from soil through ECM fungi to their host plant, fractionation against the heavier isotope (^{15}N) leaves ECM fungi and soil enriched in ^{15}N while the lighter isotope (^{14}N) is

preferentially allocated to the plants, the litter of which becomes depleted in ^{15}N (Hobbie and Colpaert, 2003; Högberg *et al.,* 1996). With increasing contribution of ECM species to the fungal community and soil organic matter increasingly originating from ECM mycelial precursors, ^{15}N abundance progressively increases in the lower layers of the organic horizon (Clemmensen *et al.* 2013; Lindahl *et al.*, 2007). In Paper IV, trenching increased ^{15}N abundance in surface litter, indicating upward redistribution of ^{15}N from the enriched N-pool normally immobilized by ECM fungi. N limited needle saprotrophs depend on upward reallocation of N to maintain high colonization and decomposition of freshly deposited litter (Boberg *et al*., 2014). Thus, the observed ^{15}N redistribution after trenching in concurrence with increased litter decomposition suggests that indeed there is competition for nutrients between saprotrophic and ECM fungi in boreal forest soils.

Competition may occur in two different ways, either directly by antagonistic interactions (interference competition) or indirectly by mutual utilisation of the same scarce recourses (exploitation competition) (Keddy, 2001).

Näsholm *et al*. (2013) demonstrated efficient exploitation competition by ECM fungi by injecting ^{15}N into forest soil. Under ambient conditions, when N-levels in the soil were low, high levels of ^{15}N were found in mycorrhizal mycelium but little in tree canopies. However, when N fertilizer had been added to the soil prior to ^{15}N labelling, the allocation of ^{15}N shifted from mycelium to tree canopies. Thus, when the mycorrhizal fungi did not have access to enough N, they could effectively compete for N with trees, but also with e.g. saprotrophic fungi. By intensify N limitation for saprotrophs, decreased decomposition rates can be expected.

In contrast, direct competition for space and resources by antagonistic interactions has been documented between saprotrophic and ECM fungi in microcosm experiments (Lindahl *et al*., 1999; 2001; 2002). These two functional groups of fungi have also been documented to be vertically separated in stratified forest soils (Lindahl *et* al., 2007; Baldrian *et al.,* 2012), indicating antagonistic interactions.

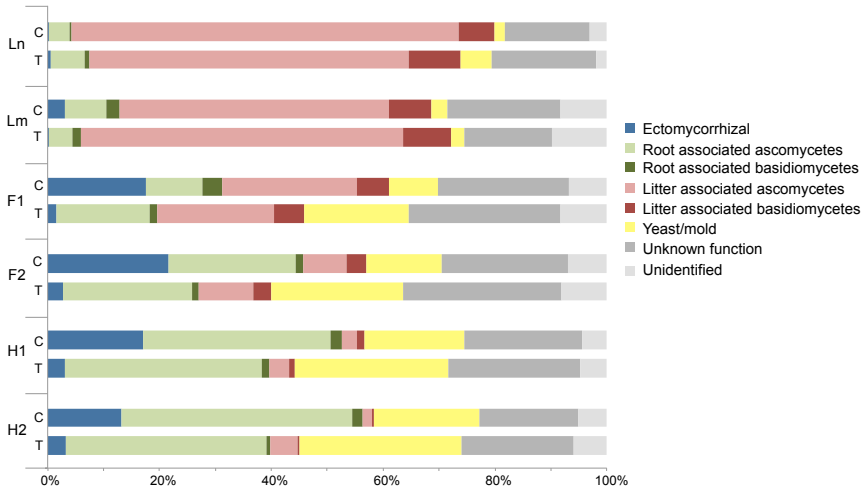

Figure 5. Relative abundance of functional groups based on sequencing of ITS2 marker in trenched (T) and control (C) plots in a boreal forest, four years after trenching. Ln – needles, Lm - mosses, F1 - upper 2/3 of F layer, F2 – bottom 1/3 of F layer, H1 - upper 1/3 of H layer, H2 - bottom 1/3 of H layer.

Exclusion of ECM fungi by trenching would then be expected to allow litter saprotrophs to expand their realized niche into deeper horizons and thereby be able to foraging for more decomposed organic matter resources. Saprotrophic fungi would thereby reallocate and mobilise N (^{15}N-enriched) from deeper horizons to surface litter (Boberg *et al*., 2014).

