Fungal communities as determinants of carbon dynamics in boreal forest soils

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Abstract

Boreal forests play an important role in the global carbon cycling, as their soils represent a substantial terrestrial sink for atmospheric CO_2 globally. In this biome, fungi are pivotal components as drivers of decomposition and nutrient cycling. Rapid development of molecular techniques increases our knowledge of fungal species distribution in different environments. However, scaling from small-scale community composition to ecosystem level processes is challenging. High throughput sequencing, enzyme assays and stable isotope analyses were used to investigate how fungal diversity and community composition responded to ecosystem fertility and forest harvesting. Radiocarbon dating and a carbon sequestration model were combined to estimate long-term carbon dynamics in soil profiles. Using data collected within a large-scale national sampling program, drivers of organic matter accumulation across the entire latitudinal range of the Swedish boreal forest were explored. Multivariate statistics and structural equation modelling were used to yield correlative relationships between environmental parameters, fungal communities and soil carbon dynamics. The re-establishing ectomycorrhizal community during stand development after clear-cutting was dominated by Atheliaceae in younger stands and by *Cortinarius* and *Russula* in older stands. The latter genera correlated positively with nutrient-mobilizing enzymes, indicating aggravated nutrient limitation. A risk that shorter rotation periods could lead to the loss of symbiosis-driven recycling of organic nutrient pools and constrain long-term forest productivity was identified. Fungal-driven enzymatic oxidation constrained belowground organic matter accumulation and promoted ecosystem fertility in an old-growth forest. Oxidative decomposition was regulated by fertility-related shifts between fungal guilds with contrasting decomposing capacities. Long-term humus build-up was driven by differences in root decomposition rates, whereas leaf litter decomposition was found to be of minor regulatory importance. Within soil profiles, carbon and nitrogen dynamics were significantly related to ecosystem fertility, root decomposition and fungal community composition. Below-ground fungal communities were important components in mediating effects of climate, soil fertility and forest management on accumulation of soil organic matter in Swedish boreal region. Overall, the results collected across different scales and ecosystem types underlined soil fungi as the principal drivers of carbon and nitrogen dynamics in boreal forest ecosystems. The thesis highlights a major potential to increase the predictive power of forest ecosystem models by including soil fungi as integrated components.

Keywords: carbon sequestration, clear-cutting, competition, decomposition, ecosystem productivity, enzymes, fungal guilds, metabarcoding, Mn-peroxidase, nitrogen cycling

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Dedication

To my parents

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 Kyaschenko, J.*, Clemmensen, K.E., Hagenbo, A., Karltun, E., Lindahl, B.D. (2017). Shift in fungal communities and associated enzyme activities along an age gradient of managed *Pinus sylvestris* stands. *ISME Journal*, 11, 863–874.

II Hagenbo, A., Kyaschenko, J., Clemmensen, K.E., Lindahl, B.D., Fransson P.* (2017). Fungal community shifts underpin declining mycelial production and turnover across a *Pinus sylvestris* chronosequence. *Journal of Ecology*, DOI: 10.1111/1365-2745.12917.

III Kyaschenko, J.*, Clemmensen, K.E., Karltun, E., Lindahl, B.D. (2017). Guild interaction within fungal communities regulates below-ground organic matter accumulation along a boreal forest fertility gradient. *Ecology Letters*, 20, 1546–1555.

IV Kyaschenko, J., Ovaskainen, O., Ekblad, A., Hagenbo, A., Karltun, E., Clemmensen, K.E., Lindahl, B.D. Back-calculating carbon and nitrogen dynamics from organic matter age profiles throughout boreal forest mor layers. (manuscript)

V Kyaschenko, J., Varenius, K., Dahlberg, A., Karltun, E., Clemmensen, K.E., Stendahl, J., Lindahl, B.D. Relationships between fungal communities and below-ground accumulation of organic matter across Scandinavian boreal forest. (manuscript)

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The contribution of Julia Kyaschenko to the papers included in this thesis was as follows:

I Participated in planning of the study. Collected samples, performed laboratory work and data analysis. Wrote the manuscript with supervisors.

II Participated in data analysis.

III Participated in designing and planning of the study. Collected samples, performed laboratory work and data analysis. Wrote the manuscript with supervisors.

IV Participated in designing and planning of the study. Performed laboratory work. Participated in data analysis. Wrote the manuscript with supervisors and input from co-authors.

V Performed data analysis. Wrote the manuscript with supervisors and input from co-authors.

Abbreviations

aP	Acid phosphatase
BG	β-1.4-glucosidase
BXD	β-1.4-xylosidase
C	Carbon
CA	Correspondence analysis
CAZymes	Carbohydrate-Active Enzymes
CBH	Cellobiohydrolase
CCA	Constrained correspondence analysis
CiBH	Chitobiosidase
DCA	Detrended correspondence analysis
ECM	Ectomycorrhiza
F1	Defined as the upper 2/3 of depth of the fragmented plant tissues in the mor layer
F2	Defined as the lower 1/3 of depth of the fragmented plant tissues in the mor layer
FACE	Free-air CO_2 enrichment
H1	Defined as the upper 2/3 of depth of more decomposed organic matter in the mor layer
H2	Defined as the lower 1/3 of depth more decomposed organic matter in the mor layer
H3	Defined as a thin slice on the transition between organic and mineral soil in the mor layer
HPLS	High performance liquid chromatography
ITS	Internal transcribed spacer
L1	Defined as structurally intact dead plant and moss litter
L2	Defined as more decomposed but still intact plant and moss litter
LAP	Leucine aminopeptidase

MAP	Mean annual precipitation
MAT	Mean annual temperature
Mn-peroxidase	Manganese peroxidase
N	Nitrogen
NAG	β-1.4-*N*-acetylglucosaminidase
NFI	National Forest Inventory
PCA	Principal component analysis
PCR	Polymerase chain reaction
SEM	Structural equation modelling
SNSI	Swedish National Soil Inventory

1 Background

1.1 The boreal forest ecosystem

Boreal forest is one of the largest biomes in the world that covers much of the landscape of Eurasia and North America. Collectively this area comprises 11% of the earth's terrestrial surface (Bonan & Shugart 1989). This biome is relatively species poor in terms of plants and its species composition is a result of a complex interaction between climatic and edaphic factors. Boreal forest ecosystems have a characteristic vegetation structure, consisting of tree and understory layers that create small-scale environmental heterogeneity and supports diverse communities of fauna and microflora (Kuuluvainen 2002). The most dominant tree species are conifers, such as spruce (*Picea*), pine (*Pinus*) and fir (*Abies*), but other tree species such as the deciduous larch (*Larix*), broadleaf birch (*Betula*) and aspen (*Populus*) are also common. Within the understory layer ericaceous dwarf shrubs (*Vaccinium myrtillus*, *Vaccinium vitis-idaea*, *Empetrum hermaphroditum*), feather mosses (*Pleurozium schreberi*, *Hylocomium splendens*) and reindeer lichens (*Cladonia* spp.) are the main components (Nilsson & Wardle 2005).

The biome is distinct by subarctic and cold continental climate with short summers, long and cold winters, and persistent snow cover. According to the traditional view, low temperatures and high soil acidity slow down the rates of nitrogen (N) mineralization, resulting in that N becomes the key growth-limiting element (Bååth & Söderström 1979; Read 1991; Tamm 1991). A continuous input of carbon (C) via needle litter and root input, compared to a more pronounced seasonal input in agricultural soils, grasslands and nemoral ecosystems, further favours N retention in soils and strengthens N limitation (Näsholm *et al*. 2013). Boreal forest ecosystems largely depend on internal recycling of N from organic stocks, implying that ecosystem C and N cycles

are tightly linked (Rastetter *et al*. 1992; Reich *et al*. 2006). In this ecosystem, evergreen species produce long-lived, thick and narrow leaves with reduced surface to limit water transpiration. As an adaptation to the short growing seasons, evergreen leaves potentially can photosynthesize already early in the spring and continue until the late fall (Lassoie *et al*. 1983). Tough leaves, together with low levels of leaf nutrient and high levels of chemical defences, help to restrain attacks by herbivores and pathogens (Mooney & Gulmon 1982). High lignin and phenol content, C:N and lignin:cellulose ratios lead to relatively slow rates of litter decomposition (De Santo *et al*. 2008; Cornwell *et al*. 2008) that, in turn, further enhance nutrient retention below ground as well as soil acidity, and together with harsh climate create a rigorous environment for both plants and soil organisms.

Podzols are the typical soils in this ecosystem and are characterized by clear vertical stratification with a distinct organic (mor) layer. High acidity disfavours earthworms, so that a scarcity of soil mixing further enhances soil stratification and formation of layers different in structure, texture and stage of decomposition within the organic topsoil.

1.2 Fungal communities in boreal forest

Variation in microbial community composition is an important predictor of ecosystem processes like organic matter decomposition and nutrient cycling (Strickland *et al*. 2009). Environmental conditions, such as availability and forms of N, pH and litter C:N ratios, are important drivers that shape microbial communities (Högberg *et al*. 2006). In boreal forest ecosystems, soil acidity and low C:N ratio of the litter favour fungi (Lundberg *et al*. 2001; Rousk *et al*. 2009) that relative to bacteria dominate the soil microbial biomass (Bailey *et al*. 2002; Buée *et al*. 2009) and, therefore, are considered to be pivotal for C and nutrient cycling. Depending on their C sources, fungi can be divided into saprotrophs, obtaining metabolic C from dead organic matter, and biotrophs, part of which form mycorrhizal symbiosis and obtain photosynthetic C from their host plants.

1.2.1 Saprotrophic fungi

Saprotrophs are free-living decomposers that usually dominate in freshly deposited litter layers (Schneider *et al*. 2012) and are the main producers of extracellular hydrolytic enzymes (de Boer *et al*. 2005; Baldrian *et al*. 2011). By production of these enzymes, saprotrophs are able to degrade biopolymers of plant cell walls (cellulose, hemicellulose and pectin), cell wall components of

fungi (chitin and glucans), and other organic substances (e.g. starch and proteins). Saprotrophic white-rot basidiomycetes are the only organisms that, by producing oxidative enzymes (lignin peroxidase and Mn-peroxidase), are capable to degrade lignin in plant litter and wood (Baldrian 2008). Some saprotrophic basidiomycetes form large mycelial networks to explore and colonize patches of new resources and are thereby well adapted to heterogeneous terrestrial environments (Boddy *et al*. 2009). During decomposition, such saprotrophs may retain and reallocate N within the litter and their mycelial networks and thereby overcome local resource limitation (Boberg *et al*. 2010; 2014).

Litter decomposition is a complex process and saprotrophic community composition changes rapidly as decomposition progresses. Fungi from the Ascomycota phylum are generally less efficient decomposers (Boberg *et al*. 2011) that selectively decompose cellulose over lignin. Such fungi have been found to dominate during early phases of litter decomposition or when disturbance creates more easily degradable substrates (Lindahl *et al*. 2010). With time (approximately within a year) they become gradually replaced by basidiomycetes (Baldrian 2008; Schneider *et al*. 2012; Voříšková & Baldrian 2013) that are capable to produce a wider variety of extracellular enzymes, compared to ascomycetes (Osono 2007; Baldrian 2008).

1.2.2 Mycorrhizal fungi

Mycorrhiza is a mutualistic symbiosis between a fungus and plant roots. Ectomycorrhizal symbiosis is formed with trees species within the families Pinaceae, Fagaceae, Betulaceae, Nothofagaceae and some others (Tedersoo *et al*. 2010). Although the proportion of ectomycorrhizal plant species is relatively low (only 2%) (Smith & Read 2008), they dominate forest ecosystems in different habitats covering large areas across the globe and, therefore, are of great ecological and economical importance. Using photosynthetically derived carbohydrates from their host trees, ectomycorrhizal fungi take up N and other nutrients from organic matter and transfer them to their host plants (Smith & Read 2008). In the generally N limited boreal forest ecosystems (Tamm 1991), N uptake via symbiotic fungi plays a decisive role in tree establishment and growth (Tedersoo *et al*. 2010).

The majority of ectomycorrhizal fungi belong to Basidiomycota, with some exceptions within Ascomycota (Smith & Read 2008). Ectomycorrhizal fungi colonize lateral roots of their host trees, forming a thick mycelial mantle around the roots and a Hartig net around the epidermal root cells (Bonfare & Genre 2010). Ectomycorrhizal fungal taxa differ in morphology of their

mycelia, which is widely used to identify and classify fungal ecological strategies with respect to substrate exploitation, resource translocation and resistance to decomposition and harsh environmental conditions (Agerer 2001).

Fungi within Ascomycota form ericoid mycorrhizal association with plants within the family Ericaceae (Smith & Read 2008). Ericoid mycorrhizal plants have a wide distribution and are especially abundant in habitats characterized by harsh environmental conditions, such as high soil acidity, nutrient limitation, low temperatures and drought (Cairney & Meharg 2003). As an adaptation to such conditions, ericoid mycorrhizal fungi usually form short melanized, long-lived and resistant hyphae within, and protruding from, the epidermal cells of the plant host (Smith & Read 2008). Dominance of root-associated ascomycetes has been proposed to lead to humus accumulation, compared with forest where ectomycorrhizal basidiomycetes dominate (Clemmensen *et al*. 2015). Given their importance in C and N cycles globally (Adamczyk *et al*. 2016), ericoid mycorrhizal fungi remain understudied (Leopold 2016), as they do not form distinct morphological structures (like the rhizomorphs present in ectomycorrhiza) that enable identification (Godzelak *et al*. 2012). Using high-throughput DNA sequencing, it has recently been shown that ericaceous plant-fungal interactions display high symbiont/host preferences (Toju *et al*. 2016) and have community network architecture similar to that of ectomycorrhizal plant-fungal interactions (Bahram *et al*. 2014).

During fungal evolution, mycorrhizal symbioses have arisen several times (Smith & Read 2008), and ectomycorrhizal fungi, together with brown rot lineages, have lost most of the extracellular enzymatic apparatus of their white rot ancestors (Kohler *et al*. 2015). Recent studies, however, suggest that under strong nutrient limitation, when nutrients are largely bound in complex organic forms inaccessible for direct plant uptake, some ectomycorrhizal genera may use energy from host-derived sugars to power nutrient mobilisation from complex organic matter (Read & Perez-Moreno 2003; Lindahl & Tunlid 2015). In this perspective, the genus *Cortinarius* has been highlighted as particularly efficient in acquiring nutrients from organic pools, as some species within this genus have retained class II peroxidase genes, which potentially enable them to decompose complex organic matter (Bödeker *et al.* 2009; 2014). It has been proposed that ectomycorrhizal fungi mine organic matter for N and other nutrients (Bödeker *et al*. 2014; Shah *et al*. 2016), thereby acting as biotrophically driven decomposers (Lindahl & Tunlid 2015). A direct involvement of ectomycorrhizal fungi in decomposition has been suggested as a possible mechanism to sustain forest productivity and mitigate ecosystem retrogression, by preventing organic matter accumulation and associated N

retention (Clemmensen *et al*. 2015; Baskaran *et al*. 2017). Using data from Swedish National Soil Inventory (SNSI) it has been shown that, across the entire boreal latitudinal range, the concentration of exchangeable manganese was the best predictor of C stock in the mor layer with a negative correlation (Stendahl *et al*. 2017). Since Mn-peroxidase is exclusively produced by Agaricomycetes (including both saprotrophs and mycorrhizal fungi) (Floudas *et al*. 2012), the results of this study highlight the central role of Agaricomycetes and their unique capacity to oxidize organic matter in boreal forest ecosystems.

It has been estimated that in boreal forest ecosystem, ectomycorrhizal mycelium constitutes a substantial part of the total fungal (80%) and microbial (30%) biomass (Wallander *et al*. 2001; Högberg & Högberg 2002). Up to 20% of net primary production is allocated to ectomycorrhizal fungi (Hobbie 2006), suggesting that ectomycorrhizal fungal mycelium is an important pathway of C into organic matter pools (Ekblad *et al*. 2013). Using both model- and empirical-based approaches, it has been shown that mycorrhizal fungi may contribute significantly to long-term C sequestration belowground (Orwin *et al*. 2011; Clemmensen *et al*. 2013), but also to retention of N in organic stocks (Näsholm *et al*. 2013; Clemmensen *et al*. 2015). Altogether, these findings emphasize the importance of fungal communities in forest C and N cycling and call for a better mechanistic understanding of factors shaping fungal community composition, diversity, spatial and temporal dynamics as well as ecophysiological properties under changing environmental conditions and disturbances.

