Ectomycorrhizal Fungal Mycelial Dynamics and its Role in Forest Soil Carbon Cycling

Andreas Hagenbo
Faculty of Forest Sciences
Department of Forest Mycology and Plant Pathology
Uppsala

Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2017

Acta Universitatis agriculturae Sueciae
2017:27

Cover: Field layer vegetation in a fifty-nine-year-old Scots pine dominated forest (top), mycorrhizal root-tip surrounded by extraradical mycelium (down), and aggregates of extraradical mycelium (right).
(Photos: Andreas Hagenbo and Julia Kyaschenko)

ISSN 1652-6880
ISBN (print version) 978-91-576-8827-9
ISBN (electronic version) 978-91-576-8828-6

Print: SLU Service/Repro, Uppsala 2017

Ectomycorrhizal Fungal Mycelial Dynamics and its Role in Forest Soil Carbon Cycling

Abstract

Most boreal tree species rely on root-associated ectomycorrhizal fungi for nutrient acquisition, in exchange the trees allocate part of their photosynthetically fixed carbon (C) to these fungi. This has a feedback on soil C dynamics as mycorrhizal fungi are important in regulating soil C cycling and storage. However, mycorrhizal fungi are often not included in C dynamics models, as mechanistic understanding of their contribution to mycorrhiza-mediated processes are largely lacking. The aim of the work described in this thesis was to address this knowledge gap by studying the contribution of mycorrhizal fungi in regulating soil C fluxes. This was done by quantifying C fluxes associated with the extraradical mycelium (ERM) of mycorrhizal fungi, and examining how the ERM contributes to variations in soil C cycling along a nemoboreal chronosequence of managed *Pinus sylvestris* forests. Production and turnover of ERM was quantified by determining ERM biomass in sequentially harvested ingrowth mesh bags and by mathematical modelling. Respiration of ERM was measured as CO_2 efflux from mesh bags, and carbon use efficiency (CUE) was calculated from ERM production and respiration rates. We assessed soil fungal communities along the chronosequence and investigated correlations between taxonomic composition and enzyme activities. The ERM standing biomass increased despite decreased production along the chronosequence. This contradiction was explained by a drastic decline in biomass turnover, from seven times to one time per year. The CUE decreased with forest age, but increased tenfold from summer (0.019) to autumn (0.200). This seasonal increase in CUE was associated with a decline in gross photosynthetic production, suggesting that variation in photosynthetic C supply regulates seasonal variations in CUE. Relative abundance of ectomycorrhizal taxa increased with forest age, and was dominated by Atheliaceae species in young forests, and by *Cortinarius* and *Russula* species in mature forests. Enzyme activities were related to community composition, and seem to be important for maintaining forest productivity, by facilitating organic nutrient mobilisation. This thesis is a first step in parameterizing mycorrhizal mycelial C fluxes to enable explicit inclusion of ERM parameters in forest ecosystem C models.

Keywords: carbon use efficiency, chronosequence, ectomycorrhiza, ergosterol, extraradical mycelium, fungal biomass, fungal community, fungal necromass, production, respiration, turnover

Author's address: Andreas Hagenbo, SLU, Department of Forest Mycology and Plant Pathology, P.O. Box 7026, 750 07 Uppsala, Sweden
E-mail: andreas.hagenbo@slu.se

Dedication

To Ala for her continuous love and support.

If a healthy soil is full of death, it is also full of life: worms, fungi, microorganisms of all kinds. …Nothing that dies is dead for very long.

Wendell Berry

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 Hagenbo, A., Clemmensen, K.E., Finlay, R.D., Kyaschenko, J., Lindahl, B.D., Fransson, P., Ekblad, A. (2016). Changes in turnover rather than production regulate ectomycorrhizal mycelial biomass across a *Pinus sylvestris* chronosequence. *New Phytologist*, DOI: 10.1111/nph.14379.

II Hagenbo, A., Hadden, D., Clemmensen, K.E., Grelle, A., Manzoni, S., Mölder, M., Ekblad, A., Fransson, P. Large seasonal variation in carbon use-efficiency of mycorrhizal fungal extraradical mycelium across a *Pinus sylvestris* chronosequence (manuscript).

III 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*, DOI: 10.1038/ismej.2016.184.

The contribution of Andreas Hagenbo to the papers included in this thesis was as follows:

I Major participation in planning, field work, lab work, data analysis and writing.

II Participation in planning, interpretation of results and writing.

III Major participation in planning, field work, lab work, data analysis and writing.