However, in our study, the vertical distribution of litter saprotrophs was unaltered by root trenching and consistently constrained to the uppermost horizons (figure 5). We conclude that litter saprotrophs were confined to the upper soil horizons, not because ECM fungi constrained their realized niche by interference competition. Rather, the shallow distribution of litter saprotrophs seems to be due to their inability to extend into the deeper horizons even in the absence of competition, i.e. a narrow fundamental niche.

3.2 Forest management (Papers I and III)

Forest management mainly focuses on the production of wood, often with clear-cutting as the main harvest regime. This has resulted in simplified forest structures and even aged stands. After clear-cutting, the habitat may no longer cover the fundamental niche of the species living there, and it may take a long time before the environment is restored. Some species, possessing traits that can cope with the new conditions may be favoured.

Effects of forest management on the ECM fungal community were investigated in Papers I and III. In both papers, clear-cutting resulted in a dramatic decrease of ECM fungal abundance and species richness. In Paper I, species composition and biomass production of ECM fungi was studied over the rotation period of managed Norway spruce stands. In this study, biomass production peaked in stands of 10-30 years old coinciding with canopy closure when tree growth is rapid and leaf area maximal (Simard et al., 2004). This finding suggests that less C is required to support ECM hyphal growth in very young and very old Norway spruce forests.

In these young stands of 10-30 years old, ECM fungal community was dominated by the fast growing *Tylospora fibrillosa*, which constituted 80% of the ECM amplicons (subjected to potential method artefact – see section 1.5.1). In forests older than 30 years, *T. fibrillosa* was gradually complemented with other species, with the consequence of a slowly increasing diversity. However, diversity continued to increase even in forests 50 to 90 years of age (figure 6).

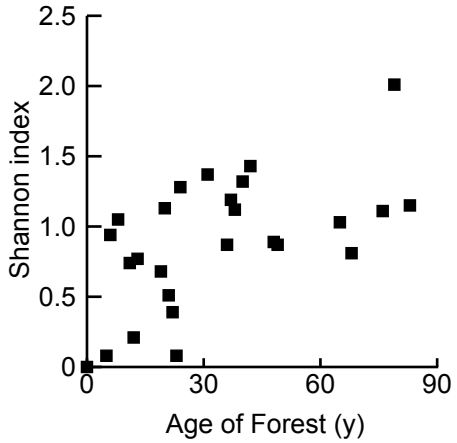

Figure 6. Shannon diversity index of ectomycorrhizal fungi in relation to age of Norway spruce (*Picea abies*) stands.

T. fibrillosa might be described as a C-strategist, being adapted to high population densities. C-strategists are characterized by efficient conversion of resources to biomass, leading to rapid growth and ecosystem dominance when resources are abundant. This is in agreement with the observed increase in dominance of *Tylospora* species in ingrowth bags in response to elevated atmospheric CO_2 concentrations (Parrent & Vilgalys, 2007), which presumably increases belowground allocation of photosynthates. The competitive advantage of *T. fibrillosa* may have declined in the maturing forest, leaving room for other species.

Further, recent studies suggest that basidiomycete community dynamics may be under strong influence of dispersal limitation with slow recruitment (Norros *et al.* 2012; Peay *et al.* 2012). Species with more efficient spore dispersal may re-establish quite fast in a clear-cut and planted forest, while for other species reestablishment might take many years. As documented in Paper II, the fungal community is strongly influenced by pH and N-availability and these parameters often increase after clear-cutting. Thus, even if efficient spore dispersal would occur, the environment could have changed, no longer having optimum conditions for the species. As the forest grow old, conditions are restored and the species may again be able to efficiently compete for space and resources.

Since clear-cutting has dramatic and long-term effects on the fungal community, retaining trees at logging may mitigate these negative impacts. Retention forestry was initiated in the early 1990s with the prospect to moderate negative harvesting impacts on biodiversity e.g. by leaving single trees, tree groups, buffering tree zones bordering lakes and wetlands, and also by leaving and creating dead wood (Fedrowitz *et al.* 2014). These actions are primarily associated with clear-cutting with the objective of "life boating" species through the regeneration phase, increasing habitat diversity and enhancing connectivity in the forest landscape.

In Paper III, the effect of tree retention on the ECM fungal community was investigated. We found a linear and positive correlation between the amount of retention trees and ECM fungal abundance and diversity (figure 7), agreeing with results from earlier studies of effects of retention trees (Luoma *et al.* 2004) and distances to trees and forest edges (e.g. (Kranabetter 1999; Kranabetter *et al.,* 1999; Kranabetter & Kroeger 2001).