1.2.3 Community ecology

In ecology, a community is an assemblage of different species in different relative abundances doing different things and co-occurring in time and space. Interactions between species within communities shape and regulate fundamental ecosystem processes, such as nutrient cycling, decomposition, organic matter accumulation and primary production. Generally, the assembly of species into a community is constrained by three main processes: dispersal limitation, environmental filtering and internal interaction (Begon *et al*. 2005). Successful establishment and subsequent survival of a fungal species is not a completely random process (neutral theory, Hubbell 2001), but could be predicted, to some degree, by unique site and weather conditions, as well as spore properties (e.g. hydrophobicity) (Peay & Bruns 2014). Dispersal limitation (Cline & Zak 2014), together with priority effects (Kennedy *et al*. 2009) has a significant effect on short-term community assembly at the

landscape level (Peay & Bruns 2014). However, environmental filtering could further shape the ecological dynamics (survival and competitive success) of newly arrived community members.

Response traits define organism suitability in the community, whereas effect traits determine community influence on ecosystem functioning (Lavorel & Garnier 2002). For example, in boreal forest, form (organic vs inorganic) and availability of N are two important environmental constrains of fungal community composition (Franklin *et al*. 2014; Sterkenburg *et al.* 2015). Fungi vary in their capacity to utilize different sources of N and tolerate different N levels (both natural and deposited). These response traits are tightly linked, as fungi efficient in inorganic N uptake ("early" fungi) (McGuire & Treseder 2010) generally are less capable of organic N utilization, but can tolerate high N levels. In contrast, "late" fungi (McGuire & Treseder 2010) are generally more efficient decomposers of organically bound N but sensitive to high levels of inorganic N. Ectomycorrhizal fungi vary in their ability to produce oxidative enzymes (Bödeker *et al.* 2009; 2014) to mobilise nutrients from complex organic matter (Read & Perez-Moreno 2003; Lindahl & Tunlid 2015). Such effect traits could have a significant effect on key ecosystem processes such as decomposition and rates of nutrient cycling. The distinction between response and effect traits is not always straightforward, as some response traits are, in fact, also effect traits. A response trait that has been selected for by environmental conditions and is highly expressed in the community could well be an effect trait. The recognition of traits that are both response and effect traits provides a useful tool to investigate ecosystem responses to environmental factors as mediated by community shifts (Koide *et al*. 2014).

Based on a theoretical framework developed by Grime (1977) and adapted for fungi by Cooke & Rayner (1984), fungi may be divided into different ecological strategies. This niche separation of fungal species is based on their differences in responses to environmental parameters (Crowther *et al*. 2014; Sterkenburg *et al*. 2015). In a stressful environment (e.g. low N availability, acidic soil, drought and low temperatures) stress-tolerant (S-strategic) fungi dominate. These fungi are characterized by slow growth and dispersal, long life span, melanised cell walls, low biomass and low mycelial turnover. The competitive (C strategists) are favoured by resource availability that enables high biomass production, and are characterized by efficient resource exploitation and reallocation over a prolonged time. In contrast to the mentioned strategies, short lived, rapidly dispersing and fast growing ruderals (R strategists) occupy disturbed habitats rich in easily available resources. This framework of ecological strategies may be used to relate fungal communities in

particular environments (response traits) to their ecophysiological properties (effect traits), and, consequently, to ecosystem processes (Koide *et al*. 2014).

Within communities, species coexist in space and time and competitive interaction between species and guilds are commonly intense (Boddy 2000; Kennedy 2010). There are two ways that species may affect their competitors negatively: interference and exploitation competition (Keddy 2001). Interference competition occurs when organisms interact directly, i.e. when one individual inhibits another, competing for space, whereas exploitation competition implies indirect interaction via resource depletion by one organism on the expense of its competitor. Competition can restrict the generally wider fundamental niche of a species (a combination of resources and conditions were a species can survive) to a narrower realized niche (a combination of resources and condition were species actually exists and can compete successfully). Similarly, competition may occur between different species assemblages, like taxonomic or functional guilds. For example, the competitive balance between fungal guilds has been proposed to shift in response to changing environmental conditions, such as climate, nutrient availability and forest management disturbances with potential effects on ecosystem C and nutrient cycling (Fernandez & Kennedy 2016).

1.2.4 Spatial separation of fungal guilds

In a stratified boreal forests soils, fungal guilds occupy different organic layers, with litter saprotrophs dominating in the freshly deposited above-ground litter, whereas mycorrhizal fungi are more abundant in the well-decomposed humus layer (Lindahl *et al*. 2007; Baldrian *et al*. 2012; Clemmensen *et al*. 2015). This vertical stratification of saprotrophic and mycorrhizal fungal guilds may be linked to their different C supply (saprotrophs obtain metabolic C from freshly fallen litter while mycorrhizal fungi rely on their host plants) (Lindahl *et al*. 2007); however, interference competition may be an additional driver of depth partitioning. It has been proposed that saprotrophic and mycorrhizal fungi have partly overlapping fundamental niches. However, since mycorrizal fungi receive metabolic C from their plant host, they have a competitive advantage over more efficient saprotrophic fungi and supress decomposers in deeper organic horizons (Bödeker *et al.* 2016). Dominance of mycorrhizal fungi with generally limited decomposer capacity (Kohler *et al*. 2015) on expense of more efficient saprotrophic decomposers has been proposed to facilitate C accumulation (a so-called “Gadgil effect”; Gadgil & Gadgil 1975; Averill *et al*. 2014; Averill & Hawkes 2016). However, evidences of mycorrhizal effects on organic matter dynamics across different forest ecosystems are inconsistent

(e.g. Brzostek *et al*. 2015; Lin *et al*. 2017), and the generality of ectomycorrhizal suppression of saprotrophic decomposition is not yet well established (Fernandez & Kennedy 2016). It has been proposed that mycorrhizal constrains on saprotrophic decomposition vary depending on environmental conditions, such as soil fertility, soil stratification and belowground C allocation (Fig. 2 in Fernandez & Kennedy 2016).

1.3 Carbon cycling in boreal forest

Forest ecosystems play an important role in global C cycling due to their large C stocks, but also due to their high potential to mitigate CO_2 emissions. Tropical and boreal forests store most of the terrestrial C, however, the nature of this C is fundamentally different: in tropical forest the majority of the C (60%) is stored in tree biomass, whereas in boreal forest it is stored in the soil (Pan *et al*. 2011). Whereas the significance of boreal soil C dynamics is little disputed, controlling factors and responses to projected climate change are highly uncertain (Hobbie *et al*. 2000; Stocker *et al*. 2013). In current ecosystem climate models C dynamics is described as a balance between above-ground CO_2 uptake by photosynthesis and below-ground loss by heterotrophic respiration, relaying on the assumption that the above-ground and below-ground biological processes can be conceptualized and analysed separately. Although this simplified approach could be useful in model design, there are accumulating evidences suggesting significant links between above- and below-ground processes, emphasizing importance of interactions between below- and above-ground processes in constructing realistic ecosystem C models (Heimann & Reichstein 2008).

The boreal region has been highlighted as one of the most sensitive regions to climate variability (Seddon *et al*. 2016). Despite numerous studies of possible feedbacks and their directions induced by climate change, no consensus has been established yet. A number of research published in high-profile journals propose opposite views of possible effects of temperature rise on C dynamics in boreal forest ecosystem, suggesting both a positive feedback (accelerated decomposition will lead to increase in productivity), and a negative feedback (productivity will exceed decomposition) (Heath *et al*. 2005; Knorr *et al*. 2005; Davidson & Janssens 2006).

1.3.1 Potential feedback between C and N

Forest ecosystems largely depend on internal recycling of nitrogen (N), and ecosystem C storage is tightly linked to the N cycle (Rastetter *et al*. 1992;

Reich *et al*. 2006). Low N availability has been found to progressively limit CO_2 fertilisation effects on plant biomass (Reich *et al*. 2006). Strong N limitation can also influence decomposition of soil organic matter negatively, so that slow turnover of below-ground organic pools could lead to low N availability, resulting in a feedback that further constrains ecosystem fertility and productivity (Wardle *et al.* 2012; Clemmensen *et al.* 2013). In contrast, in more fertile forests, rapid turnover of below-ground organic pools enable redistribution of N from soils to plant biomass, leading to high primary production and a sustained above-ground sink for C (as trees have higher C:N ratios than soil) (Rastetter *et al*. 1992; Norby & Zak 2011). It is important to establish the bidirectional mechanistic links between belowground decomposition and ecosystem fertility, as their interplay enables feedback interactions, which regulate organic matter accumulation, ecosystem fertility, productivity as well as responses to disturbances.

1.3.2 Forest C balance and disturbances

Stand-replacing disturbances, both natural (fires, pest outbreaks) and anthropogenic (clear-cutting), as well as subsequent forest regeneration, play an important role in forest C dynamics, although it is difficult to assess the magnitude and direction of these processes (Jandl *et al*. 2007). Using net ecosystem C exchange, it has been shown that during the first few years after tree harvest, the forest is usually a net C source (Magnani *et al*. 2007; Nave *et al*. 2009). With increasing age and gradual tree regeneration, the forest becomes a net C sink with a broad maximum in maturing forests, however, the strength of the C sink usually declines as forest becomes older (Magnani *et al*. 2007).

For a long time, old-growth forests were assumed to be C neutral (Kira & Shidei 1967), but a recent analysis of C flux estimates showed that old-growth forests steadily sequester C for hundreds of years and a large quantity of this C will return to the atmosphere in case of disturbance (Luyssaert *et al*. 2008). It was further proposed that in old-growth forests the above-ground biomass increase is more or less continuous, as losses of individual trees (due to lightning, wind storm, insect or fungal attacks) are replaced by recruitment of new seedlings waiting for favourable conditions. Relatively slow release of C from dead wood decomposition is, therefore, compensated by faster regeneration of young trees (Luyssaert *et al*. 2008). Such small-scale disturbances create a patchy landscape of habitats with different ages, vegetation types and soil properties, and the understanding of driving factors regulating C and N dynamics in those habitats is largely lacking.

1.4 Shifts along gradients

Environmental parameters are usually spatially and temporarily variable, and multiple variables are often highly correlated. Therefore different gradients, both natural and anthropogenic, constitute a useful tool for investigation of community responses to environmental changes. Distribution of biomes along global environmental gradients has been related to the dominant mycorrhizal associations (i.e. ecto-, ericoid- and arbuscular mycorrhiza) and nutrient cycling characteristics (Read & Perez-Moreno 2003). Different mycorrhizal associations may, in turn, have opposite effects on fundamental ecosystem properties, nutrient retention and C sequestration (Phillips *et al*. 2013; Averill *et al*. 2014; Clemmensen *et al*. 2015; Lin *et al*. 2017). Measurements on fungal community variation along different environmental gradients provide important information on fungal niche separation. Inclusion of novel understanding and data on the composition and activity of fungal communities may enable better predictions on ecosystem directional development under changing environmental conditions (Crowther *et al.* 2015).

1.4.1 Natural fertility gradients

Fungal communities are known to respond to different gradients, such as nitrogen deposition gradients (Lilleskov *et al*. 2001; 2002; Kjøller *et al.* 2011), a leaf degradation gradient (Peršoh *et al*. 2013), soil fertility gradients (Nilsson *et al.*, 2004; Toljander *et al*. 2006) and global climatic patterns (Tedersoo *et al*. 2014). Nitrogen availability has been highlighted as a main regulator of conifer mycorrhizal communities across complex environmental gradients (Cox *et al*. 2010), and soil fertility (a combined measure of pH, inorganic N and litter C:N ratio) has been found to be the main predictor of fungal community composition in soils and litters of boreal old-growth forests (Clemmensen *et al.* 2015, Sterkenburg *et al*. 2015). While trying to establish main drivers of community composition, it is important to consider that the importance of different factors may vary greatly depending on scale (Willis & Whittaker 2002). On a local scale (<10 m), fungal community composition might be driven by disturbance, recruitment of new individuals and competition for space and resources, while on a larger scale, climatic and edaphic factors might be the better predictors (Lilleskov & Parrent 2007; Tedersoo *et al*. 2014; Hiiesalu *et al*. 2017).

1.4.2 Forest age gradient

Forest management affects fungal communities mainly by changing vegetation, nutrient availability, soil structure and chemistry and litter quantity and quality (Jurgensen *et al.* 1997; Andersson & Östlund 2004). In particular, clear-cutting leads to drastic changes in soil microbial communities (Hartman *et al.* 2012). Not surprisingly, clear-cutting has detrimental short-term effects on ectomycorrhizal fungi, but with increasing forest age and forest regeneration, mycorrhizal communities recover and mycelial production reaches its peak in young stands (10-30 years) during stand canopy closure (Simard 2009; Wallander *et al*. 2010). Recently, it has been shown that mycelial production and turnover is highest on younger sites (Hagenbo *et al.* 2017) and decline with forest age. Ecosystem responses to clear-cutting disturbances are mechanistically complex and there is a lack of studies that evaluate fungal taxonomic and functional diversity during the entire stand development, e.g. from recent clear-cuts to old-growth forests.

1.5 Methods in fungal ecology

1.5.1 Molecular identification of fungal communities

Methodological limitations have constrained understanding of fungal communities and their ecological roles, as available methods, such as DNA fingerprinting and cloning, had a limited capacity to identify fungi at the species level, complicating assignment to functional guilds (Matheny *et al*. 2006). Recent advances in large-scale molecular methods (Lindahl *et al*. 2013; Nguyen *et al*. 2015) enabled profiling of fungal community with reasonably high resolution. In our projects we used single molecule real time (SMRT) sequencing from Pacific Biosciences as previous generation platforms (IonTorrent and Illumina) are biased to sequence length differences by preferential sequencing of shorter amplicons. The internal transcribed spacer (ITS) region of the ribosome-encoding operon (Gardes & Bruns, 1993) has been accepted as a standard fungal barcode, as it has higher interspecific variation compared to its intraspecific variation (Schoch *et al*. 2012). The development of new fungus-specific primers (fITS7, gITS7 and fITS9) (Ihrmark *et al.* 2012; Clemmensen *et al*. 2016) allows amplification of the ITS2 region, which is much shorter (250-350 base pairs), compared to the ITS1, 5.8S and ITS2 fragment amplified using the ITS1f-ITS4 primer combination (Ihrmark *et al.* 2012). Shorter PCR fragments, in turn, increase

PCR efficiency, reduce the number of PCR cycles required, reduce chimera formation (Kanagawa 2003) and reduce problems with discrimination against longer PCR fragments. The primer fITS9 selects strongly in favour of fungi but mismatch with most plant templates, whereas gITS7 amplifies many plants (except conifers) but also yields the most diverse amplicon communities (Ihrmark *et al*. 2012). In this thesis both primer combinations gITS7-ITS4 and fITS9-ITS4 were elongated with unique identification tags enabling control over tag switching during high-throughput sequencing (Carlsen *et al*. 2012).

In this thesis sequences were analysed using the bioinformatics pipeline SCATA (https://scata.mykopat.slu.se). Sequences passing quality control were clustered into species hypotheses (a term used for a taxa discovered in clustering on different similarity thresholds (Kõjalg *et al*. 2013)) using single-linkage clustering with a 1 or 1.5% threshold distance for sequences to enter clusters. Names were assigned to species with at least 98% similarity to identified reference sequences in the UNITE database (Abarenkov *et al*. 2010).

These novel molecular methods enable more detailed and less biased identification of complex fungal communities to the species level and improve our knowledge of niche separation of fungal species in relation to their ecological strategies (Crowther *et al.* 2014; Sterkenburg *et al*. 2015). Molecular identification of fungal taxa based on DNA extracts is a useful tool, as it enables identification of fungi directly from environmental samples, irrespective of fungal morphology and growth stage (Schoch *et al*. 2012). We can now address questions about how communities of soil fungi on a finer spatial scale respond to environmental drivers (such as soil fertility) in unmanaged old growth forest and how intense forestry (such as clear-cutting) shifts fungal community composition during stand development in managed forest.