Abbreviations

C	Carbon
CCA	Canonical correspondence analysis
CO_2	Carbon dioxide
CUE	Carbon use efficiency
DCA	Detrended correspondence analysis
ERM	Extraradical mycelium
Eqn	Equation
GPP	Gross primary production
ITS	internal transcribed spacer of rDNA
N	Nitrogen
P	Phosphorus
PCR	Polymerase chain reaction
yr	Year

1 Background

1.1 The carbon cycle of forest ecosystems

In the global carbon (C) cycle, photosynthetic fixation of CO_2 from the atmosphere and release of CO_2 via respiration are the two main driving processes regulating the transfer of C between atmosphere and biosphere (Fig. 1). In terrestrial ecosystems soil respiration represents an important C efflux and is equivalent to 10 times the amount of CO_2 released by fossil fuel burning (Raich & Potter, 1995; Grace & Rayment, 2000). In forest ecosystems, soil respiration is the main pathway by which C is returned to the atmosphere (Schlesinger & Andrews, 2000). Carbon enters the soil primarily via litter input represented by above- and belowground plant residues. However, root-associated fungi have recently been shown to contribute substantially to the soil C input; Clemmensen *et al.*, (2013) found that as much as 50-70% of the C accumulated in humus layers, along a postfire chronosequence of boreal forests stands, had entered the soil pool via roots and associated fungi, rather than via aboveground litter deposition. Soil constitutes the largest terrestrial C pool, exceeding the combined total storage of vegetation and atmosphere (Batjes, 1996).

As storage of C in soil may mitigate the rising atmospheric CO_2 concentrations from anthropogenic emissions (Batjes, 1996), it is important to understand the factors regulating soil C dynamics. In forest ecosystems, soil fungi are pivotal for C and nutrient cycling as they are largely responsible for organic matter decomposition and for plant nutrient acquisition (Smith & Read, 2008). Despite their importance, fungal-mediated soil processes are still poorly understood, and this represents a critical bottleneck restricting ecosystem C models from accurately and quantitatively predicting changes in soil C dynamics, in response to forest management and environmental change.

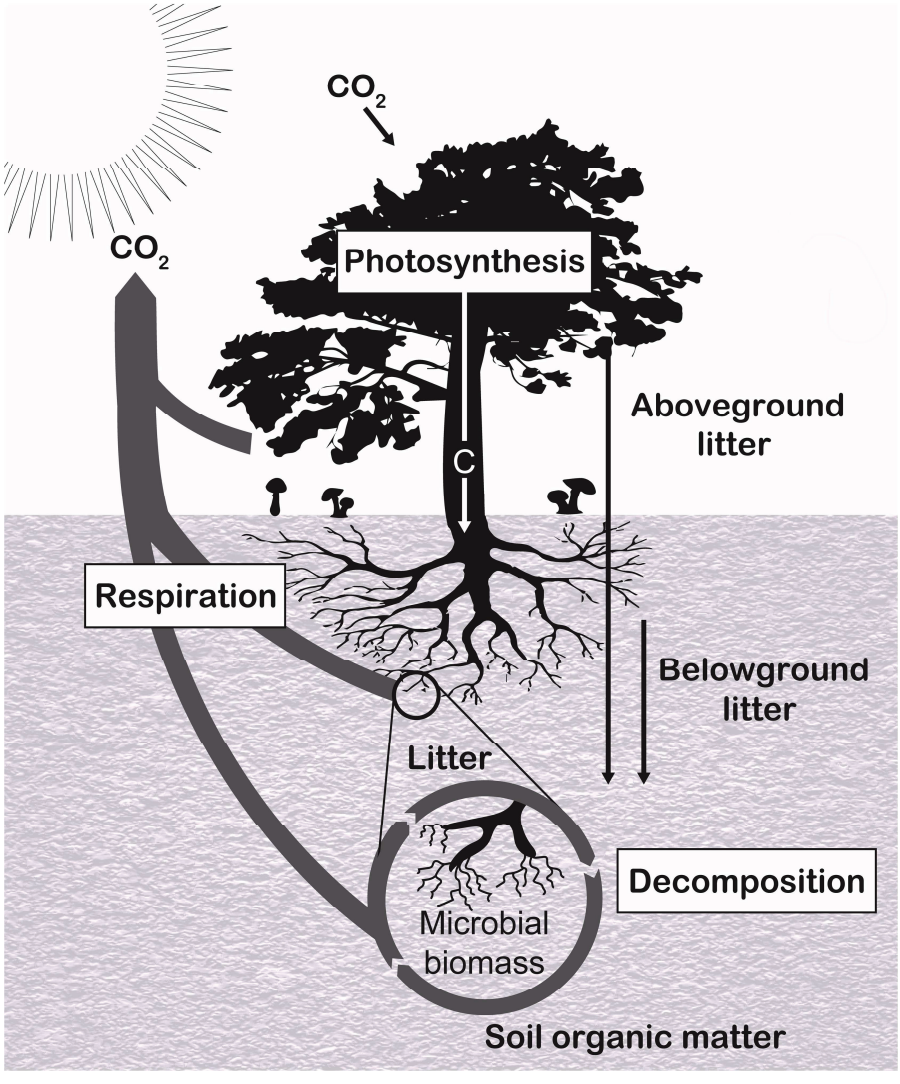

Figure 1. The carbon cycle of forest ecosystems. This figure illustrates the main carbon (C) fluxes in a forest ecosystem. Boxes represent central processes and arrows represent C fluxes. Litter, microbial biomass and soil organic matter represent soil C pools. Plants use light energy to fix CO_2 from the atmosphere to produce carbohydrates used in growth. Fixed C may enter the soil via plant belowground C allocation and via above- and belowground litter inputs. Regardless of the pathway, all soil C is part of the soil C cycle, which involves decomposition of litter and transfer of C between organisms. Carbon in the form of CO_2 returns to the atmosphere via respiration by above- and belowground plant components and by soil microorganisms.