When retaining at least 30% of the trees, there were still ECM-fungi present (even though with a lower biomass) in almost all (85%) samples and no clear difference in community composition compared with unlogged plots was found. However, the clear-cut plots had a different fungal community and only ECM present in half of the samples.

By leaving retention trees, the dramatic shift in community composition documented in Paper I, may be avoided. Assembly of ECM communities has been shown to be affected by priority effects, where early colonizers are at a competitive advantage (Kennedy & Bruns 2005; Kennedy *et al.,* 2009). Thus, mycelial individuals life-boated through the clear cut phase on retained trees, may persist by priority even though not best adapted to the new conditions of young planted forest.

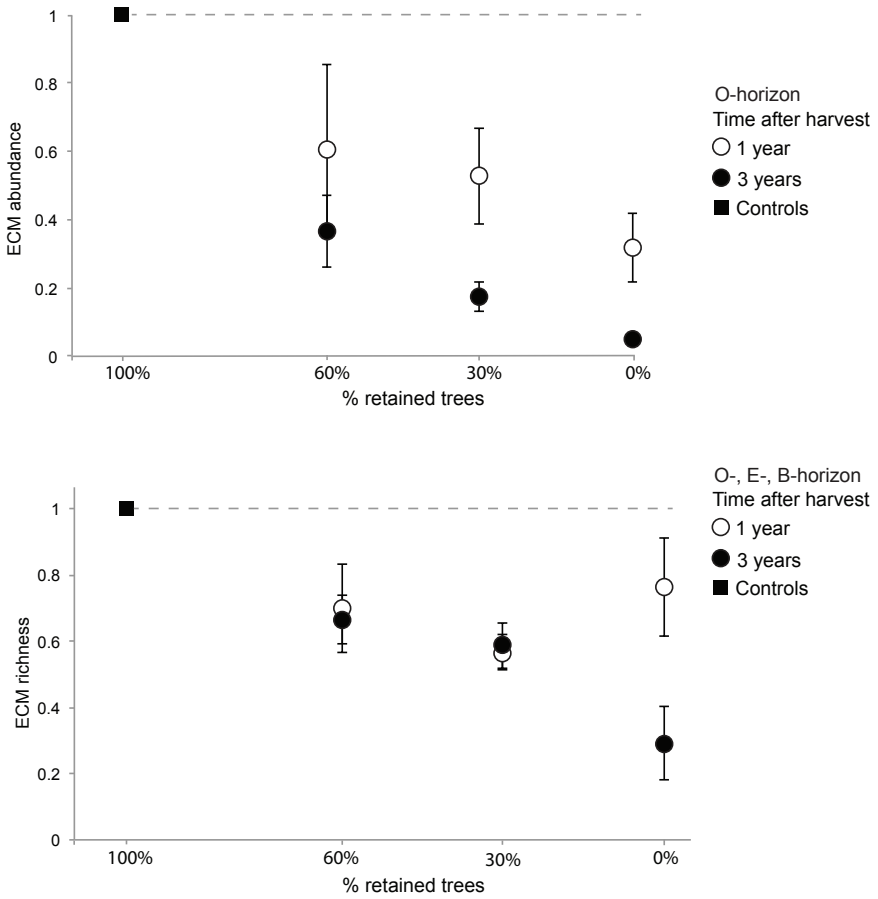

Figure 7. a) Relative abundance of ectomycorrhizal fungi in the O-horizon and b) Ectomycorrhizal fungal species richness in a pine forest plots with 60%, 30% and 0% of trees retained at harvest. To account for variation between years, abundances and species richness are expressed in relation to unlogged plots. Open circles represent samples collected one year after cutting (2011) and closed circles represent samples collected three years after cutting (2013).

3.3 Ectomycorrhizal decomposition (Paper IV)

So far, the focus has been on response traits i.e. how the environment affects the fungal community. In turn, fungi may affect its habitat and environmental processes at different scales by possessing different effect traits.

One important factor that has the potential to affect nutrient and C cycling at a global scale is in what way ECM fungi affect soil organic matter decomposition. Since ECM biomes represent a consistent global net sink for atmospheric CO_2 (Pan *et al.*, 2001; Averill *et al.*, 2014), the knowledge gap

around this topic contributes uncertainty to current projections of global C cycling and resulting climate change (Finzi *et al*. 2014).