1.5.2 Fungal biomass

Species relative abundance might not give the correct picture about fungal species in different samples, as it does not represent the actual fungal biomass. Ergosterol is the primary sterol of the cell membrane of fungi and is generally used as a marker of living fungal biomass (Nylund & Wallander 1992; Ekblad *et al*. 1998). Similar to chitin, the ergosterol content has been found to differ between different fungal species (Bermingham *et al*. 1995). However, when comparing with a qPCR approach targeting DNA markers, the use of ergosterol appeared to be more reliable for fungal biomass estimation (Baldrian *et al*. 2013). Using the established high performance liquid chromatography (HPLS) methods and a rough conversion factor of 3 mg ergosterol per mg of

fungal biomass (Salmanowicz *et al*. 1989) yield fast results. In our projects, we used the ergosterol content of soil and litter samples to estimate living fungal biomass and relate it to the relative abundance of fungal species and guilds as analysed by DNA markers. To capture ectomycorhizal fungal community and quantify biomass of extraradical mycelium (Ekblad *et al*. 2013; Wallander *et al*. 2013), ingrowth mesh bags filled with sand (to discriminate against saprotrophs) were used.

1.5.3 Stable isotopes

Isotopes are variants of an element that differ in the number of neutrons. Carbon has three isotopes (^{12}C, ^{13}C and ^{14}C), whereas N has two isotopes (^{14}N and ^{15}N). Isotope ^{14}C is radioactive and its use in research is described in section 1.5.5. The other natural isotopes of C and N are stable and occur in approximate proportion of 99:1 (^{12}C:^{13}C and ^{14}N:^{15}N respectively). The amounts of the different isotopes can be measured by mass spectrometry and be compared to standards – Pee Dee Formation belemnite ($CaCO_3$) for C and atmospheric N_2 for N:

$$\delta E = [(R_{sample}:R_{standard}) - 1],$$

where E is an element (C or N) and R is the ratio of ^{13}C:^{12}C or ^{15}N:^{14}N in the sample or standard.

Stable isotope analyses may provide additional information about biogeochemical mechanisms of ecosystem C and N cycling (Ehleringer *et al*. 2000; Robinson 2001), as different biological, chemical and physical processes lead to fractionation between heavier and lighter isotopes. Therefore, analyses of changes in the ratio between stable isotopes of C and N (^{13}C:^{12}C and ^{15}N:^{14}N) enable tracing of matter and energy flows through biological systems and evaluation of details of soil and ecosystem processes (Tiunov 2007). Compared to other techniques (culture studies, stable isotope labelling), measurements of isotopic natural abundances are advantageous because they are non-invasive, and therefore reflect processes under actual soil conditions (Hobbie & Hobbie 2008).

In forest ecosystems, the relative content of ^{13}C ($\delta^{13}C$) of soil organic matter in most profiles increases with soil depth by 1-3‰ (Boström *et al*. 2007). Similarly, the age of soil organic matter age increases with depth (Trumbore 2000). Therefore it is generally assumed that the observed depth-dependent increase in $\delta^{13}C$ is related to organic matter formation processes (Billings & Richter 2006). The differential enrichment in $\delta^{13}C$ among ecosystems has been explained by four different mechanisms (Ehleringer *et al*.

2000): 1) burning of ^{13}C-depleted fossil fuels leading to lower $\delta^{13}C$ in younger material closer to the surface (Trolier *et al*. 1996), 2) preferential microbial respiration of ^{13}C-depleted compounds leading to increased $\delta^{13}C$ in microbial biomass, 3) preferential microbial decomposition of ^{13}C-depleted litter and soil organic matter leading to accumulation of recalcitrant material with higher $\delta^{13}C$ and 4) progressive incorporation of ^{13}C-enriched microbial residuals into the soil matrix. Ectomycorrhizal mycelium is generally ^{13}C-enriched as a result of fractionation during the transfer of C from the host to the fungus (Högberg *et al*. 1999).

In boreal forests, where N cycling is tight (with little or no external inputs and small losses), $\delta^{15}N$ usually increases with increasing soil depth (Högberg *et al*. 1996). Similar to $\delta^{13}C$, there are several possible mechanisms behind this pattern. Preferential transfer of ^{15}N-depleted N from mycorrhizal fungi to their hosts (Högberg *et al*. 1996; Clemmensen *et al*. 2013) is considered as one of the main factors. This leads to accumulation of ^{15}N-depleted plant litter on the surface, while ^{15}N-enriched organic matter of fungal origin accumulates at depth (Hobbie & Ouimette 2009). Secondly, preferential decomposition of ^{15}N-depleted compounds and preservation of ^{15}N-enriched compounds (assuming no or little soil mixing) could also explain the general accumulation of ^{15}N-enriched organic matter deeper in the soil profile. Finally, nitrification and denitrification processes in soils could contribute to soil ^{15}N enrichment, but might be of less importance in N-limited systems (Hobbie & Ouimette 2009).

In our projects, we tried to explain the enrichment in stable isotopes (^{15}N and ^{13}C) from factors such as soil properties and fungal species composition. In addition to DNA based methods and enzyme analysis, which provide a picture of the fungal community and its activity in a soil at the time of sampling, stable isotopes measurements reflect the integrated result of C and N flows across decades. Using a natural fertility gradient, we investigated possible influences of forest nutrient status on isotopic fractionation in soil profiles, in order to draw conclusions about fungal ecology and long-term N and C dynamics in the ecosystems.

1.5.4 The C:N ratio

The carbon-to-nitrogen (C:N) ratio of soil organic matter is used to describe patterns of N retention, immobilization and mineralization. Together with soil pH, the C:N ratio is an important determinant of microbial community composition (Högberg *et al*. 2007). Since fungi can tolerate relatively wide C:N ratios in their tissues, they grow more efficiently than bacteria on low-N (high C:N) substrates (Robertson & Groffman 2007). As a result of progressing

decomposition and C respiration the C:N ratio is commonly decreasing with soil depth (Berg & McClugherty 2003). It has been found, however, that in boreal forest humus the C:N ratio increased significantly in deeper organic layers (Lindahl *et al*. 2007). Since mycorrhizal fungi dominated in the studied soil profiles, the deeper increase in C:N ratio was attributed to selective N removal, driven by plant allocation of recently fixed C to mycorrhizal fungi. At the scale of forest types, it has been shown that the fungal community composition correlates well with soil acidity and C:N ratio, shifting from ascomycete-dominated communities in the nutrient poor (high C:N) forest sites to higher abundance of basidiomycetes in the rich (low C:N) sites (Sterkenburg *et al*. 2015). Thus, shifts in C:N ratios through soil profiles and across environmental gradients seem to be important in shaping mycorrhizal communities and their activity, but not much is known about further effects of the C:N ratio on organic matter dynamics.

1.5.5 Radiocarbon dating

Radiocarbon (^{14}C) is one of the tools to study long-term dynamics of soil C pools, and could be used as an indicator of seasonal and annual changes in the sources of respired C (Trumbore 2000). ^{14}C is measured direct by counting the number of ^{14}C and ^{12}C atoms in a sample via accelerator mass spectrometery. ^{14}C is a cosmogenic nuclide that is constantly created in the atmosphere, however, during nuclear weapons testing in the early 1950s, the ^{14}C:^{12}C ratio in Northern Hemisphere atmospheric CO_2 nearly doubled. After the Test Ban Treaty of 1963 the amount of ^{14}C in the atmosphere has declined due to absorption by terrestrial and aquatic C pools (Peterson & Fry 1987) and the bomb-produced ^{14}C became useful as a universal isotope tracer of CO_2 exchange between global C reservoirs (Levin & Hesshaimer 2000). As detailed records of the amounts of ^{14}C in the atmosphere since the bomb peak are available, age measurements of recent C (number of years since photosynthetic fixation) in organic soil layers can be made with high accuracy (Trumbore 2009). Combining the ^{14}C signal from fine soil layers with data about C pools, litter production and decomposition, C flows can be estimated (Franklin *et al*. 2003) and related to fungal community composition as well as environmental variables. Recently, ^{14}C analysis was used to determine the age of fixed C in the soil profiles of forest islands (Clemmensen *et al*. 2013). Data on C distribution in vertical soil layers were incorporated into a mathematical model that estimated the proportion of younger root-derived C (compared to older litter-derived C) in the deeper soil profiles. Parameterization of the model using ^{14}C measurements showed that root-mediated C input was the major

driver of long-term carbon sequestration in those study system (Clemmensen *et al*. 2013). In this thesis, we further developed this C sequestration model and estimated long-term (>100 years) decomposition rates for root-derived C in old-growth coniferous forest. The average turnover rate of the humus C pool was assessed against a null-hypothesis predicted from one-year mass loss of above-ground litter.

1.5.6 Extracellular enzyme activities

By producing extracellular enzymes, microorganisms degrade complex biopolymers, such as cellulose and chitin, into simple metabolic compounds, such as glucose and amino acids. Hydrolases (enzymes that catalyse the hydrolysis of chemical bonds) and oxidative enzymes (catalyse oxidation) are two major groups of extracellular enzymes. Saprotrophs are the main producers of hydrolytic enzymes, such as cellulases, proteases and chitinases (Baldrian *et al*. 2011). Cellulose of dead plant biomass is decomposed by cellulases that cleave the β-1.4 bond in the cellulose chain. Hemicellulose has more complex structure and its decomposition usually involves a wide range of hydrolytic enzymes such as cellulases, xylosidases, glucosidases and others. Wood-degrading fungi are able to produce chitinolytic enzymes to degrade their own mycelium as well as dead mycelium of primary colonizers, thereby facilitating efficient utilization of scarcely available N (Lindahl & Finlay 2005). It has also been shown that a wide range of ectomycorrhizal fungi has the genetic potential to produce N-acetylglucosaminidases that is a part of the chitin-degrading machinery (Lindahl & Taylor 2004). Further, some mycorrhizal fungi have been found to produce chitinases, presumably to obtain nutrients from dead fungal tissues (Buée *et al.* 2007). With progressing decomposition, the proportion of non-hydrolysable organic pools increase (Berg & McClaugherty 2014) and oxidation by extracellular peroxidases and phenol oxidases is needed to progress with decomposition of structurally more complex organic matter (Hatakka 1994; Sinsabaugh 2010). Oxidative enzymes evolved specifically within the Agaricomycetes (Basidiomycota) and are essential for decomposition of complex, phenolic macromolecules, such as lignin (Floudas *et al*. 2012). Being able to degrade lignified tissues, litter-degrading Agaricomycetes are generally more efficient decomposers than ascomycetes, which largely lack ligninolytic capacity (Boberg *et al*. 2011). During their early evolution from saprotrophic ancestors, most ectomycorrhizal groups (similar to litter-decomposing basidiomycetes, the majority of ectomycorrhizal fungi belong to the Agaricomycetes) lost their capacity to decompose organic matter (Kohler *et al*. 2015). However, some

ectomycorrhizal taxa have retained the ability to oxidaze complex organic matter in similar manners to brown- and white-rot wood decomposers (Bödeker *et al*. 2009; 2014; Rineau *et al*. 2012; Shah *et al*. 2016), thereby acting as biotrophically driven decomposers (Lindahl & Tunlid 2015).

Boreal forest ecosystem accumulates dead organic matter on the soil surface, resulting in distinct vertical soil stratification, and accumulation of non-hydrolysable organic matter at depth (Lindahl *et al*. 2007). With progressing decomposition, enzyme activities are expected to shift from prevalence of hydrolytic enzymes in freshly fallen litter to dominance of oxidative enzymes in deeper layers. Enzyme assays of bulk organic material collected at different depths and related to fungal communities in the substrate therefore provide valuable information on the ecophysiology of various parts of the fungal community and increase our understanding of links between nutrient availability, fungal community, and ecosystem processes.

Enzyme assays are designed to simulate the environmental enzyme activity in laboratory settings. In this thesis hydrolytic enzymes were measured fluorometrically by adding enzyme-specific substrates to soil slurries. After one hour of incubation the release of a fluorescent dye in an enzyme-catalyzed reaction was measured using a microplate fluorometer with 365 nm excitation and 450 nm emission filters. The activity of oxidative enzymes was quantified spectrophotometrically by measuring light absorbance at 420 nm (for laccase) and 590 nm (for Mn-peroxidase) as a beam of light passed through sample solution. Laccase activity was measured by the ABTS (2.2-azino-bis(3-ethylbenzoline-6-sulphonate) oxidation. Mn-peroxiadse was measured by the DMAB (3-dimethylaminobenzoic acid) and MBTH (3-methyl-2-benzothiazolinone hydrazine hydrochloride) oxidation. The actual activity was calculated as a difference between activities in the assay mixture with and without EDTA (ethylenediaminetetraacetic acid).

1.5.7 Statistical methods

To investigate correlations between several fungal species and environmental variables multivariate statistics were used. Correspondence analysis (CA) is used to visualize differences in fungal communities across different samples, as species are not related to explanatory variables (Ramette 2007). The analysis is based on species and sample scores along the ordination axis scores that are assigned based on the weighted averages of the species relative abundances and the weighted averages of samples where these species occur. To offset artificial patterns in data representation, usually referred to as “horse shoe” or

“arch” effect, detrending is applied. The analysis is therefore called detrended correspondence analysis (DCA).

In canonical correspondence analysis (CCA) CA is complemented by a linear regression analysis and is used to test the significance of correlative relationships between fungal communities and selected explanatory variables. For this, Monte Carlo permutation test is applied.

Including correlating variables into linear regression could introduce bias in regression coefficient variance. Principal component analysis (PCA) could be used to combine all significantly correlated variables into one variable that contains most of the important information. PCA transforms a number of correlated variables into a smaller number of uncorrelated variables called principal components. The first principal component accounts for most of the variation in the data set and is usually used to derive an index that describes the variation in all combined variables.

Interactions between fungal community and the environment as well as between different environmental variables usually are rather complex. Structural equation modelling (SEM) has been shown to have a capacity for testing multiple hypotheses (Grace 2006). SEM represents a useful tool to illustrate complex networks of possible interactions but also to test direct and indirect relationships (Eisenhauer *et al*. 2015). In this thesis we used piecewise SEM (Lefcheck 2016) in which a diagram of hypothesised relationships is translated into a set of linear regressions that are tested individually (Lefcheck 2016). The method has a number of limitations, one of which is impossibility of reciprocal relationships evaluation in the same model (Lefcheck 2016).

2 Objectives

The overall aim of this thesis was to obtain a better mechanistic understanding of how fungal communities take part in the regulation of ecosystem processes, such as nutrient cycling and organic matter accumulation, in boreal forest soils. The studies were designed to investigate how climate, stand properties and forest age shape fungal communities and their functional properties. Observed shifts along gradients in environmental drivers were further linked to organic matter dynamics and ecosystem fertility.

The specific objectives were to:

I Evaluate the effect of clear-cutting on fungal communities and their associated enzyme activities and assess how the observed changes could be coupled to long-term succession during stand development in a pine-dominated forest stand chronosequence (Paper I).

II Assess if changes in mycelial dynamics during development of even-aged forest stands is related to shifts in fungal community composition and examine inherent biases in the ingrowth mesh bag technique, which is a commonly used method to assess production of ectomycorrhizal mycelium in soils (Paper II).

III Investigate how the interplay between functional guilds and fungal-specific oxidative enzyme activities relates to organic matter accumulation across a fertility gradient in old-growth coniferous forest (Paper III).

IV Determine long-term (>100 years) decomposition rates and C and N dynamics in organic profiles of old-growth coniferous forest (Paper IV).

V Explore drivers of organic matter accumulation in the mor layer of Swedish boreal forests (Paper V).

3 Project descriptions

3.1 Fungal communities and associated enzyme activities in aging forest stands (Paper I)

In Paper I fungal community composition, enzyme activities and fungal biomass were evaluated across a chronosequence of ten forest stands dominated by *Pinus sylvestris* and ranging in age from 1 to 158 years (Figs 1-4). All selected stands were located in the Uppsala County, central Sweden (60°05′ to 60°10′ N; 17°29′ to 17°54′ E), unfertilized and established after clear-cutting (stands <50 years old). The more or less even-aged structure of older stands (>80 years old) was obtained by selective thinning (the history of the 59-years old stand is uncertain).

In October 2012, nine soil cores were randomly collected down to mineral soil or rock in each of ten forest stands in a 10×10 m grid pattern. After removal of all green plant parts, each core was split into 3 layers: litter (slightly decomposed plant material), fragmented litter (moderately decomposed plant material) and humus (highly decomposed plant material) and materials from respective layers were pooled within each site. Extractable pools of inorganic N, soil pH and organic content were determined on humus samples. Fungal biomass was estimated using ergosterol analysis (more details in 1.5.2) and fungal community composition was assessed using PacBio sequencing of ITS2 amplicons (more details in 1.5.1). Sequences were clustered into species hypothesis (hereafter referred to as species), which were further taxonomically identified and assigned to functional groups. All samples were assayed for activities of selected hydrolytic enzymes: cellobiohydrolase (CBH), β-1.4-glucosidase (BG), β-1.4-xylosidase (BXD), leucine aminopeptidase (LAP), chitobiosidase (CHiB), β-1.4-*N*-acetylglucosaminidase (NAG), acid

Figure 1. A replanted clear-cut. Photo: Julia Kyaschenko.