1.2 The boreal forest ecosystem

Boreal forest, extending across the northern parts of Europe, Asia and North America, covers 11% of Earth's land surface and act as a global sink for atmospheric CO_2 (Bonan & Shugart, 1989). Boreal forests store 32% of the global forest C stock, and 60% of this C is stored belowground (Pan *et al.*, 2011). The climate is characterized by generally cold conditions and by large temperature variations between seasons (Bonan & Shugart, 1989). Winters are typically long with sub-zero temperatures, whereas summers are relatively short and moderately warm. The plant community of the boreal forest biome is typically composed of an overstory dominated by coniferous trees and an understory of Ericaceous shrubs, which together produce recalcitrant litters. Poor litter quality together with the cold climate restricts microbial decomposition and results in organic matter accumulation in the soil (Swift *et al.*, 1979). Hence nutrients are largely immobilized in the soil and thus unavailable for uptake by plants and microorganism, and as a consequence boreal forests are often nutrient limited with particularly low availability of nitrogen (N). Boreal forest plants have adapted to these nutrient poor conditions by associating with mycorrhizal fungi.

Historically natural wildfires have been the primary agent of disturbance in boreal forests, often resulting in stand-replacement and resetting of succession (Bonan & Shugart, 1989). Today firefighting efforts suppress the wildfires, and large areas are instead subjected to management in which clear-cutting represents the primary stand-replacing disturbance that resets the succession. Forest management reshapes the forests' structure, and in Scandinavia most forest areas are managed as evenly aged stands. This change in age structure is likely paralleled by changes in fungal community composition (Hynes & Germida, 2012) with potential feedbacks on soil C cycling and storage (Clemmensen *et al.*, 2015).

1.3 Mycorrhizal fungi

Since boreal forests are N limited, the plant community is highly dependent on root associated mycorrhizal fungi – mainly ecto- and ericoid mycorrhizal - for their N acquisition. Both ascomycetes and basidiomycetes, which are the two largest fungal phyla, form ectomycorrhizal associations with plant roots. In this symbiosis, specific types of fungi penetrate plant roots and form a hyphal network (Hartig net) surrounding the root cells, and embed the root in a mantle from which the extraradical mycelium (ERM) extends into the soil (Fig. 2,3). The ERM forage for growth-limiting soil nutrients, which are transferred to the host plant in exchange for photosynthetically fixed carbohydrates. Most land

plant species and families associate with different types of mycorrhizal fungi for improved nutrient uptake (Wang & Qiu, 2006), and in boreal forests almost all tree fine root tips are colonised by ectomycorrhizal fungi (Taylor *et al.*, 2000), and at least one-third of the microbial biomass is constituted by the ERM of ectomycorrhizal fungi (Högberg & Högberg, 2002). The amount of C allocated from the host plant to the mycorrhiza is thought to represent 10-50% of total plant fixed C (Simard *et al.*, 2003), and is partitioned into growth of fungal structures (mantles, Hartig net and mycelia), respiration and exudation (mainly dominated by low molecular weight organic compounds), *i.e*. organic acids, amino acids and simple sugars (van Hees *et al.*, 2005). Although a major part of the C allocated to the mycorrhizal fungi is released via respiration and exudation (Högberg *et al.*, 2001; van Hees *et al.*, 2005), a significant fraction is directed to production of ERM (Ekblad *et al.*, 2013). Whereas production of mycorrhizal ERM constitutes an important pathway of C into soil (Godbold *et al.*, 2006; Clemmensen *et al.*, 2013), it is also evident that ERM may affect soil C storage negatively, through its activity in decomposition (Moorhead & Sinsabaugh, 2006; Lindahl & Tunlid, 2015). Similarly to decomposer fungi, some groups of ectomycorrhizal fungi produce oxidative and hydrolytic enzymes which are used to mobilise nutrients from recalcitrant soil organic matter (Read & Perez-Moreno, 2003; Lindahl & Tunlid, 2015). Since production and turnover of ERM link above- and belowground C fluxes and represent significant processes for soil C input and storage (Baskaran *et al.*, 2016), their explicit incorporation into ecosystem C cycling models has been called for (Chapin *et al*., 2009; Deckmyn *et al*., 2014). To enable inclusion in models and assess the importance of these processes, we need to extend our understanding of the role of mycorrhizal fungi in soil C cycling.