In Paper IV, as mentioned, we found increased decomposition rates of surface litter in the absence of ECM fungi. Thus, ECM fungi hampered decomposition, probably due to an indirect exploitation competition for N rather than through direct interference competition.

In contrast, ECM fungi may also directly act to stimulate degradation of organic matter by acting as decomposers, in order to obtain nutrients (Lindahl & Tunlid, 2015). We found hydrolytic enzymes and laccases to decrease sharply with depth (c.f. Snajdr *et al*., 2008), in line with a shallow distribution of litter saprotrophs. However, the activity of peroxidases, including basidiomycete specific Mn-peroxidases (Floudas *et al*., 2012), was evenly distributed throughout the entire organic horizon. Thus, below the surface zone of relatively freshly deposited aboveground litter, further decomposition of more decomposed organic matter largely seemed to depend on oxidative mechanisms. When ECM fungi were excluded, there was an almost complete loss (91% decrease) of manganese peroxidase activity (figure 8) showing that ECM fungi not only have the potential to decompose complex organic matter (Bödeker *et al*., 2009; Lindahl & Tunlid, 2015), but actually were the principal drivers of humus degradation in this system.

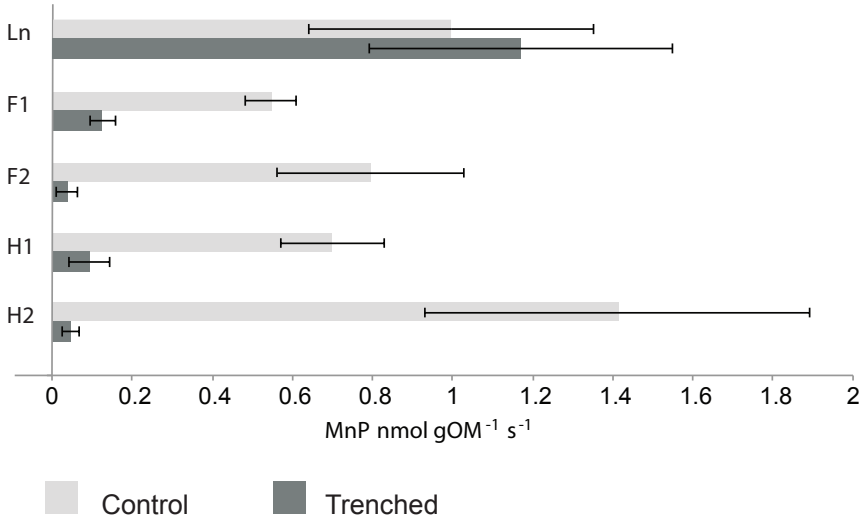

Figure 8. Activity of manganese peroxidase in organic soil profiles of trenched and control plots in a boreal forest, four years after treatment.

With access to host-derived sugars, mycorrhizal fungi are well suited to perform co-metabolic oxidation of complex, humified organic matter. However, ECM fungi are not saprotrophs that decompose SOM to retrieve C for their metabolism, as indicated by the low activity of hydrolytic enzymes in ECM dominated horizons. Rather, the primary benefit of ECM decomposition is likely to be mobilization of N locked up in non-hydrolysable organic complexes (Lindahl and Tunlid, 2015).

Taken together, we found that ECM fungi *both* competed with free-living decomposers (the Gadgil effect), thereby reducing decomposition of surface litter, *and* that ECM fungi themselves acted as decomposers and contributed directly to OM decomposition in deeper, more decomposed humus layers.

Just as in our study, where the pool of C was 15 fold bigger in the F1-H2 horizons compared to the litter horizons, boreal forest C pools in deeper soil horizons are generally much larger than litter stores, and processes in the root-zone rather than litter decomposition rates regulate over-all C storage (Clemmensen *et al*., 2013). This suggest that direct decomposition by ECM fungi is of greater importance than the Gadgil effect in regulating C storage in organic soil horizons of boreal forests and that the overall role of ECM fungi is to facilitate decomposition rather than suppressing it. However, ECM fungi also contribute significantly to C input in deeper soil horizons (Clemmensen *et al*., 2013), which could be seen as higher soil organic matter in the deep humus layers in the trenched plots of our study. Depending on ecosystem properties, the net balance between C input and C decomposition may vary between forests.