Figure 2. A pine-dominated forest stand (34 years old). Photo: Julia Kyaschenko.

phosphatase (aP) and oxidative enzymes: Mn-peroxidase (MnP) and laccase (more details in 1.5.6).

Correlation between stand age and community composition was evaluated for statistical significance using canonical correspondence analysis (CCA). Co-variation in community composition and enzyme profiles in individual layers was assessed for significance using a Mantel test and further explored by a correlation matrix. Effects of forest age on abundance of specific functional groups of fungi were tested using mixed effects linear models. Fungal community variation was visualized using a detrended correspondence analysis (DCA) sample plot and a CCA species plot, and correlation between enzyme profiles and fungal taxa was illustrated using principal component analysis (PCA) (more details in 1.5.7). Using space-for-time substitution, we were able to establish fungal community responses (at both taxonomic and functional levels) to harvest disturbances and follow re-establishment of fungal communities with increasing forest age. Further, we were able to statistically couple enzyme profiles to fungal community composition in different organic layers.

3.2 Fungal community composition and mycelial dynamics in aging forests stands (Paper II)

The study in Paper II was conducted in the same chronosequence as above, except that the two youngest stands (1 and 5 years old) were excluded from the analyses. Mycelial production was estimated using sand-filled 50-µm mesh ingrowth bags (Wallander *et al.* 2001). In each forest, mesh bags were incubated at the interface between the fragmented litter and humus layers from October 2012 to November 2014 over different periods (ranging from 16 days up to 2 years). After harvest, sand from the bags was freeze-dried and ground to fine powder with mortar and pestle. Sub-samples were subjected to ergosterol analysis (more details in 1.5.2) and sequencing of ITS2 amplicons (more details in 1.5.1). Mycelial turnover was estimated using an exponential decay model (Ekblad *et al*. 2016), as presented in Hagenbo *et al*. (2017). For comparison of fungal community composition in mesh bags with that of the surrounding soil (at the interface between the fragmented litter and humus layers), data obtained in Paper I was used.

CCA was used to test if total and ectomycorrhizal fungal communities correlated with mycelial turnover, forest age and their interaction. Variation of fungal community composition in mesh bags and soil was illustrated using DCA (more details in 1.5.7). To investigate similarity in overall distribution of fungal guilds in soil and mesh bags of different incubation times, ergosterol-weighted averages of relative abundances of fungal guilds across all forest stands were established.

Figure 3. A pine-dominated forest stand (59 years old). Photo: Julia Kyaschenko.

Figure 4. A pine-dominated forest stand (100 years old). Photo: Julia Kyaschenko.

3.3 Fungal regulation of organic matter stocks in an old-growth coniferous forest (Paper III)

In Paper III we investigated drivers of organic matter accumulation along a fertility gradient in an old-growth coniferous forest. Further, we examined how shifts in fungal functional groups, throughout soil profiles and across the fertility gradient, mediated the connection between organic matter oxidation and ecosystem fertility. For this, we selected eight sites in a coniferous old-growth forest reserve (Fiby Urskog) to represent a local fertility gradient, as reflected in vegetation types, N availability and soil acidity (Fig. 5). Fiby Urskog is a nature reserve 15 km west of Uppsala, characterized by a long history of free development (it has not been clear-cut or burned since the 18th century), and consists of a mosaic of smaller patches of different forest types.

In October 2012, ten soil cores were collected in a 10×10 m plot down to mineral soil of rock on each of the eight forest sites. Each core was divided into seven fine layers (litter 1-2, fragmented litter 1-2 and humus 1-3; Fig. 6), and each layer was pooled within a plot. For each plot, understory vegetation composition (species coverage) and standing tree biomass (biometrics) were recorded, and production of above-ground litter (litter traps), moss (through-

Figure 5. Study sites in the nature reserve Fiby Urskog representing low-fertility pine-dominated plots (left panel) and high-fertility spruce-dominated plots (right panel). Photos: Julia Kyaschenko

growth nets) and soil mycelium (ingrowth bags) as well as litter decomposition rates (litter bags) were estimated. Soil pH and extractable inorganic N were analysed on additional composite samples assembled from 3 random locations on each plot. All pooled samples were subjected to selected enzyme analyses (mentioned in section 3.1; more details in 1.5.6), ergosterol analysis (more details in 1.5.2) and fungal community analysis (PacBio sequencing of ITS2 amplicons; more details in 1.5.1). Sequences were clustered into species hypotheses (hereafter referred to as species), which were further taxonomically identified and assigned to functional groups.

To account for covariance between significantly correlated variables, the first axis of a PCA of inorganic N content, pH and litter C:N was used as a fertility index. Similarly, a vegetation index was derived from understory plant, moss and tree species abundances, and a productivity index was derived from total standing tree biomass, total litter production and mycelial production. Relationships between organic matter stocks (total and N) and their potential predictors, plot-specific Mn-peroxidase activities and environmental drivers, organic matter stocks and the fertility index as well as between Mn-peroxidase activities and mycelial production were evaluated for statistical significance using linear regressions. Correlations between environmental drivers and fungal community composition as well as between Mn-peroxidase activity and the Agaricomycetes community were validated using CCA. Effects of fertility on relative abundance of different functional groups were evaluated using mixed effects linear models. The same procedure was used to investigate further correlations between Mn-peroxidase activity and proportions of different functional groups (total Agaricomycetes, ectomycorrhizal or litter basidiomycetes and Agaricomycetes saprotrophs).

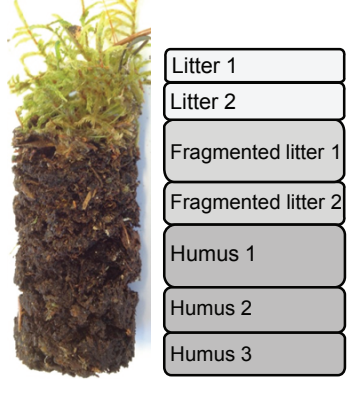

Figure 6. A schematic illustration of soil core subdivision into organic layers. Photo: Julia Kyaschenko.

Correspondence analysis (CA) was used to visualize variation in fungal community composition at species level and DCA was used to visualize fungal community composition at the genera/orders level (more details in 1.5.7). This approach enabled us to identify the main regulators of organic stocks as well as establish fungal drivers of ecosystem fertility, linked to their enzyme production and capacity to recycle N from belowground organic stocks to growing trees.

3.4 Carbon and N dynamics in the mor layer of an old-growth coniferous forest (Paper IV)

In Paper IV we analysed bomb-produced ^{14}C (more details in 1.5.5) to estimate C mass and age throughout the soil organic profiles studied in Paper III. To describe the accumulation of non-decomposed C originating from above-ground (litter fall) and below-ground (roots) sources, we used a C sequestration model developed by K. Clemmensen, O. Ovaskainen and B. Lindahl (Clemmensen *et al*. 2013). By parameterization of this model with data on C pools and their age (obtained by ^{14}C analysis), long-term decomposition rates as well as root-mediated C input was estimated. Further, we tested if differences in C and N dynamics across plots could be related to ecosystem fertility, fungal community composition and enzyme activities, as analysed in Paper III.

To evaluate C and N dynamics across plots, the average C and N mass per "cohort" was calculated. A cohort outlines a physical space that contains litter fall deposited during a single growing season. The model estimates the number of cohorts in each sampled layer based on the organic matter age, as estimated by ^{14}C analysis, accounting for recent C input via roots. Based on the estimated number of cohorts in each sampled layer, the age, mass and N content of organic pools, originating from litter fall or root growth, could be estimated for each layer. Effects of fertility, cohort age and their interaction on C and N stocks in each layer were evaluated using mixed effects linear models.

To further investigate C and N dynamics in the mor layer, stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) and C:N ratios (more details in 1.5.3 and 1.5.4) were measured throughout soil profiles and compared with corresponding values in litter, moss, roots and mycorrhizal mycelium.

Effects of ecosystem fertility, organic matter age (calculated on a cohort basis) and their interaction on C and N isotopic signatures and C:N ratios were evaluated using mixed effects linear models.

3.5 Regulation of organic matter stocks in the mor layer of Swedish boreal forests – a large-scale perspective (Paper V)

This study is based on data collected in 2014 and 2015 in conjunction with the Swedish National Forest Survey and the Swedish Forest Inventory, which have re-monitored ca 30 000 permanent plots systematically distributed across the country every 5-10 years since 1983 (Fridman *et al*. 2014).

Within this dataset, the sample plots were stratified according to: location, forest age, tree species, humus type, soil pH and moisture. The final data set consisted of 81 mor/moder humus samples from 58 pine-dominated and 23 spruce-dominated forests with a moderate pH range (3.2–4.3), situated 60° to 68.1° north and 12.3° to 23.8° east. Measurements of tree basal area (m^2 ha^{-1}) were used as a proxy for primary production. In addition, yearly stand increment (m^3 ha^{-1} $year^{-1}$) for each stand was calculated from the difference in woody volume for the last 5 years (Fridman *et al*. 2014). Mean annual temperature (MAT) and precipitation (MAP) for each forest were extracted

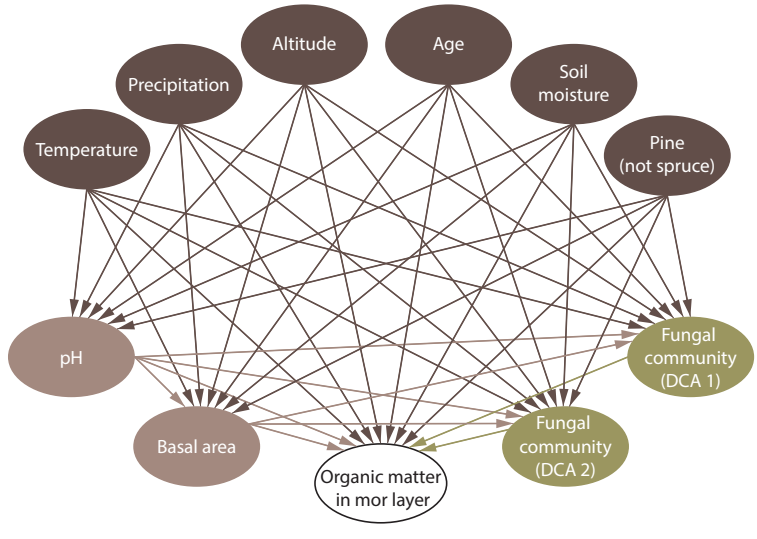

Figure 7. Tested direct and indirect predictors of mor layer soil organic matter of 81 Scandinavian boreal forests (>60 years) as analysed by piecewise structural equation modelling. The amount of organic matter stored in the humus layer of the studied forests could be directly constrained by climate (temperature and precipitation), altitude, dominant tree species (pine) and tree biomass (basal area) as well as soil properties (pH and soil moisture). Same parameters could indirectly affect organic matter stocks by shaping fungal community composition (represented by detrended correspondence analysis axis 1 (DCA1) and 2 (DCA2) scores).

from the Swedish Meteorological and Hydrological Institute database over the period from 2003 to 2012.

All samples were subjected to fungal community analysis (PacBio sequencing of ITS2 amplicons; more details in 1.5.1). Sequences were clustered into species hypotheses (hereafter referred to as species), which were further taxonomically identified and assigned to functional groups.

The relative importance of potential environmental factors and fungal community composition as statistical predictors of organic matter stocks in the mor layer (Fig. 7) was tested using piecewise structural equation modelling (SEM) using the function psem in the piecewiseSEM package (Lefcheck *et al*. 2016) (more details in 1.5.7). Due to methodological limitations bidirectional correlations were not tested. *Post-hoc* correlations between relative abundances of the most abundant fungal species and organic matter stocks were performed using generalised linear models.

4 Results and discussion

4.1 Factors shaping fungal communities

In this thesis shifts in fungal community composition were assessed across different scales: in a single forest (Papers III and IV), in a regional forest age gradient (Papers I and II) and across boreal Scandinavia (Paper V). Furthermore, the main drivers of fungal community composition were explored depending on the different settings: in forests altered by clear-cutting practices (Papers I and II), in an old-growth nature reserve (Papers III and IV) and in mature forests (Paper V). Ecosystem fertility (soil pH, inorganic N and litter C:N ratio) was identified as the main direct factor shaping fungal community composition in mature and old-growth forests, supporting previous observations (Sterkenburg *et al*. 2015). In a chronosequense of managed forests, fungal community correlated strongly with stand age. However, co-varying changes in pH and inorganic N levels along the gradient suggested their potential involvement also in mediating fungal community responses to stand age. A better understanding of species responses to environmental changes is essential, as those responses are connected to species activities and properties that, in turn, could influence ecosystem processes and properties (Koide *et al*. 2014). In this thesis, shifts in fungal communities along different gradients (depending on response traits) were linked to their activities and their potential effect on ecosystem development (effect traits), in particular nutrient cycling and organic matter accumulation.

4.1.1 Forest management

Clear-cutting implies drastic changes on forest ecosystem by altering nutrient availability, vegetation composition, micro-climate and water availability. It is well known that such stand replacing harvesting eliminate ectomycorrhizal

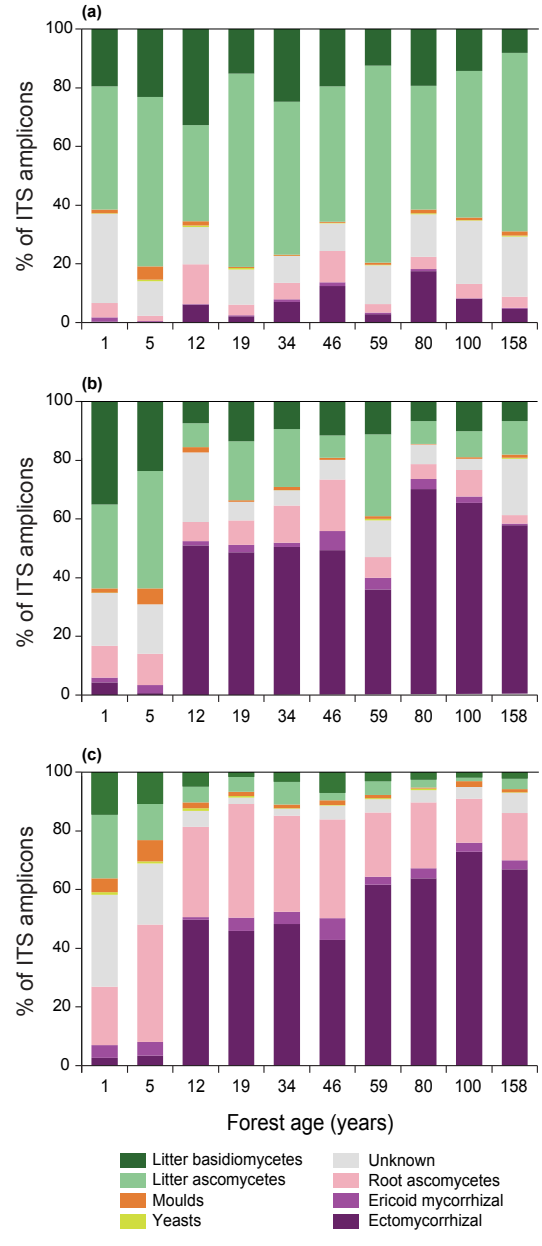

Figure 8. Distribution of fungal functional groups in different organic layers: (a) litter (b) fragmented litter (c) humus, of 10 *Pinus sylvestris* forest stands of different ages (1–158 years), as estimated by PacBio sequencing of amplified ITS2 markers. Abundances are given as percent of the identified amplicon sequences (accounting for 92% of total sequences).

fungi and promote opportunistic and saprotrophic fungi (Hartman *et al*. 2012), which together with enrichment disturbance could lead to increased N mineralization (Kreutzweiser *et al*. 2008) and organic matter loss (Magnani et *al*. 2007). However, re-establishment of the pre-disturbance community is less studied, yet important due to its potential effects on long-term C sequestration, nutrient cycling and forest productivity.