Figure 2. Mycorrhizal root-tip surrounded by extraradical mycelium grown in peat. Photo: Andreas Hagenbo.

Figure 3. Aggregates of extraradical mycelium grown in peat. Photo: Andreas Hagenbo.

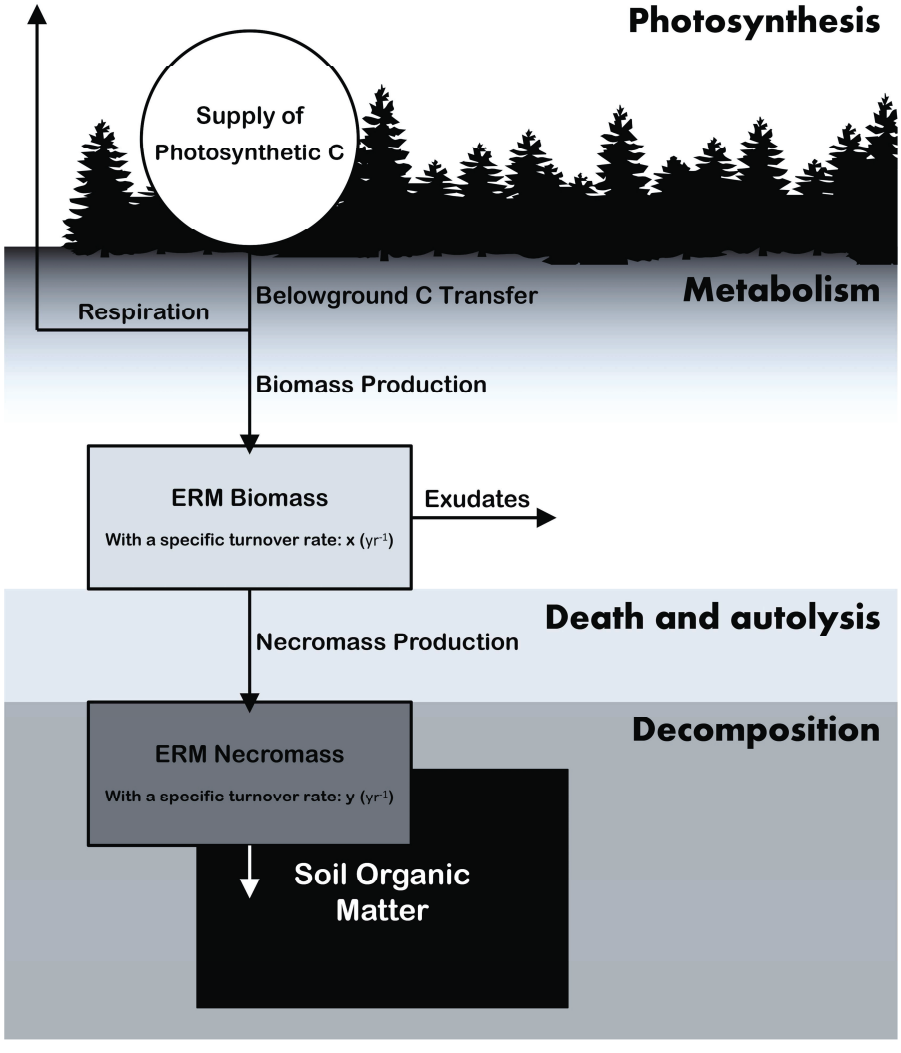

Figure 4. Schematic diagram of soil carbon (C) fluxes and pools associated with the extraradical mycelium (ERM) of mycorrhizal fungi. Arrows represent fluxes and solid boxes represent pools. The main processes regulating respective C fluxes and pools are written in bold at the right-hand side. Plant transfer of C to belowground supports the production of ERM which contributes to input of C to the ERM biomass. Production of ERM necromass is determined by the rate of ERM biomass turnover and the ERM biomass size, and represents a flow of C from the ERM biomass to ERM necromass pool. The EMR necromass is a constituent of the soil organic matter pool, and the turnover rate of necromass determines the retention time of C in the necromass.

1.3.1 Conceptual model of mycorrhizal mycelial carbon fluxes

The transfer of C through the mycorrhizal fungal ERM network can be conceptualised as three different C pools; ERM biomass, ERM necromass (recently dead biomass) and soil organic matter; and five different C fluxes; belowground C transfer, respiration, biomass production, necromass production, and exudation (Fig. 4). Pools are represented by amounts and fluxes are represented by rates of transfer. The supply of C available for transfer to mycorrhizal fungi (1) is determined by the amount of C photosynthetically fixed by the host plant, and the transfer rate belowground (2) which constrains the ERM biomass production (3) and respiration rates (4). Transfer of C belowground sustains the production of ERM, resulting in input of C into ERM biomass (5). The proportion of allocated C that is invested in growth is often referred to as the carbon use efficiency (CUE), and it is defined as the ratio between C incorporated into biomass and total C uptake (del Giorgio & Cole, 1998). Low CUE translates to a large fraction of allocated C being lost via respiration, rather than incorporated into biomass, and high CUE translates to a larger C fraction being incorporated into biomass, and less C being released via respiration.