Most models concerning C cycling in forest ecosystems use soil temperature and moisture as the main drivers of soil organic matter decomposition. However, it has been argued that plant C allocation to roots and rhizosphere microbes is a major driver of SOM decomposition with a significant impact at ecosystem scales (Fontaine *et al.,* 2007). Based on meta-analysis and mathematical models, Finzi *et al*. (2014) showed that rhizosphere processes are a widespread, quantitatively important driver of SOM decomposition and nutrient release. Our results reinforce the importance of integrating roots and rhizosphere microorganisms, in particular ECM fungi, in C cycling models.

3.4 General discussion

The different Papers of this thesis have concerned fungal response traits and how fungal communities are shaped, but also, how fungi affect their environment via effect traits (Koide *et al*., 2013).

Combining the results from Papers II and IV, a pattern regarding soil fungi and their effect on C sequestration in different habitats emerges. In Paper II, we found a clear shift from a fungal community dominated by ascomycetes to a community dominated by basidiomycetes with increasing pH and N-availability in the forest. The shift was observed in both litter and soil samples.

Among litter saprotrophs, basidiomycetes are considered especially important for the degradation of recalcitrant plant material, because of their production of ligninolytic enzymes (Osono & Takeda, 2002). Ascomycetes, on the other hand, have generally much lower decomposition capacity (Boberg *et al*., 2011).

In Paper IV, we found that ECM fungi (mainly basidiomycetes) were the principal drivers of humus degradation in the studied system. Thus, in both litter and humus layer, fungi capable of decomposing recalcitrant SOM increased in forests with higher N-availability and pH.

Hypothetically, C sequestration should be strongly influenced by this shift in decomposing capability of the fungal community (exemplified in figure 9). In forests dominated by ascomycetes, litter degradation should accordingly be low, and with the humus layer dominated by ericoid mycorrhiza, degradation of the deeper humus layer should be slow. Consequently, in this type of habitats, C sequestration should be high, as observed by Clemmensen et al. 2015. Due to the low decomposition rates, nutrients will be locked into complex organic compounds, aggravating the N-limited conditions. This feedback should worsen the position for the basidiomyceteous fungal community. In forests with slightly better conditions in terms of higher pH (around pH 4.5) and higher N-availability, a shift towards basidiomycetes and ECM fungi will occur. The ECM community composition may shift to be represented by species with long-medium exploration types (e.g. *Suillus* and *Cortinarius* spp.), which possess the ability to oxidize organic matter (Bödeker *et al*., 2009; 2014; Shah *et al*., 2015) restricting C sequestration to a minimum with a feedback on increased ecosystem productivity (Clemmensen *et al.,* 2015).

When pH and N-availability is further increased, the ECM community will become more and more dominated by short and smooth exploration types of ECM fungi (Lilleskov *et al*., 2002; 2010). ECM fungi with these exploration types do not typically have oxidative enzymes (Hobbie & Agerer, 2009). Together with increased organic matter input (roots and litter) due to improved plant growth under N-rich conditions, C sequestration should increase. In a meta-analysis, Janssens *et al*. (2010) found that N deposition hampers organic matter decomposition, and thus increases C sequestration. Even further along the gradient, when broad leave forests with an understory vegetation of herbs

and grasses replace the boreal forest, the fungal community will be dominated by arbuscular mycorrhiza, and soil fauna and bacteria will gradually replace fungi as the principal drivers of organic matter turn-over. As found by Averill *et al.* (2014), these forests have significantly lower C sequestration (per soil N) than boreal forests, mainly due to more easily decomposable plant litter.

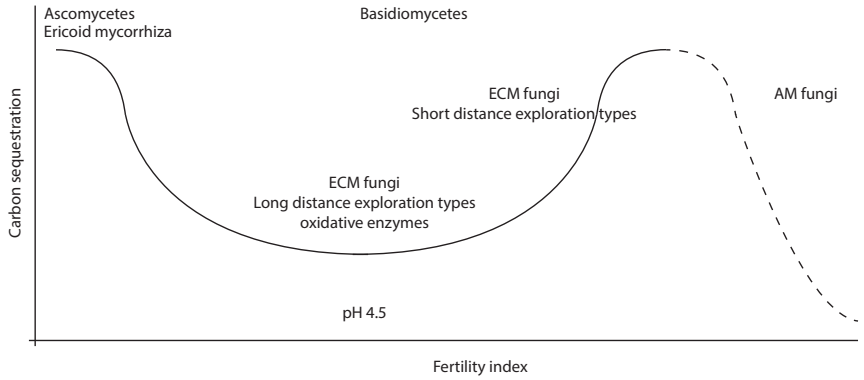

Figure 9. Hypothetical relation between C sequestration and dominating fungal guilds along a soil fertility gradient. Remains to be tested against empirical data.