In Paper I we evaluated effects of tree harvest and stand development on fungal communities and their enzymatic activities. Forest age had a dramatic effect on fungal communities in all organic layers, with the strongest effect on fungal communities in all organic layers, with the strongest effect in the humus layer. In line with our expectation, the relative abundance and diversity of ectomycorrhizal fungi increased during stand development, on expense of litter saprotrophs (Fig. 8). Although abundances of fungal guilds were assessed in relative terms, stable ergostorol concentrations (e.g. fungal biomass) across the gradient suggest that this shift could be interpreted in absolute terms. Clear-cutting almost completely eliminated ectomycorrhizal fungi and it took more than 60 years for their species richness to recover. In line with previous observations (Wallander *et al*. 2010), our results further highlight ectomycorrhizal fungi as being particularly sensitive to tree harvesting.

Ectomycorrhizal community composition changed steadily with increasing forest age, from dominance by *Piloderma* and *Tylospora* spp. in the young forests to dominance by *Cortinarius* and *Russula* spp. in the oldest forests (Fig. 9). The dominance by Atheliaceae (*Piloderma* spp. in particular) corresponds to developmental stages with the highest standing fine root biomass (Konôpka *et al*. 2015) and maximum ecosystem C sink (Magnani *et al*. 2007). In contrast, increased age-related N limitation and soil acidity favoured communities dominated by *Cortinarius*, *Cenococcum* and *Russula* spp. Species within the genus *Cortinarius* are usually found in old-growth forests (Dahlberg 2001), and extensive clear-cutting practices may potentially have negative long-term effects on its population, but it is not yet possible to evaluate.

Fungal communities correlated significantly with quantitative enzyme activity patterns (Fig. 10). As expected, activities of hydrolytic enzymes correlated positively with saprotrophic ascomycetes within Pleosporales and Hypocreales in the upper litter layer and *Mycena* spp. in more decomposed litter. Within ectomycorrhizal taxa, species belonging to *Cortinarius*, *Cenococcum* and *Russula* genera correlated positively with nutrient-acquiring enzymes, such as β-1.4-N-acetylglucosaminidase (NAG), acid phosphatase (aP) and Mn-peroxidase. These correlations were found to be significant in more decomposed litter, where saprotrophic *Mycena*, Helotiales and Pleosporales correlated negatively with aP and Sordariales and Pleosporales

correlated negatively with NAG. These results support the idea that ectomycorrhizal fungi play an important role in nutrient acquisition from complex organic matter. In particular, positive spatial correlation between Mn-peroxidase and the genus *Cortinarius* corroborates previous observations (Bödeker *et al*. 2014) and further highlights fungi within this genus as important degraders of organic matter in boreal forest.

Although forest age did not correlate with enzyme activities, the significant correlation between fungal communities and enzyme activities, as well as between fungal communities and forest age, implies that both enzyme activities and fungal communities shifted during stand development. As the distribution of enzymes within soil profiles and landscapes could be highly patchy and dynamic (Baldrian 2014), more intense sampling could potentially have provided better statistical support for direct links between forest age and enzyme activities.

Clear-cutting has a strong and long-term effect on fungal community composition, diversity and associated enzyme activities. These shifts are likely to have a major impact on ecosystem processes, such as nutrient cycling and C sequestration. Management efforts to maintain ectomycorrhizal diversity and preservation of genera particularly efficient in enzymatic recycling of organic nutrient pools (such as *Cortinarius* and *Russula*) may, potentially, alleviate age-related decline in N availability and hereby improve plant productivity at

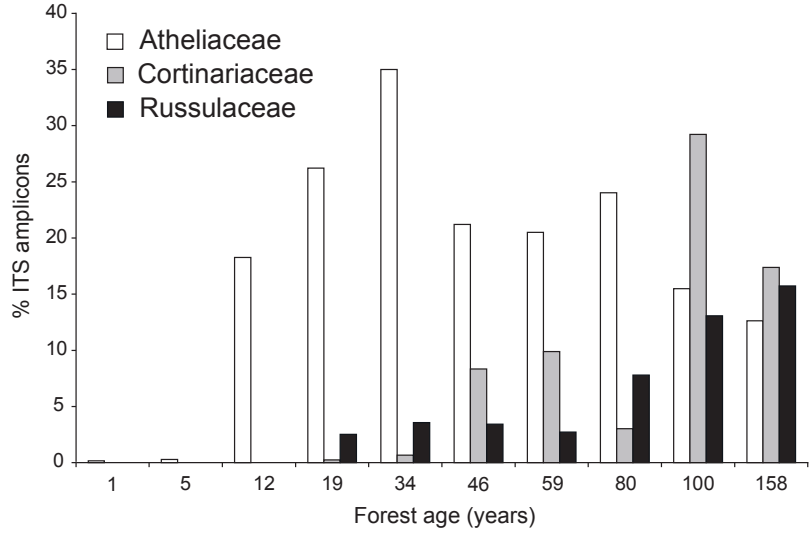

Figure 9. Distribution of the most dominant ectomycorrhizal fungal families in the humus layer of 10 *Pinus sylvestris* forest stands of different ages (1–158 years), as indicated by PacBio sequencing of amplified ITS2 markers.

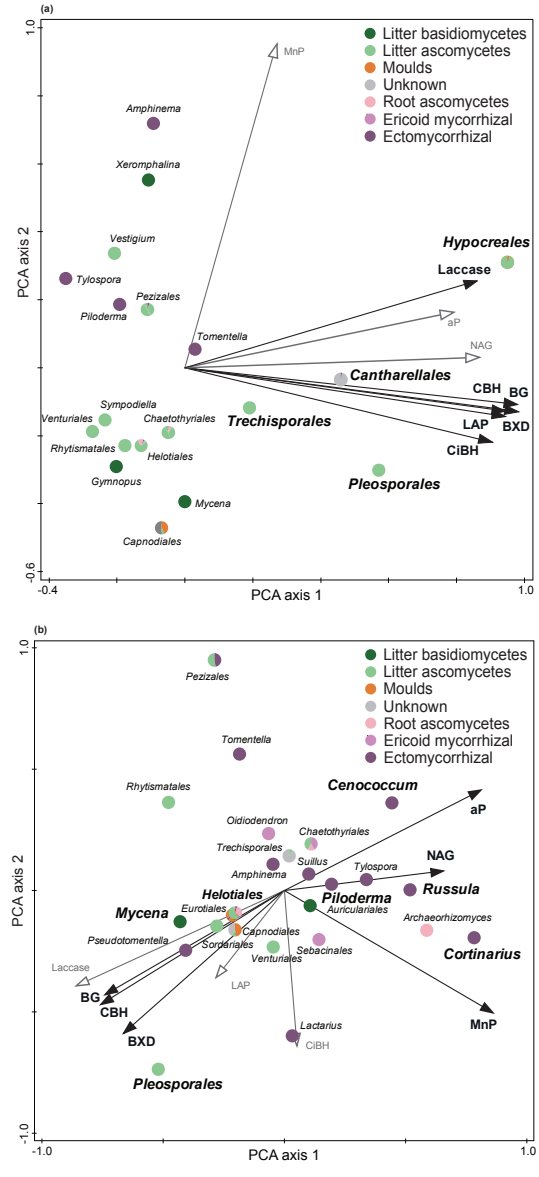

Figure 10. Similarity in enzyme profiles among fungal genera/orders, based on principal component analysis of their specific enzyme activities, calculated as abundance weighted averages. The figures are based on enzyme activity analyses and PacBio sequencing of amplified ITS2 markers from (a) litter and (b) fragmented litter of 10 *Pinus sylvestris* forest stands of different ages (1–158 years). Only genera accounting for more than 1% of total amplicons in two or more samples are shown. Genera/orders and enzymes with significant positive correlations are highlighted by bold text and black vectors, respectively. aP, acid phosphatase; BG, β-1.4-glucosidase; BXD, β-1.4-xylosidase; CBH, β-D-cellobiohydrolase; CiBH, chitobiohydrolase; LAP, leucine aminopeptidase; MnP, Mn-peroxidase; NAG, β-1.4-Nacetylglucosaminidase.

later stages of stand development. By removal of *Cortinarius* spp., forestry could potentially facilitate below-ground C stocks in older forest stands (as will be discussed in section 4.5).

4.1.2 Communities in soil versus mesh bags

In Paper I we showed that fungal communities undergo taxonomic and functional shifts along forest stand development. Differences between mycorrhizal fungal species in mycelial morphology and substrate exploration strategies (Agerer 2001) could potentially affect overall mycelial production, turnover and biomass. Using the same chronosequence, it was recently estimated that fungal biomass increased with forest age, although mycelial production rates declined with stand development (Hagenbo *et al*. 2017). These patterns were explained by a rapid reduction in turnover rates, from 7 to 1 times per year, as forests get older (Hagenbo *et al*. 2017). Since these estimates were obtained using sand-filled ingrowth mesh bags, the direct connection between shifts in the soil community (Paper I) and turnover estimates remained to be established. Further, to identify potential biases of mesh bags as a commonly used method to estimate growth of ectomycorrhizal extraradical mycelium (Ekblad *et al*. 2013; Wallander *et al*. 2013), in this study, communities in bags incubated over different periods (ranging from 16 days up to 2 years) were compared with those in soil.

With increasing forest age, there was a significant shift in fungal community composition also in mesh bags (Paper II). Ectomycorrhizal fungal community composition correlated significantly with biomass turnover estimates, corroborating our original hypothesis that changes in production and turnover would be related to fungal community shifts, rather to intraspecific growth plasticity (Fig. 11). With increasing forest age and fungal biomass most pronounced shift in fungal communities was found within ectomycorrhizal basidiomycetes so that species within genera *Suillus* and family Atheliaceae (*Piloderma* and *Tomentella*) dominated mesh bags incubated in early stage forests with high turnover, whereas species within *Amphinema*, *Cenococcum*, *Russula* and *Cortinarius* genera dominated in in-growth bags within older forests with low turnover.

Comparison of fungal communities in mesh bags and the surrounding soil (Paper II) revealed highly biased patterns of mycelial ingrowth into mesh bags. The bias was related to both time of incubation and forest age (Figs 12a and b). We found that species within genera commonly found in old-growth forests, e.g. *Cortinarius* and *Russula* (Dahlberg 2001; Lilleskov *et al*. 2002), seemed to be greatly under-represented in the mesh bags (Fig. 12 c). As observed

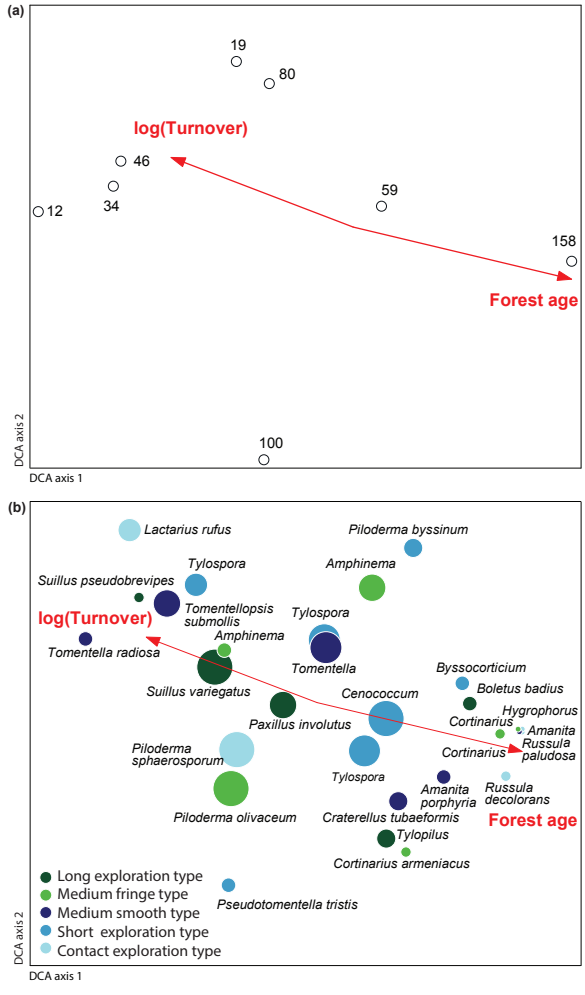

Figure 11. Variation in ectomycorrhizal (ECM) fungal community composition in ingrowth mesh bags of 8 *Pinus sylvestris* forest stands of different ages (12 – 158 years), as visualised by a sample plots (a) and a species plot (b) of a detrended correspondence analysis (DCA) based on PacBio sequencing of amplified ITS2 markers. DCAs were based on 51 ECM fungal species. Each sample in (a) represents an ergosterol weighted average community of bags incubated during three deferent periods (97, 109 and 389 days). In the species plot (b), only 30 most abundant ECM fungal species are shown and circles are colour coded according to ECM exploration type with area indicating relative abundance. Arrows represent supplementary environmental variables plots in ordination space. Axes 1 and 2 explained 28.2 and 12.2 %, respectively, of the total inertia of 1.2.

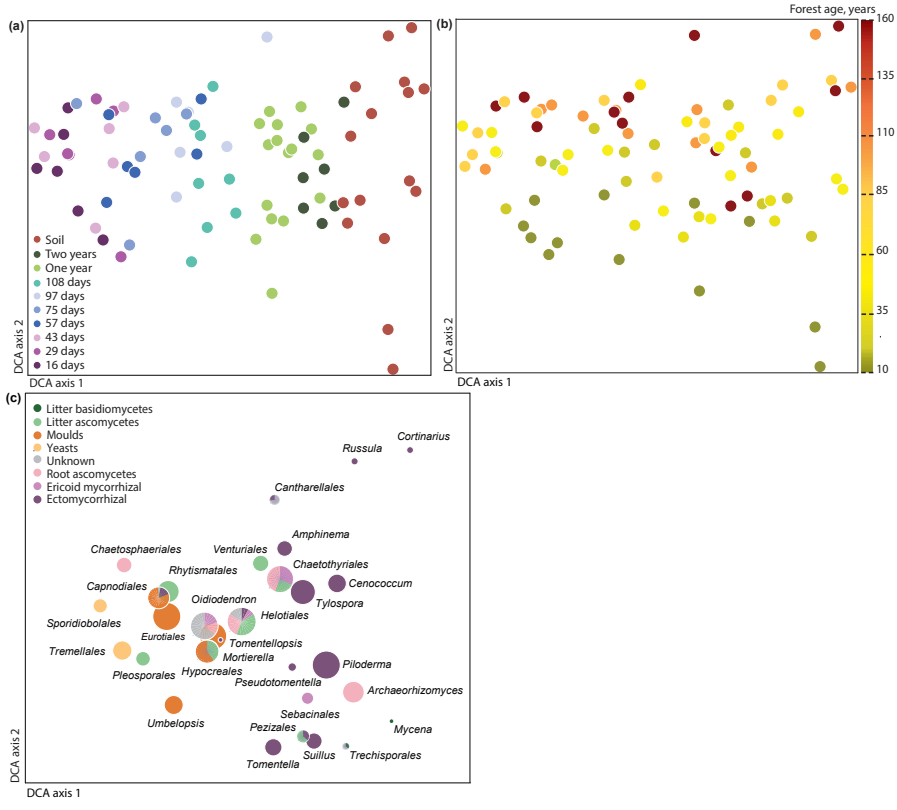

Figure 12. Variation in fungal community composition in soil and ingrowth mesh bags of 8 *Pinus sylvestris* forest stands of different ages (12–158 years), as visualised by sample plots (a, b) and a species plot (c) of a detrended correspondence analysis (DCA) based on PacBio sequencing of amplified ITS2 markers. DCAs were based on 77 fungal genera/orders. Circles are colour coded according to: (a) sample origin and incubation time of ingrowth mesh bags; (b) forest age; (c) functional groups with area indicating relative abundance. In the species plot (c), only the 30 most abundant orders/genera are shown. Axes 1 and 2 explained 14.6 and 6.3%, respectively, of the total inertia of 1.8.

previously, certain species within genus *Cortinarius* seem to be involved in decomposition of organic matter via production of oxidative enzymes (Bödeker *et al*. 2009; 2014). The mycelia of these fungi are well adapted to long-distance exploitation within the soil matrix and could potentially avoid (or grow through) the mesh bags, which are devoid of organic resources. In contrast, *Russula* spp. that produce very short mycelia that grow in close contact with the substrate and could therefore be rare in the mesh bags. It is not clear how the mesh-bags discrimination against particular ectomycorrhizal fungi (species within *Cortinarius* and *Russula*) could have affected the obtained biomass turnover estimates (Hagenbo *et al*. 2017).