Although some of the C allocated to mycorrhizal fungi may be lost via release of exudates (6), *e.g.* as organic acids, amino acids and simple sugars (van Hees *et al.*, 2005), most of the biomass C is likely transformed into necromass C through the death of the biomass. Thus, necromass production (7) represents a flux from biomass to the necromass pool (8), which is an integrated part of the soil organic matter pool (9). In this conceptual model, the process of biomass turnover is defined as necromass production, and the rate of turnover is not a flux, instead it is an inherent property of the biomass pools. The necromass production rate depends on two factors; biomass size and biomass turnover rate. A large necromass production is only possible when the biomass is large and/or when the rate of biomass turnover is high. When biomass is at steady state, the total biomass production is equal to the total production of necromass. Similar to the biomass pool, the necromass pool has a specific turnover rate, which depends on its decomposability and on the activity of the soil microbial community.

Since the mycorrhizal ERM network links above- and belowground C fluxes and represents a significant factor for regulating soil C cycling and storage (Clemmensen *et al.*, 2013; Baskaran *et al.*, 2016), their explicit incorporation into ecosystem C cycling models has been called for (Chapin *et al.*, 2009; Deckmyn *et al.*, 2014). Up to now mycorrhizal fungi have been widely ignored in forest soil models (Deckmyn *et al* 2014). Quantitative estimates of the mycorrhizal C fluxes are still lacking, and the role of

mycorrhizal mycelial C fluxes in soil C cycling is poorly understood. The main focus of the thesis is to address this knowledge gap by assessing the quantitative contributions of respiration, production and turnover of ERM biomass and necromass under field conditions. This will be one step in parameterizing mycorrhizal mycelial C fluxes to enable their inclusion in ecosystem C models predicting responses of mycorrhiza-mediated processes to management and environmental changes.

1.4 Potential drivers of mycorrhizal fungal carbon fluxes

As mycorrhizal fungi are dependent on C from their host plants, factors influencing photosynthetic C fixation and belowground C transfer are likely to affect mycorrhizal C fluxes. In this thesis the aboveground photosynthetic C supply, the transfer of C belowground and fungal community composition are considered as potential regulators of mycorrhizal C fluxes.

1.4.1 Photosynthetic carbon supply

The gross assimilation of photosynthetic C has been described as having an immediate effect on mycorrhizal C fluxes (Ekblad *et al.*, 2013; Wallander *et al.*, 2013), since mycorrhizal fungi depend on their host plant for supply of C (Smith & Read, 2008). Therefore, factors regulating plant photosynthesis (*e.g.* atmospheric CO_2 concentration, humidity, availability of water, light and nutrients) are likely to affect the C availability of mycorrhizal fungi. The gross assimilation of photosynthetic C represents the total uptake of atmospheric C by the ecosystem and is referred to as gross primary production (GPP) in ecosystem ecology. Gross primary production increases with foliage area (Lindroth *et al.*, 2008), hence GPP tends to be low in young forest and reaches a peak in intermediately aged forests, when the leaf area is maximised, and then gradually declines as forests matures (Tang *et al.*, 2014). However, high GPP may not necessarily translate directly into a high C availability for the mycorrhizal fungi, since GPP is partitioned within the plant, and invested into different plant components and processes, and the specific proportion of GPP allocated belowground determines the amount of C potentially available for the mycorrhizal fungi. The partitioning of GPP belowground has been shown to vary across a wide range of forest types and conditions (Litton *et al.*, 2007). However, the underlying mechanisms regulating how GPP is partitioned and translocated within the forest and how it varies with forest age are not well established.