4 Conclusions and future prospects

Studying soil fungal communities involves many difficulties, primarily because soil fungi mainly live under ground. Recent methodological advances in molecular biology, for example high-throughput sequencing of molecular markers (Lindahl *et al*., 2013; Nguyen *et al.,* 2015), enable at least semi-quantitative descriptions of entire fungal communities in e.g. a soil sample with unprecedented capacity and resolution. Making use of this new technique, ecological questions can begin to be addressed, which, due to methodological limitations, until know have not been easily studied.

The work in this thesis focused on how different factors shape soil fungal communities, but also aimed at a better understanding of how soil fungi affect environmental processes, such as C cycling and sequestration.

Fungal community composition was, with some statistical certainty, found to be predictable from environmental parameters. Predictions were valid at the level of species, but also on higher taxonomic levels, as well as for broad functional groups. At the phylum level there was a clear shift from a community dominated by S-strategic ascomycetes in the low fertility end of the gradient to a community with increased contribution of C-strategic basidiomycetes at higher fertility.

Further, the impact of forest management on the soil fungal community was investigated. ECM fungi were found to potentially disappear with complete clear-cutting, and although ECM mycelial production was found to resume within some years after clear-cutting, reestablishment of ECM diversity take several decades. However, a clear positive relationship between the amount of retention trees and ECM fungal species richness and abundance was demonstrated. Although the ECM fungal biomass will be significantly reduced, retaining trees at logging has the potential to maintain the most abundant fungal taxa and counteract local extinctions of rare species.

When studying the effect of ECM fungi on soil organic matter decomposition, it was found that ECM fungi *both* compete with free-living decomposers (the Gadgil effect), thereby reducing decomposition of surface litter *and* that ECM fungi themselves act as decomposers, directly contributing to organic matter decomposition in deeper, more decomposed humus layers. Since deeper soil horizons generally contain much larger C stores compared to litter, and processes in the root-zone rather than litter decomposition rates regulate over-all C storage (Clemmensen *et al*., 2013), direct decomposition by ECM fungi is likely of greater importance in regulating over-all C storage. However, soil C sequestration is not just a function of C losses during decomposition, but also depends on rates of C input to the soil. Although trenching largely seemed to disrupt enzymatic oxidation in the rooting zone, four years without root-mediated C input still decreased C pools in the lower horizons, indicating a positive net balance between root-mediated C inputs and losses, and confirming the importance of roots and associated fungi as a source of soil organic matter (Clemmensen *et al.,* 2013).

When applying the results in this thesis to ecological processes, it is important to recognize the great functional variation between different ECM fungi, with major differences in enzyme production capacities (Kohler *et al*., 2015) and colonization of organic soil substrates (Agerer, 2001). While high-throughput sequencing is a powerful tool to assess the microorganisms that are present in a given habitat, the functional and genetic aspects are still missing. For many of the retrieved sequences, a match in databases to a known species or functional affiliation could not be found. This makes it difficult to appraise what role a specific fungal community will play in relation to ecosystem processes.

A next step in investigations of fungal communities is the use of metatranscriptomics, where a snapshot of the composition and relative abundance of actively transcribed genes is provided. By measuring all transcribed genes (e.g. enzyme-coding) in an environmental sample, information about the metabolic diversity, activities, and community interactions among fungal species and in their interactions with plants and bacteria may be obtained (Kuske *et al.,* 2015). By using metatranscriptomics in a gradient similar to Paper II, not only the composition of a fungal community could be assessed but also the fungal community function, providing an empirical basis to the hypothetical discussion in the previous section (section 4.4).

A final conclusion on the applicability of the results of this thesis work: support for tree retention as a means to moderate short-term and potentially also long-term negative effects of logging on the ECM fungal abundance and

diversity was found. While abundant species may be maintained at low levels of tree retention, infrequent species may be lost even at 60% of the trees retained. The Swedish forest stewardship council (FSC) standard requires 5% retention trees, and at this level was a loss of 75% of the species indicated.

Further, the results in this thesis work suggested ECM fungi to be the principle decomposers of boreal forest humus layers, and fungal communities were found to be predictable with some statistical certainty, this reinforces the importance and ability of integrating rhizosphere microorganisms, in particular ECM fungi, in forest ecosystem models.

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