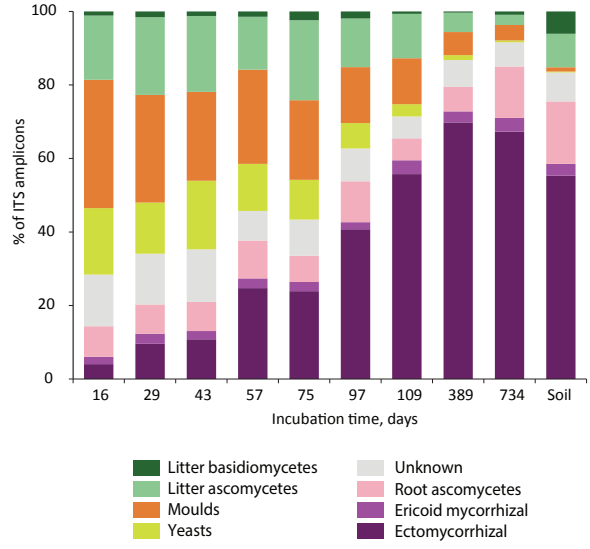

Figure 13. Distribution of fungal functional groups in mesh bags of different incubation time and soil of 8 *Pinus sylvestris* forest stands of different ages (12–158 years), as estimated by PacBio sequencing of amplified ITS2 markers. Abundances are given as percent of the identified amplicon sequences (accounting for 92% of total sequences).

As incubation time of the mesh bags increased, their community composition became more similar to that in the soil (but still missing *Cortinarius* and *Russula* spp.) (Fig. 13). During the first 2-3 months of incubation, moulds and yeasts dominated bag communities, whereas ectomycorrhizal fungi accounted for only around 20% of the amplicons (typically around 50% in the soil). The observed high relative abundance of fungal opportunists could be due to disturbance effects during bag installation. After one year of incubation communities in the mesh bags were more similar to soil communities with respect to guild composition, suggesting that longer incubation time might be needed for accurate community description. In mature forests with high abundance of organiophilic mycorrhizal species, mesh bag incubation techniques must be used with caution.

4.1.3 Ecosystem fertility and productivity

In Paper III we conducted detailed observations and measurements of different environmental parameters that were further used as a proxy for ecosystem productivity and fertility (as described in section 3.3; Fig. 14a). Only the fertility index correlated significantly with fungal community composition,

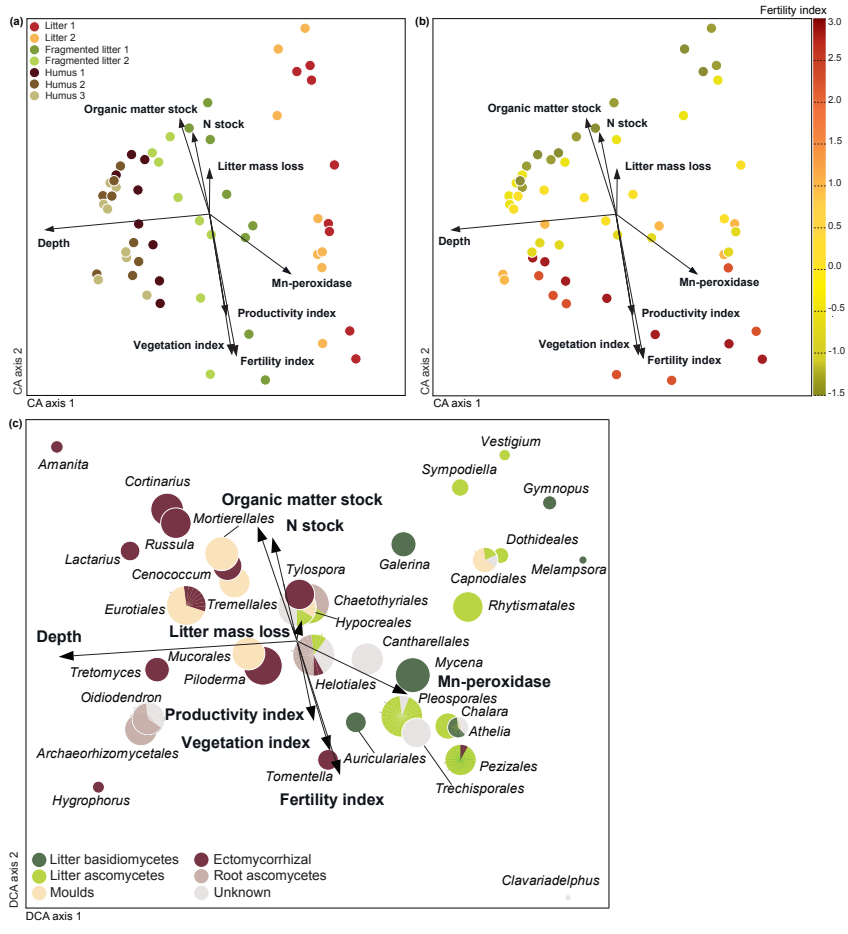

Figure 14. Variation in fungal community composition in organic soil profiles of 8 old-growth coniferous forest sites, as visualised by (a and b) sample plots of a correspondence analysis (CA) and (c) a species plot of a detrended correspondence analysis (DCA) based on PacBio sequencing of amplified ITS2 markers. The CAs were based on 1144 fungal species and the DCA was based on 79 fungal genera/orders, but for clarity only the 36 most abundant taxa are shown. Circles are colour coded according to: (a) sample origin in the soil profile (b) a fertility index and (c) fungal guilds with area indicating relative amplicon abundance. Dark brown and green colours in (c) correspond to Agaricomycetes. Vectors represent environmental variables plotted in ordination space. Vegetation, fertility and productivity indices are ordination scores of the first axis of the separate principal component analyses: vegetation index (abundance of understory plant and moss species, tree species composition), fertility index (soil inorganic N, pH and litter C:N ratio), productivity index (tree biomass and litter, moss and mycelial production). CA axes 1 and 2 explained 8.6 and 5.3%, respectively, of the total inertia of 7.8 (a and b) and DCA axes 1 and 2.explained 22.5 and 5.4%, respectively, of the total inertia of 1.6 (c).

both on species and genus levels (Fig. 14b). In Paper V, fertility (pH) and tree growth (tree basal area) were complemented with soil physical properties (soil moisture), climate (mean annual temperature and precipitation) and altitude as potential predictors of fungal community composition. Soil pH and dominant tree species were found to be the strongest predictors of fungal community. Fungal community composition also correlated significantly with tree basal area, altitude and precipitation.

Our results corroborate previous observations of fungal communities in old-growth forests (Sterkenburg *et al*. 2015) and global fungal diversity patterns (Tedersoo *et al*. 2014), which highlighted soil acidity as a key limiting factor for ectomycorrhizal species distributions. In both papers (III and V) we found that basidiomycetes over-all are favoured by higher N availability and pH, whereas strong nutrient limitation and soil acidity promoted ascomycetes fungi. The effect of soil fertility was obvious in all organic layers (Paper III) (Fig. 14c). Expect for moulds (Paper III) and litter ascomycetes (Paper V), there were significant species shift within all fungal guilds with increasing fertility. Although the fertility effect seems to be consistent, the different scales represented by the measurements should be considered. Soil pH and N availability were assessed on the same soil cores as fungal community composition, and we found a strong correlation between these variables. In contrast, productivity and climate were estimated on much larger scales and showed no or weak correlation with community composition. Data collected on the same spatial scale would enable establishment of more direct links between environmental drivers and fungal communities.

4.2 Fungal guilds interactions

4.2.1 In managed forests

Clear-cutting eliminated ectomycorrhizal fungi for at least 10 years and opened a niche for saprotrophic fungi, which occurred in high abundance also deeper in the soil profiles in the two youngest forests (ergosterol levels were not much lower in those forests) (Paper I) (Fig. 8). A positive correlation between presumed saprotrophs within Pleosporales and Hypocreales in the upper litter layer and *Mycena* in the deeper layer with hydrolytic enzymes involved in holocellulose and protein degradation provided indirect evidences for stimulated saprotrophic processing of C and N from organic matter (Fig. 10). Combined, higher litter quality and quantity and the loss of mycorrhizal suppression (that is the “Gadgil effect”) could have facilitated access of

saprotrophs to low C:N ratio substrates in deeper layers, accelerate rates of N mineralization and further elevate soil pH after clear-cutting (Smolander *et al*. 1998). Activated saprotrophic decomposition might explain the high C losses that are generally observed during the first decade after harvest-related disturbances (Peltoniemi *et al*. 2004; Magnani *et al*. 2007).

After about a decade, ectomycorrhizal fungi seem to have re-established and remained constant in high proportions throughout stand development (as indicated by the lack of significant correlation between guild relative abundance and the layer×stand age interaction, when tested in 12-158 years old forests), whereas there was a significant negative effect of forest age on litter basidiomycetes (also when tested on the same sub-set of forests). It seems likely that competitive suppression of saprotrophs by mycorrhizal fungi is restored directly after arrival of the early mycorrhizal fungi and remained throughout stand development. This could potentially decrease decomposition rates (Averill & Hawkes 2016) and facilitate organic matter accumulation (Averill *et al*. 2014). In the absence of major disturbances, retention of N in accumulating organic pools could lead to ecosystem retrogression (Magnani *et al*. 2007; Clemmensen *et al*. 2013), unless mycorrhizal fungi may provide direct access to organic N for their plant symbionts. As discussed in section 4.1, in the older forests, *Cenoccocum* and *Russula* correlated positively with enzymes involved in N and phosphorus mobilization and *Cortinarius* correlated positively with Mn-peroxidase (Fig. 10). As these genera become more abundant with increasing forest stand age, their positive correlations with nutrient acquisition enzymes suggest that they may sustain plant productivity and delay ecosystem retrogression (Clemmensen *et al*. 2015; Baskaran *et al*. 2017).

4.2.2 In an old-growth forest

In Paper II we found that the balance between saprotrophic and mycorrhizal Agaricomycetes shifted along a local fertility gradient. With increasing ecosystem fertility the proportion of saprotrophic Agaricomycetes increased, and they also proliferated deeper into the soil profile, at the expense of ectomycorrhizal fungi. Furthermore, saprotrophs had a particularly strong effect on Mn-peroxidase activity in the deeper organic layers – enzymes that correlated significantly (negatively) with organic matter stocks along the gradient (Fig. 15). These results suggest that the two fungal guilds compete for limiting N, in line with the Gadgil hypothesis (Gadgil & Gadgil 1975; Orwin et al. 2011; Averill *et al*. 2014; Averill & Hawkes 2016; Fernandez & Kennedy 2016), and that increased N availability releases ectomycorrhizal competition

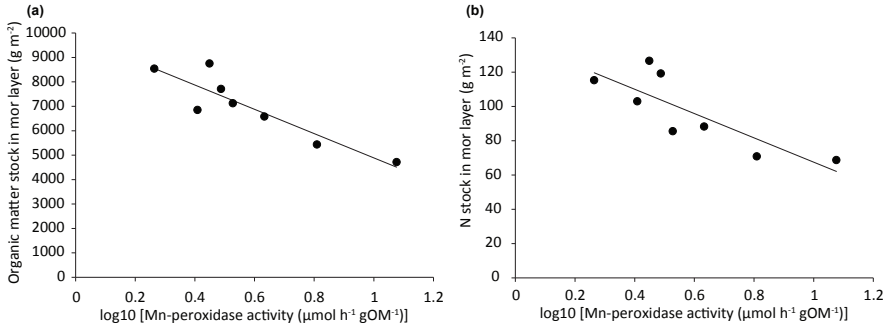

Figure 15. Relationships between: (a) organic matter and (b) N stocks (g m^{-2}) and Mn-peroxidase activity (integrated across layers as mass weighted averages) in the mor layer of 8 old-growth coniferous forest sites. (a) y= –4970x+9858; R^2=0.82; *P*=0.002; (b) y= –71x+138; R^2=0.67; *P*=0.01.

and enable habitat sharing by the two guilds. Increased saprotrophic oxidation of deeper organic layers seems to reduce the amounts of organic matter and and drive ecosystem productivity by preventing N retention in complex organic pools.

Some ectomycomycorrhizal fungi (e.g. certain *Cortinarius* spp.) have been found to possess peroxidase genes, enabling them to contribute to organic matter decomposition (Bödeker *et al*. 2014). Our results, however, indicate that mycorrhizal fungi generally are less efficient decomposers, compared to saprotrophs that possess the whole suit of extracellular enzymes (Colpaert & van Laere 1996; Kohler *et al*. 2015; Bödeker *et al*. 2016). Previously it has been reported that forests dominated by ectomycorrhizal fungi (*Cortinarius* spp. in particular) have significantly reduced organic matter stocks, compared to ascomycete-dominated forests (Clemmensen *et al*. 2015). Our findings contradict this observation. However, our study was conducted in more fertile system, spanning across a wide gradient in fertility, and the research site is at the southernmost border of the true boreal region.

4.2.3 In Scandinavian mature forests

In mature (>60 years old) forests distributed across the Scandinavian boreal region (Paper V), root ascomycetes (as a guild) correlated significantly (negatively) with soil pH and marginally significantly (positively) with organic matter stocks in those forests. Further, occurrence of certain species within this guild correlated with high organic matter accumulation. These results go in line with previous observations suggesting that root ascomycetes contribute to long-term C sequestration below ground (Clemmensen *et al*. 2015). In contrast,

basidiomycetes (both saprotrophic and mycorrhizal) increased with higher soil pH. Although the over-all relative abundance of basidiomycetes did not correlate significantly with organic matter stocks, individual correlations between C stocks and species presences/absence data revealed that two *Cortinarius* spp., present in 11 forests out of the total 81, correlated negatively with C stocks. These observations corroborate previous results that highlight particular *Cortinarius* spp. as potential producers of oxidative enzymes involved in organic matter decomposition (Bödeker *et al*. 2014; Clemmensen *et al*. 2015).

We did not find any support for an important "Gadgil effect" in the cross-Scandinavian sampling, e.g. that increased N availability would release mycorrhizal suppression and facilitate saprotrophic decomposition (Paper III). Such patterns have been observed along local fertility gradients with wide variation in environmental parameters to enhance the chances of pinpointing underlying mechanisms (Paper III) (Högberg *et al*. 2006) or in analyses spanning across different biomes and mycorrhizal types (Averill *et al*. 2014; Cheeke *et al*. 2017).

To summarize, our results obtained from three different studies provide evidence supporting the hypothesis that saprotrophs and mycorrhizal fungi have overlapping fundamental niches, and that ectomycorrhizal fungi under undisturbed condition with a constant flow of plant C may compete for limiting N and constrain the realized niche of saprotrophs, thereby suppressing saprotrophic decomposition (Averill & Hawkes 2016; Bödeker *et al*. 2016). As ecosystem fertility increases, the competitive pressure from mycorrhizal fungi may be released, enabling decomposition by saprotrophs that proliferate into deeper organic layers (Paper III). Clear-cutting disturbance eliminated ectomycorrhizal fungi for at least a decade, stimulating saprotrophic decomposition and presumably also C loss. Mycorrhizal re-establishment restored competitive suppression of saprotrophs by mycorrhizal fungi (Gadgil & Gadgil 1971), presumably hampering decomposition (Averill & Hawkes 2016) and leading to organic matter accumulation (Magnani *et al*. 2007) (Paper I). However, in mature forests across boreal Scandinavia (Paper V), we did not find support for fertility-related shifts between mycorrhizal and saprotrophic guilds. Our results rather suggest that ectomycorrhizal decomposition (particular in *Cortinarius* spp.) could be a valid strategy to maintain plant productivity under N-limited environments with restricted saprotrophic decomposition and N mineralization. In other words, the "*Cortinarius* effect" is likely to be conditional on the "Gadgil effect", as predicted by the model of Baskaran *et al*. (Fig. 3c, 2017).