1.4.2 Belowground carbon transfer and its dependence on nitrogen availability

Plant transfer of C belowground represents a significant soil C input, influencing both soil C cycling and C storage, and determining the amount of C available for mycorrhizal fungi - and hence the fungal biomass production (Hobbie, 2006; Fransson *et al.*, 2007). In boreal forest ecosystems the majority of GPP (about 65%) is allocated belowground to roots and root-associated ectomycorrhizal fungi (Litton *et al.*, 2007; Gill & Finzi, 2016). This large belowground C transfer seems to be coupled to N limitation, since fertilisation experiments in boreal forests demonstrate that transfer of C to belowground decreases when the N limitation is alleviated (Axelsson & Axelsson, 1986; Högberg *et al.*, 2010). In boreal forests N availability typically declines with forest development, as progressively more N is locked up in recalcitrant soil organic matter (Bauhus *et al.*, 1998; DeLuca *et al.*, 2002). This decreased N availability seems to be coupled with an increase in respiration by root and ectomycorrhizal fungi (DeLuca *et al.*, 2002; Goulden *et al.*, 2011), which implies that the belowground C transfer increases with forest age (Goulden *et al.*, 2011). However, rather than an increased belowground C transfer with forest age, the apparent increase in respiration could be the result of a decrease in the CUE of roots and mycorrhizal fungi. A forest age-related decrease in CUE of roots and mycorrhizal fungi would result in a relatively smaller proportion of the C uptake being incorporated into biomass and a greater proportion being released via respiration. In response to decreased N availability with forest age (DeLuca *et al.*, 2002; Ward *et al.*, 2014; Blaško *et al.*, 2015), it is possible that the CUE of mycorrhizal fungi would decline with age, as the CUE of microorganisms has been shown to decrease with restricted access to nutrients (Devêvre & Horwáth, 2000; van Hees *et al.*, 2005; Manzoni *et al.*, 2012). Furthermore, as mycorrhizal fungi exploit organic matter and invest resources in enzymes mobilizing N from recalcitrant soil organic matter (Bödeker *et al.*, 2014; Lindahl & Tunlid, 2015), it could be expected that mycorrhizal fungi would invest progressively more resources in energy demanding enzyme production with increasing forest age, leading to a reduction in the mycorrhizal CUE.

Across a wide range of forest types belowground C transfer has been shown to be tightly linked with aboveground plant production (Litton *et al.*, 2007), and laboratory studies have extended this correlation to mycorrhizal fungi (Hobbie, 2006). It is a universal phenomenon that above- and belowground forest production declines with age, after a mid-successional maximum (Ryan *et al.*, 1997; Pregitzer & Euskirchen, 2004; Tang *et al.*, 2014). This is true for both managed and natural forest ecosystems (Pregitzer & Euskirchen, 2004). The underlying mechanisms for this decline are still unclear but most evidence

suggests that reduced photosynthetic performance due to decreased leaf-area, limited soil nutrient supply, and limited stomatal conductance caused by the increased hydraulic resistance of taller trees, are the main responsible factors (Ryan *et al.*, 1997). If belowground C transfer is related to plant production, belowground C transfer might reach a maximum in mid-successional forests, after which belowground C transfer successively declines with increasing forest age and development (Ryan *et al.*, 1997). A mid-successional peak in belowground C transfer coupled with a decline in CUE with forest age should hypothetically result in rapid ERM production in young and intermediately aged forests. Some evidence is consistent with this hypothesis as the accumulation of ERM biomass has been shown to reach a mid-successional maximum in nemoboreal *Picea abies* forests (Wallander *et al.*, 2010).

1.4.3 Mycorrhizal fungal community composition

Ectomycorrhizal community composition changes during succession related to forest development (Twieg *et al.*, 2007; Wallander *et al.*, 2010; Sun *et al.*, 2015). In line with the theories of Cooke and Rayner (1984) the community seems to undergo a successional shift from a dominance of ruderal to more competitive and stress tolerant species as forest age increase. Ruderal species are favoured by disturbance and high resource availabilities; competitive species predominates under relatively undisturbed conditions and high resource availabilities, and stress tolerant species are adapted to harsh environments where other species fail to establish and/or survive (Cooke & Rayner, 1984). Agerer (2001) classified ectomycorrhizal fungi into contact-, short-, medium- and long-distance exploration types, based on the amount and differentiation of their ERM. The growth form of medium- and long-distance exploration types typically produce large aggregates of ERM, whereas contact- and short-distance exploration types produce no or very small ERM networks.

As mycorrhizal fungal species differ in life-strategies and growth forms, it is likely that ERM-associated C fluxes differ among mycorrhizal species and communities. Thus a shift in community structure is likely paralleled by changes in ERM-associated soil C fluxes with potential feedbacks on soil C pools (Koide *et al.*, 2014; Clemmensen *et al.*, 2015). However, C fluxes associated with ERM are poorly quantified, and understanding how a particular community composition would influence those C fluxes is even less well investigated. Hence knowledge of how specific mycorrhizal communities may affect ERM-associated C fluxes is needed in order to establish a mechanistic understanding of how mycorrhizal fungi regulate and contribute to soil C cycling.

Based on community mapping of fungal traits related to growth form, life-strategy and cell-wall biochemistry it is possible to predict how certain communities may affect ERM-associated C fluxes (Koide *et al*., 2014). For example species forming large fungal biomass structures such as cords or extensive ERM networks could potentially impose a greater C cost on the tree, as more energy would be required to support the growth and maintenance of a large mycorrhizal biomass (Rygiewicz & Andersen, 1994). However it is uncertain if a large biomass truly indicates a high C demand by the fungus as the growth and turnover rates seem to be the primary factor determining the C demand of mycorrhizal fungal species (Koide *et al*., 2014). In addition, differences in fungal resource demand (*e.g*. for C and nutrients) are related to different life strategies and habitat preferences. In line with the theories of Cooke & Rayner (1984), rapid ERM biomass production and turnover can be expected in nutrient rich and recently disturbed system where ruderal species dominate. In contrast, slow biomass production and turnover can be expected in more stable and nutrient limited, conditions dominated by long-lived competitive and stress-tolerant species. Irrespective of whether a forest is natural or managed, in young recently re-established boreal forests, ruderal species should be abundant as the degree of disturbance and N availability is typically highest at the beginning of the succession (DeLuca *et al.*, 2002). In mature forests where the degree of disturbance is generally lower and N availability is more restricted, strong priority effects should promote more long-lived species with slower N usage, which may result in an increased abundance of competitive and stress-tolerant species with slow turnover. Hypothetically, progressive N limitation coupled to a mycorrhizal community shift may translate into decreasing production and turnover of ERM with increased forest age.

The mycorrhizal fungal community composition may also be important for the turnover of necromass. Analogous to plant litter, the degradability of fungal necromass has been shown to vary among fungal species in laboratory experiments (Koide & Malcolm, 2009; Wilkinson *et al.*, 2011), and relate to differences in cell-wall biochemistry and N content (Koide & Malcolm, 2009; Fernandez & Koide, 2014). A high abundance of mycorrhizal fungal species producing recalcitrant necromass may thus lead to a high proportion of necromass being preserved in the soil, due to a slow rate of necromass turnover (Koide & Malcolm, 2009; Fernandez & Koide, 2014). Furthermore, slow necromass turnover seems to feedback on soil C storage as variations in mycorrhizal fungal community composition have been shown to be associated with variations in long-term soil C storage (Averill *et al*., 2014; Clemmensen *et al*., 2015). Several stress-tolerant mycorrhizal species, associated with late

successional forests (Clemmensen *et al.*, 2015), have been shown to produce ERM resistant to decomposition (Fernandez & Koide, 2014). Clemmensen *et al.* (2015) showed that mycorrhizal species with stress-adapted traits largely contribute to the soil C accumulation through the production of recalcitrant necromass. Despite evidence of necromass driving soil C accumulation, the rate of necromass turnover is unknown.

Although soil C storage seems to partly depend on the accumulation of mycorrhizal ERM necromass (Godbold *et al.*, 2006; Clemmensen *et al.*, 2013), it is also evident that ERM may affect soil C pool negatively through its role in decomposition (Moorhead & Sinsabaugh, 2006; Lindahl & Tunlid, 2015). As mentioned previously, the major fraction of the N pool in boreal forest is locked up in soil organic matter. To mobilise this N (as well as other mineral nutrient), some mycorrhizal fungal species are equipped with nutrient mobilizing enzymes which degrade organic complexes in the soil (Lindahl & Tunlid, 2015). Since mycorrhizal species differ in their enzymatic profiles and activities, community changes are likely to have a direct effect on the enzymatic degradation of soil organic complexes, and on the mobilisation of N. By combining profiling of mycorrhizal fungal communities and enzyme analyses, it should be possible to assess environmental drivers of the enzyme expression of mycorrhizal fungi.

1.5 Methods for quantifying mycelial fungal biomass

A first step in assessing C fluxes associated with mycorrhizal ERM is to separate the ERM from the soil matrix; this is commonly done by incubating ERM ingrowth mesh bags in the soil. The fungal biomass in mesh bags can be quantified by using four main types of techniques; 1) direct microscopic observation, 2) selective respiratory inhibition, 3) quantification of ribosomal DNA, and 4) quantification of specific cell-wall and cell membrane components (Joergensen & Wichern, 2008; Baldrian *et al.*, 2013; Wallander *et al.*, 2013). For the work in this thesis the fungal biomass markers ergosterol and chitin were analysed. These are two of the most commonly used techniques for fungal quantification.

1.5.1 Ingrowth mesh bags

In a field setting it is possible to estimate the production and turnover of ERM by incubating ingrowth mesh bags (Wallander *et al.*, 2013). Mesh bags are made from fine nylon mesh (<50 μm) which allows for hyphal ingrowth, but prevents tree roots from entering. The bags are commonly installed at the interface between the organic and mineral soil layers. In boreal forest soils this should result in maximised ingrowth as mycorrhizal fungi dominate this part of the soil profile (Lindahl *et al.*, 2007). The incubation time in the field is often several months, whereupon the accumulation of ERM biomass is quantified to estimate total production of ERM biomass during that period. Sand lacking organic C, is typically used as a growth substrate to discriminate against non-mycorrhizal fungal ingrowth. As mycorrhizal fungi rely on their plant host for their supply of C, they can more easily colonize C free substrates than non-mycorrhizal fungi (*e.g*. saprotrophic fungi). Trenching experiments, as well as analysis of DNA composition and ^{13}C abundance, have demonstrated that sand-filled mesh bags are dominated by mycorrhizal fungi (Wallander *et al.*, 2001; Kjøller, 2006; Parrent & Vilgalys, 2007; Wallander *et al.*, 2010).