4.3 Root decomposition estimates

Using a natural fertility gradient in an old-growth coniferous forest we found that fungal community and associated Mn-peroxidase activity were the best predictors of organic matter stock variation, whereas ecosystem fertility and productivity, as well as hydrolytic enzyme activities and litter decomposition rates were of minor importance (Paper III). We reasoned that these observations could be explained by the Gadgil effect – mycorrhizal suppression of saprotrophic decomposition in the deeper organic layers, though the conclusions were based entirely on correlations. To get a more detailed overview of C and N dynamics in those plots, we fitted a mathematical model to the collected data, complemented with organic matter dating by bomb ^{14}C analyses (more details in 3.4). This approach is based on back-calculation of long-term decomposition from measurements of stocks, rather from short-term decomposition tests. Extrapolating decomposition of fallen litter from one-year measurements, we were able to derive decomposition rates also for root-litter, based on the mass vs. age distributions in the soil profiles (purely organic mor layer). We found that decomposition rates of above-ground litter were similar across the fertility gradient, and mathematical extrapolation indicates that approximately 10% of deposited litter should remain after 10 years decomposition. In contrast, estimated decomposition rates of root litter were substantially higher in more fertile plots compared to less fertile plots, corresponding to approximately 20% and 40% of mass remaining, respectively, after 10 years (Fig. 16). These findings may be related to the higher abundance of saprotophic genera and associated Mn-peroxidase activity deeper in the profiles (the root zone) of more fertile plots. Our findings further highlight that short-term decomposition of above-ground litter is of minor importance in explaining variation in long-term humus build-up, whereas differences in root decomposition rates are the principal drivers of organic matter accumulation, confirming the findings of Clemmensen *et al*. (2013) from a northern boreal setting. Although this analysis relied on a constant relationship between above- and below-ground litter production across the gradient, our estimates of root decomposition in forest sites of contrasting fertility provide a basis for actual quantification of the mycorrhizal suppression of saprotrophic decomposition (Fernandez & Kennedy 2016). We conclude that root litter contribute substantially to organic matter accumulation compared to fallen litter, and that this contribution may increase even more by suppression of saprotrophic activities in the root zone.

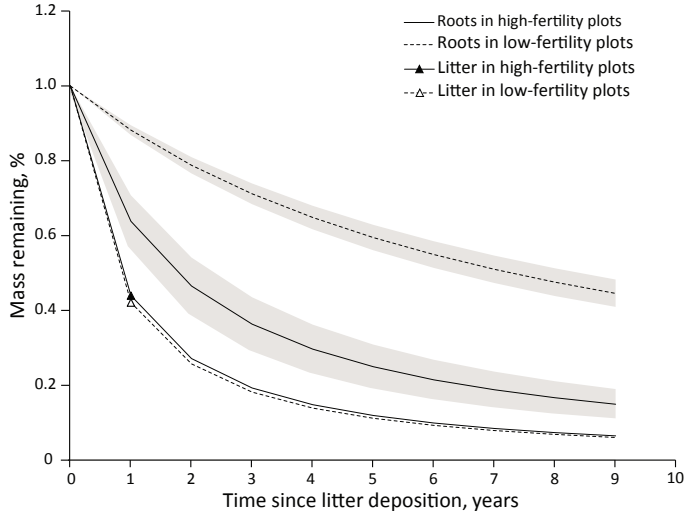

Figure 16. Rates of litter and root mass loss in low- (broken lines) and high-fertility (solid lines) coniferous forest plots simulated by the decomposition equation of Bosatta & Ågren (2003). Litter decomposition rates were extrapolated from litterbags incubated over one year in the soil. Root decomposition rates were estimated by a carbon sequestration model (Clemmensen *et al*. 2013) fitted to data on organic matter mass and age. Shaded areas represent ±SE; triangles represent one-year fallen litter decomposition rates for high- (black) and low-fertility (white) plots

4.4 Carbon and N dynamics in soil organic profiles

Organic matter dynamics was estimated by partitioning of C and N stocks onto cohorts, each corresponding to the litter fall from a single growing season. Using space-for-time substitution, we back-calculated organic matter dynamics for 150 years, with the decomposing cohorts of fallen litter gradually moving downwards through the organic profile and mixing with root-litter. Different patterns were observed for C and N, as well as for low- and high-fertility plots (Fig. 17). The amounts of C and N per cohort initially declined with time (depth) during the first 20 years of decomposition. Thereafter, N amounts increased significantly during 40 years (at the transition between the F and H layers) indicating that the organic matter during this decomposition phase was a sink for N rather than a source (Fig. 17b). In contrast, C pools per cohort were quite stabile during this phase, indicating a balance between below-ground inputs and decomposition. After about 60 years (in the H layer), cohorts declined significantly with time (soil depth) with respect to both C and N.

Since C:N ratio in the soil profile of low-fertility plots declined significantly with depth (Fig. 18c), the observed increase in N amounts could

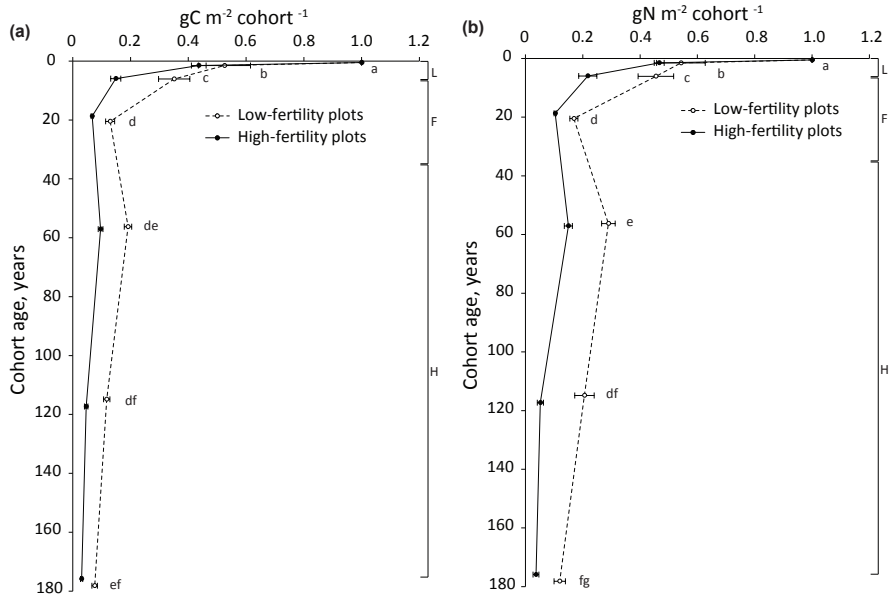

Figure 17. Dynamics of carbon (C) (a) and nitrogen (N) (b) in litter cohorts in low- (white dots, broken lines) and high-fertility (black dots, solid lines) coniferous forest plots. One cohort corresponds to the litter fall from a single growing season into which root litter is progressively deposited. Organic matter stocks were partitioned on cohorts using a carbon sequestration model (Clemmensen *et al*. 2013) fitted to data on organic matter mass and age. The estimates that share the same letters are not significantly different between age classes (layers). All data points are means ±SE (n=5 for low-fertility plots, n=2 for high- fertility plots). The intervals on the left indicate transition between the main organic layers sampled. L, litter; F, fragmented litter; H, humus.

be an indication of N retention in the F-H layers in those plots. The identification of a N sink at the F-H transition, where root density and proliferation of mycorrhizal mycelium is at maximum (Clemmensen *et al*. 2013), fits well with the previous proposal that myccorrhizal fungi under N-limited conditions may further intensify tree nutrient limitation, transferring only a minor fraction of acquired N to the host and retaining most in their own mycelia (Näsholm *et al*. 2012). Overall, N turnover in the low-fertility plots was incomplete, progressively driving the ecosystem into retrogression (paper III; Clemmensen *et al*. 2013; 2015). In contrast, in high-fertility plots, the F-H N sink was less pronounced, whereas C:N ratios increased significantly in the deeper layers. These observations suggest that in high-fertility plots, the well-decomposed humus in the lower parts of the profile is primarily used as a N source, counteracting N retention. The relatively slower C release (as indicated by the increasing C:N) could be interpreted as a bypass product of "N mining"

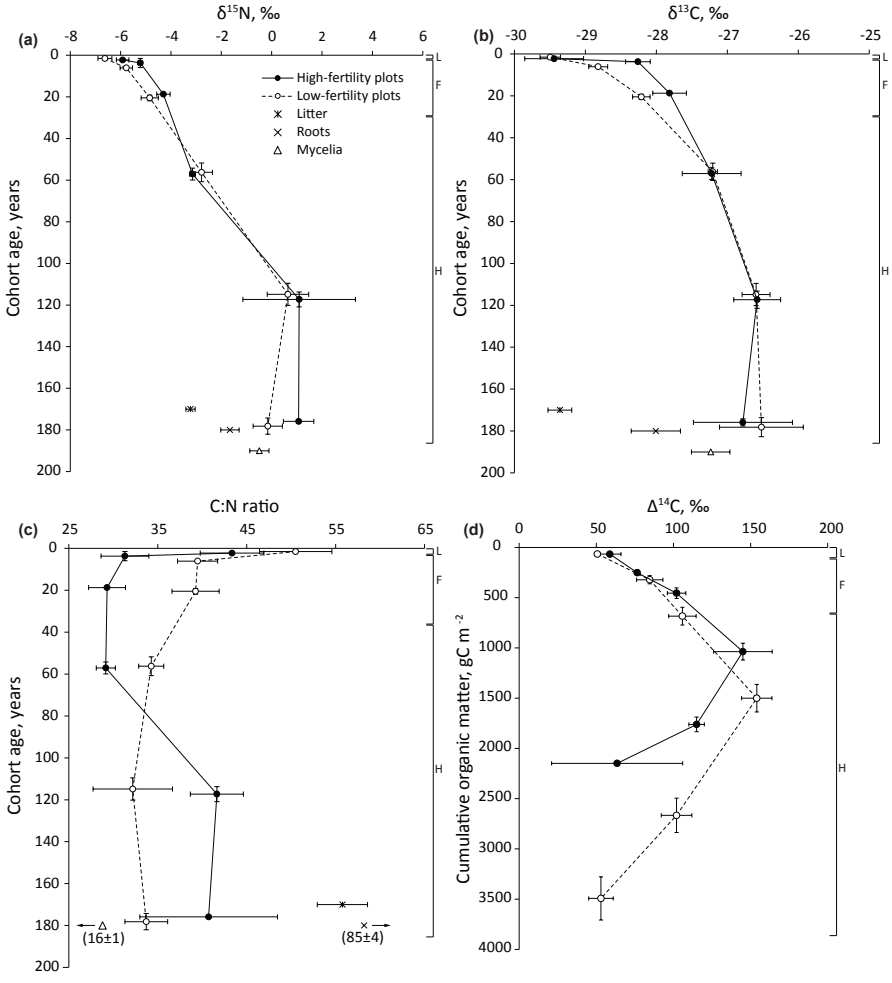

Figure 18. Stable isotopes signatures (a) $\delta^{15}N$, (b) $\delta^{13}C$ and (c) C:N ratio in soil organic profiles of low- (white dots, broken lines) and high-fertility (black dots, solid lines) coniferous forest plots. One cohort corresponds to the litter fall from a single growing season into which root litter is progressively deposited. The number of cohorts in each layer was estimated by a carbon sequestration model (Clemmensen *et al*. 2013) fitted to data on organic matter mass and age. The data points are means ±SE (n=5 for low-fertility plots and n=2 for high-fertility plots). For reference, $\delta^{15}N$, $\delta^{13}C$ and C:N ratio are given for litter (above-ground litter fall), mycelia (ingrowth mesh bags) and roots (<2 mm). The data points are means across all plots ±SE (n=7). The intervals on the left indicate transition between the main organic layers sampled. L, litter; F, fragmented litter; H, humus.

(Högberg *et al*. 2006; Orwin *et al*. 2011; Phillips *et al*. 2013; Paper III). In the low-fertility plots, C:N did not increase with depth, indicating more retention than "mining" for N.

The abundance of ^{13}C increased significantly with increasing age (depth) of the organic matter (Fig. 18c), in line with the generally observed decrease in $\delta^{13}C$ ratios of modern atmospheric CO_2 due to fossil fuel use (the so-called "Suess effect") (Keeling 1979). We also found a significant increase in $\delta^{15}N$ with increasing age (Fig. 18a), as usually found with increasing soil depth (Högberg *et al*. 1996). This pattern has mainly been attributed to preferential transfer of ^{15}N-depleted N from mycorrhizal fungi to their plant hosts (Högberg *et al*. 1996; Clemmensen *et al*. 2013), so that ^{15}N-depleted plant litter accumulates at the surface, while ^{15}N-enriched organic matter of fungal origin accumulates at depth (Hobbie & Ouimette 2009). In Clemmensen *et al*. (2013) a steeper ^{15}N-gradient was observed on large islands (representing younger, more productive ecosystem with smaller organic matter stocks) compared to less steep ^{15}N-gradient on small islands (representing older, less productive ecosystem with large organic matter stocks). This difference was ascribed to more active N cycling through the mycorrhizal pool, counteracting C accumulation on large islands. In our project we did not observed similar patterns as no significant effect of fertility on ^{15}N signature was found. Possibly our fertility gradient was not strong enough to capture potential differences in ^{15}N enrichment patterns observed by Clemmensen *et al*. (2013) in the island settings. As previously described in Paper III, the more fertile plots were characterized by higher abundance of saprotrophs at depth, and resulting mineralization and direct N uptake by roots would not necessarily be reflected in ^{15}N signature shifts.

4.5 Fungal regulation of forest C dynamics – an emerging paradigm?

Soil organic matter comprises a substantial amount of terrestrial C (Lal 2008), and a better understanding of the mechanisms regulating C dynamics and sequestration in forest ecosystem is therefore particularly important. Traditionally, litter quality has been proposed as the most important predictor of decomposition rates across ecosystems (Cornwell *et al*. 2008; Zhang *et al*. 2008). Litters with low lignin-to-N ratios have been shown to decompose more quickly initially, but as decomposition progresses, other chemical, biological and physical controls take over. Despite numerous studies, no consensus has been established concerning the effects of climate and local environmental factors on different (labile or resistant) organic matter pools (Kirschbaum 1995; Liski *et al*. 1999; Fang *et al*. 2005; Fierer *et al*. 2005; Knorr *et al*. 2005). Instead, the emerging view of persistence and stability of soil organic matter suggests both environmental and biological factors to be important drivers of C

sequestration at ecosystem level (Schmidt *et al*. 2011; Lehmann & Kleber 2015). The different studies described in this thesis highlight soil fungi as pivotal mediators of plant-soil interactions in boreal forests. Further, our findings emphasise the importance of fungal traits (relative to plant traits) in explaining organic matter accumulation below ground in different ecosystem types (Fig. 19).

Recently it was found that the concentration of exchangeable manganese was the strongest predictor of C storage in mor layer of Swedish forests, compared to variation in other factors, such as climate and tree biomass (Stendahl *et al*. 2017). Since manganese plays a central role in the mechanism of Mn-peroxidase production – a lignin-degrading enzyme that uniquely evolved within the Agaricomycetes (Floudas *et al.* 2012) – the results of this study suggest that fungal-mediated decomposition plays a key role in forest soil C dynamics. Using fungal community data obtained from permanent plots within the Swedish National Forest Survey and the Swedish Forest Inventory (limited to boreal forest stands older than 60 years with an intermediate pH range; Paper V), we found that neither climatic nor edaphic factors or productivity could explain local variation in organic matter stocks. Instead, fungal community composition was singled out as the strongest predictor of mor layer C stocks (Fig. 20). Although data on extractable manganese was not available for those samples, the negative correlation between the presence of

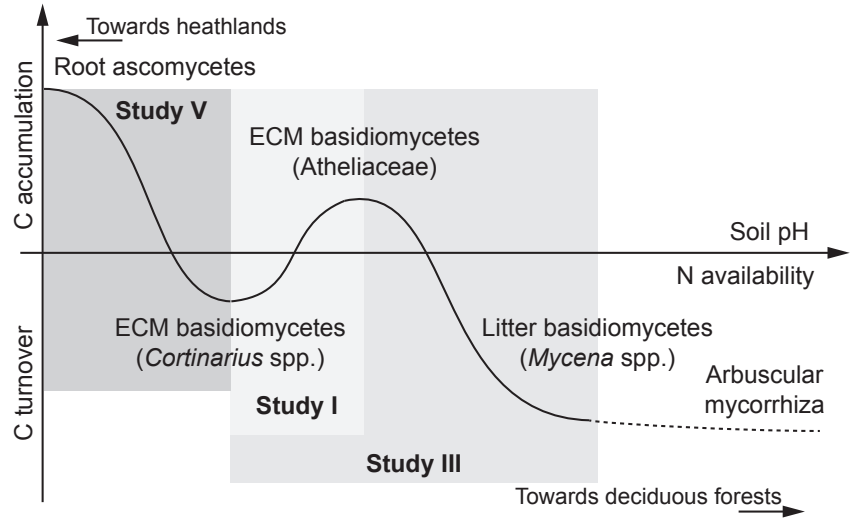

Figure 19. The different studies described in the thesis emphasize the importance of fungal communities in explaining carbon (C) accumulation in different forest ecosystem types. ECM, ectomycorrhiza.

two *Cortinarius* spp. and C stocks indirectly supports the idea of the importance of fungal-mediated decomposition in boreal forest soils (see section 4.2.3). The fact that *Cortinarius* spp. are mycorrhizal opens up for interesting links and feedbacks between trees, symbiotic fungi and below-ground organic stocks (Lindahl & Tunlid, 2015; Baskaran *et al*. 2017).

Mn-peroxidase activity was the only variable that correlated significantly (negatively) with organic matter stocks along a boreal forest fertility gradient (Paper III). In contrast, biomass production, litter decomposition rates or hydrolytic enzyme activities failed to explain variation in organic matter stocks between those plots. These findings further underline the importance of Agaricomycetes in regulating organic matter stocks in boreal forest. Higher oxidation rate was observed on more fertile plots, congruent with smaller organic matter stocks on those plots. These results go in line with previous observations suggesting a narrower ecological niche of Agaricomycetes than ascomycetes in terms of nutrient availability and soil acidity (Sterkenburg *et al*.

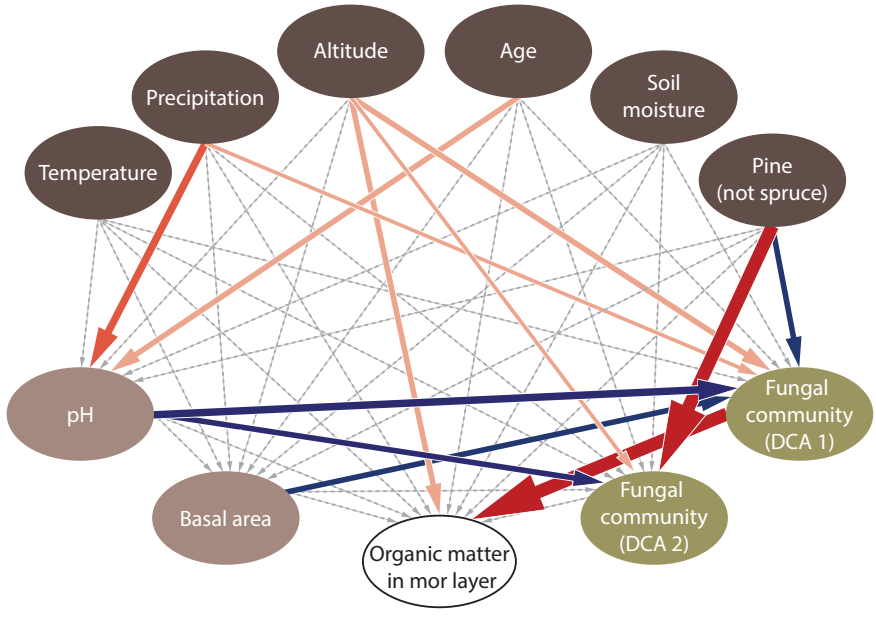

Figure 20. Tested direct and indirect predictors of mor layer soil organic matter of 81 Scandinavian boreal forests (>60 years) as analysed by piecewise structural equation modelling. Filled coloured arrows indicate significant correlations, whereas dashed grey arrows indicate non-significant correlations. Positive effects indicated by red and negative by blue colours. Colour intensity corresponds to levels of significance. Arrows size represents standard estimate of each significant predictor.

2015). In free-air CO_2 enrichment (FACE) experiments it has been observed that increased primary productivity does not necessarily imply increased ecosystem C stocks (Norby & Zak 2011). Higher rates of microbial mineralization of soil organic matter have been proposed to offset C sequestration under those conditions (Billings *et al*. 2010, Dieleman *et al*. 2010). Our observations provide further evidence suggesting that the counterintuitive relationships between fertility, productivity and organic matter stocks were primarily regulated by taxonomic and functional shifts in fungal community composition (see section 4.2.2).

Tree harvest has a dramatic effect on forest C dynamics by turning the disturbed forest area into a temporary CO_2 source for at least a decade (Law *et al*. 2003; Magnani *et al*. 2007). The gradual post-harvest transition from C source to C sink usually occurs in parallel to tree re-establishment. The sink strength reaches its peak at the time of canopy closure and gradually decline, as the forest gets older (Law *et al*. 2003; Magnani *et al*. 2007). Our results from Paper I suggest that shifts in fungal communities, fungal guild interaction and associated alterations in enzymatic activities might explain the generally observed changes in C dynamics throughout stand development (see section 4.2.1). The findings highlight certain groups of fungi (species within genera *Cortinarius* and *Russula*) as particularly sensitive to forestry, and since the two oldest forests in our gradient had never been clear-cut, the long-term effect of clear-cutting on these genera cannot be established yet. Shorter rotations could potentially prevent arrival of *Cortinarius* spp. and reduce associated organic matter oxidation leading to increase in the below-ground C sequestration capacity of the forest ecosystem. Similarly, N fertilization could also decrease organic matter decomposition rates and increase soil C stocks (Janssens *et al*. 2010), as some ectomycorrhizal taxa, including *Cortinarius* and *Russula* are especially sensitive to high inorganic N inputs (Lilleskov *et al*. 2001). In the short term, such management practices could increase the strengths of the forest C sink. However, in the long run, loss of fungal taxa efficient in maintaining ecosystem productivity could potentially lead forest into a N limitation trap (Frankling *et al*. 2014) and may also eliminate or even reverse the expected positive effect of tree regeneration on C sequestration due to decreasing above-ground C fixation. It is also important to consider the fate of the wood removed by clear-cutting, as more C would be sequestered for a longer time if wood is used for construction compared to products with short lifespan (Eriksson *et al*. 2007).

4.6 Methodological considerations

4.6.1 Large scale versus precision

A proper system description requires numerous measurements, which, due to time and resource limitation, are generally conducted on smaller scales, e.g. soil cores, litter traps, ingrowth mesh bags. The observations obtained from these smaller units are then extrapolated to higher levels, e.g. forest stands, ecosystems, biomes. This generalization, however, relies on an assumption that the number of smaller units and their sampling strategies were efficient enough to represent the whole system. In Paper I, enzyme activities (measured at soil core level) did not correlate significantly with forest age (estimated at stand level). However, the significant correlation of fungal communities (measured at soil core level) and forest age, on the one hand, and the significant correlation between enzyme activities and fungal communities (measured on the same soil cores), on the other hand, provide indirect evidence of shifts in enzyme activities with stand development. Since enzyme activities have been found to be particularly patchy within soils (Baldrian 2014) but also horizontally across the stands, more intense sampling could potentially help to capture the direct link between stand properties and enzyme activities.

In Paper V, fungal community composition, soil pH and organic matter content were measured on the same soil cores, and statistical modelling indicated the strongest correlation between these variables, compared to other variables measured on much larger scales (e.g. climate and productivity) that had no or weak correlation with fungal community composition and organic matter stocks. Such mixing of data obtained on different scales into a single statistical analysis is inevitable considering the span of the study, and aggregation of data at the same spatial scale would facilitate establishment of more direct links between environmental variables, fungal community composition and organic matter stocks.

4.6.2 Sampling strategies

In all of the studies included in the thesis (except for Paper V), pooling of biological samples was implemented. Physical pooling provides some obvious advantages, such as reduced experimental costs and preparation times. In addition it can also compensate for a limited amount of collected material and mitigate variation between individual samples. Abundant fungal species seem to be well captured by the physical pools (Song *et al*. 2015). Furthermore, physical pools generally have higher richness when compared with each

individual samples (Song *et al*. 2015), though, computational pooling of individual samples has been found to yield higher species richness than pooling of physical sample (Branco *et al*. 2013; Song *et al*. 2015). Rarefaction of computational pools to the sequence depth of the smallest pooled sample has been found to diminish differences in community structure in the two differently pooled samples (Branco *et al*. 2013). Taken together, these observations suggest that choice of sampling strategy should account for both the efforts and potential benefits. In our projects we investigated the most abundant fungal species, and sample pooling seemed to be a good strategy, whereas an individual-sample approach could be more appropriate in diversity studies focusing on rare species.

4.6.3 Unaccounted biotic and abiotic factors

All our findings rely entirely on correlations and more controlled manipulative tests would be needed to strengthen our conclusions and corroborate proposed underlining mechanisms. Further, we focused only on fungal communities and their potential contribution to ecosystem processes, whereas potential involvement of bacteria in enzyme activities, as well as other fungal-related mechanisms, e.g. the Fenton reaction (Shah *et al*. 2016), were not considered in our experimental designs. Globally, it has been shown that coniferous forest soils have particularly high fungal:bacteria ratios, correlating with the high C:N ratio (Fierer *et al*. 2009). Further, metaproteomics has identified fungi as the main producers of extracellular enzymes in an ectomycorrhizal forest (Schneider *et al*. 2012), and a recent study of forest soil metatranscriptomes by Hesse *et al*. (2015) found that fungi dominated transcripts of well-known CAZyme families, whereas bacterial transcripts were of lower abundance or missing. Finally, Mn-peroxidases – one of the key enzymes in our studies – evolved specifically in fungi (Floudas *et al.* 2012). Taken together, we think that these observations provide a solid base for the fungi-centric hypotheses tested in our studies.

Besides environmental variables measured in our studies, other factor such as N deposition, host specificity, soil type, moisture and temperature could potentially contribute to shifts in fungal communities, their biomass and activities as well as organic matter stocks. Although these parameters could have increased the amount of explained variation in fungal communities (usually <10% of total variation), the main drivers, such as soil pH and N availability seem to be constant across broader gradients and explain a significant part of fungal community variation.

4.6.4 DNA-based species identification

In all the projects we based our analysis on molecularly identified fungal communities. However, ITS-based species abundances may be highly under- or overestimated as ITS copy number per ng DNA is highly variable (Baldrian *et al*. 2013). Thus species abundance data obtained from the PCR-based analyses of the ITS region should be inferred with cautions. By complementing the abundance of genetic markers by estimates of biomarkers such as ergosterol (more details in 1.5.2), more precise estimates of fungal biomass could be obtained. Another limitation of using purely molecular methods to study soil fungi is the absence of discrimination in the technique between living and dead biomass as well as between active and dormant organisms (Bridge & Spooner 2001). In our projects we used in vitro enzyme activities estimates as a proxy for fungal involvement in organic matter decomposition, however, this approach does not resolve species-specific enzymes production rates. A way forward would be to use metatranscriptome sequencing – a rapidly growing technique with high potential to advance our knowledge of community metabolisms in different environments (Hesse *et al*. 2015; Kuske *et al*. 2015).

5 Conclusions and future perspectives

In order to build ecosystem models able to better predict future carbon (C) budgets at local, national and global levels, we need a better understanding of drivers and mechanisms regulating organic matter accumulation. In this thesis we tested the hypothesis that fungal community composition is a pivotal factor in boreal forest ecosystems and play a decisive role in regulating C and nitrogen (N) dynamics in soil organic profiles. We found correlative support for this hypothesis in all of the component projects, opening up for new ideas to be confirmed in manipulative experiments. Our results suggest that shift in balance between functional guilds of fungi may have a major influence on organic matter stocks. While this conclusion is consistent with a growing body of work on this topic, experimental manipulation of the presence or abundance of the different functional guilds is needed to support the suggested mechanisms behind variation in organic matter stocks. In contrast to DNA-based method that characterizes genomic potential of the community, more advanced RNA-based techniques would potentially reveal both the taxonomic composition and the biochemical activity of the community.

Short-term litterbag incubations have frequently been used as a standard method to estimate litter decomposition rates across ecosystems, and such estimates have been used to parameterize ecosystem models, although the extrapolation of these estimates into long-term decomposition rates is rather uncertain. Estimation of root decomposition is quite difficult methodologically, and long observations times are required to assess plant root storage. In this thesis we did an attempt to parse apart the contribution of above- and below-ground litter decomposition to long-term humus build-up using a mathematical C sequestration model. Our results suggest that root-associated processes play a more important role in organic matter dynamics then decomposition of above-ground plant litter. More observations from other ecosystem types would be needed to get a better understanding of C and N dynamics under different environmental settings.

In this thesis we took advantage of recently launched DNA-based identification of soil fungal communities within a large-scale national sampling program to investigate potential factors correlating with organic matter stock in selected forests within the Swedish boreal region. Although our results further supported fungal regulation of humus build-up and highlighted the importance of mycorrhizal fungi as decomposers, the inclusion of data from different forests types would be needed to obtain a better understanding of the links between decomposition and its environmental drivers.

Overall, the results presented in this thesis reveal that the composition and enzymatic activities of fungal communities are important determinants of C and N dynamics in boreal forest soils and it is urgent to incorporate fungi as central components of ecosystem models, to enable better prediction of responses to global change.

6 Popular science summary

Boreal forests play an important role in global carbon (C) cycling, as they represent one of the largest terrestrial sink for C globally. Fungi are essential components in these forests as, compared to bacteria, they are able to better tolerate soil acidity and low nutrient availability, generally observed in these forests. Fungi vary greatly in diversity and functions, and recent advances of DNA-based methods for species identification allow us to study the entire fungal communities belowground. By assessing fungal activities (e.g. measuring rates of different enzymes that fungi produce to degrade organic matter) it is possible to further investigate how different species are involved into organic matter decomposition and nutrient cycling.

Environmental variables, such as climate, soil fertility and nutrients availability have been shown to have a strong affects on fungal community composition. Furthermore, forest management practices impose dramatic changes on forest ecosystems and fungal communities. A majority of the forests in Sweden are clear-cut, and mycorrhizal fungi (e.g. those living in symbiosis with tree roots; mycorrhizal fungi receive C from their host trees in return for nutrients), are particularly vulnerable to such practices. In this thesis the effects of climate, stand properties and forest age on fungal communities composition and their function (enzyme activities) were investigated. We used enzyme assays and isotope analysis (a technique that provide integrated results of C and nitrogen (N) flows across decades) to link observed shifts in fungal communities and their activities to C and N dynamics and ecosystem fertility.

By studying forest stands ranging from recent clear-cuts to old-growth forests, it was found that tree harvesting eliminated mycorrhizal fungi but favoured saprotrophic fungi (e.g. free-living fungi that obtain C and N from dead organic matter), efficient in organic matter decomposition. Re-establishment of the mycorrhizal community initiated a decade after tree harvest, however the effect of this disturbance lasted for at least 50 years. Observed patterns in fungal communities and their activities correlated well

with variation in C fluxes during the rotation period. Our findings indicate that certain mycorrhizal fungi could be particularly sensitive to forestry, and there is a major risk that shorter rotation periods could lead to declining populations of these fungal taxa and associated functions related to maintaining forest growth in older stands.

To investigate how interactions between saprotrophic and mycorrhizal fungi and fungal-specific enzyme activities relate to organic matter turnover, forest plots representing a natural fertility gradient within an old-growth coniferous forest were selected. The data describing ecosystem C fluxes (i.a. litter production and decomposition rates) that are generally considered to be the main factors regulating C accumulation were collected. Fungal community composition and enzyme activities both along the fertility gradient, but also in vertical soil profiles were analysed. It was found that the only variable that could explain the observed differences in C and N stocks along the studied gradient was the activity of Mn-peroxidase – a fungus-specific enzyme that can degrade even the most resistant components of litter and soil organic matter. High activity of this enzyme seems to decrease the organic stocks substantially. It was further found that fungal community composition changed with increasing fertility, in concert with Mn-peroxidase activity. The obtained results suggest that the alterations in balance between saprotrophs and mycorrhizal fungi and related enzyme activities play an essential role in regulating C and N dynamics.

To obtain a better understanding of the mechanisms behind observed C and N dynamics in the selected fertility gradient, but also to parse apart the importance of above-ground (leaf litter) and below-ground (root litter) components in the long-term build-up of C and N stocks, a mathematical model was fitted to the data on organic matter mass and age (assessed by radiocarbon dating). According to model estimates, C imported through the roots constituted a substantial proportion of the organic stocks, whereas C originating from above-ground plant litter was of less importance for long-term humus build-up. These results further emphasised the importance of below-ground processes in long-term humus build-up, rather than above-ground litter quantity and quality.

Finally, it was tested if the patterns of fungal involvement in organic matter accumulation observed in a single forest stand would also hold on the larger scale of the entire Swedish boreal region. The data on fungal community composition was combined with the data on climate, soil properties and tree growth in a single analysis. It was found that fungal community composition was the best predictor of organic matter stocks, whereas climate and soil properties were of minor importance, or had an indirect effect via their

influence on the fungal community. Combined, obtained results showed that fungi are important components of boreal forest, as their community composition and activity play a crucial role in nutrient cycling and organic matter accumulation.

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