1.5.2 Ergosterol and chitin as fungal biomass markers

Ergosterol is a sterol found almost exclusively in the cell-membranes of living fungi, and is often used as a fungal biomarker since it degrades rapidly after cell death (Nylund & Wallander, 1992). Ergosterol is the dominating sterol in most asco- and basidiomycetes, however in some other fungal taxonomical groups such as Glomeromycota (forming arbuscular mycorrhiza), ergosterol may either be lacking or absent (Olsson *et al.*, 2003). Ergosterol frequently correlates very well with other methods used for quantifying fungal biomass (Wallander *et al.*, 2013), and ergosterol assays largely represent a standard in the research community for estimating fungal biomass in soils and mesh bags. Chitin is a polymer of N-acetyl-D-glucosamine present in the cell-walls of all fungi (Lezica & Quesada-Allué, 1990), and is similar in function to cellulose in higher plants. In soil most of the chitin is derived from fungi, but microarthropods may also contribute as chitin is abundant in their exoskeletons. However, their contribution should be small as the biomass of microarthropods is typically below 0.5% of the fungal biomass (Beare *et al.*, 1997; Simpson *et al.*, 2004). Analysis of chitin has been used extensively previously to estimate fungal biomass, but now ergosterol is mostly preferred as chitin content appears to be relatively stable in dead fungal tissues (Ekblad *et al.*, 1998).

2 Objectives

The studies in this thesis concern the role of the ectomycorrhizal fungal ERM and soil fungal community composition in regulating soil C fluxes and storage and contributing to ecosystem functioning. Projects aimed at quantifying C fluxes associated with ectomycorrhizal fungal ERM, to allow their inclusion in future ecosystem C models, which may increase the capacity of models to predict responses of mycorrhiza-mediated processes to management and environmental changes.

The specific objectives were to:

I. Quantify the production and turnover of ectomycorrhizal fungal ERM, and examine how they together regulate the standing ERM biomass under field conditions (paper I).
II. Establish whether ectomycorrhizal fungal ERM dynamics change with forest development, in response to variation in C allocation pattern and soil N availability (paper I).
III. Estimate the CUE of ectomycorrhizal ERM and whether it changes with forest development and season (paper II).
IV. Estimate the contribution of ectomycorrhizal ERM respiration to total soil respiration and whether this contribution changes with forest development and season (paper II)
V. Estimate the proportion of GPP allocated to the ectomycorrhizal ERM and whether the allocation to ERM varies over the season (paper II).
VI. Assess whether a change in soil fungal community composition and enzyme activities occurs with forest development and if potential compositional changes are coupled to ERM dynamics, nutrient cycling and C storage (paper III).

3 Materials and Methods

3.1 Description of the chronosequence

All thesis projects were conducted during 2012-2014 across a chronosequence consisting of ten managed *Pinus sylvestris* L. stands ranging in age from 1 to 158 years, located in Uppsala County, central Sweden (Fig. 7). Stands were selected from a forest stand database including 2260 separate *P. sylvestris* stands using ARCMAP 9.3 (Esri, Redlands, CA, USA). All selected stands were managed, unfertilized, evenly aged, with similar water availability and site/fertility index. In stands less than 50 years old the even age structure was obtained by re-planting after clear-cutting, whereas the even age structure in stands more than 50 years old was obtained by selective thinning. History of the 59-year-old stand is uncertain. The distance between stands was selected to be at least 0.2 km to enable separate stands to be treated as independent replicates, but within 28 km of each other to avoid major differences in climate and geology.

The soil in the chronosequence is classified as an Umbric podzol (FAO, 1998) and is composed of sandy, loamy till with many boulders. Although all stands were dominated by *P. sylvestris*, *Picea abies* (L.) Karst. became an increasingly abundant component of the understory vegetation with increasing stand age. In younger stands narrow-leaved grasses, such as *Deschampsia flexuosa* L. and *Festuca ovina* L., dominated the field layer vegetation, but with increasing age, grasses were replaced by Ericaceae plants, such as *Vaccinium myrtillus* L. and *V. vitis-idea* L. In older stands there was some occurrence of herbs (*e.g*. *Linnea borealis* L.) and ferns (*e.g*. *Pteridium aquilinum* (L.) Kuhn). The mean annual temperature is 5.4 °C and the annual precipitation is 520 mm (Lindroth *et al.*, 1998). The length of the growing season (mean daily temperature above 5 °C) is typically 180 days, beginning in mid-April and ending in mid-October.

Figure 5. Twelve-year-old Scots pine (*Pinus sylvestris*) forest, representing one of the younger stands in the chronosequence. The field layer vegetation is dominated by grasses (*i.e. Deschampsia flexuosa* and *Festuca ovina*). Photo: Julia Kyaschenko.

Figure 6. Thirty-four-year-old Scots pine (*Pinus sylvestris*) forest representing the mid-successional stage of the chronosequence. Ericaceous plants (*i.e. Vaccinium myrtillus* and *V. vitis-idea*) are starting to replace the grasses. Photo: Julia Kyaschenko.

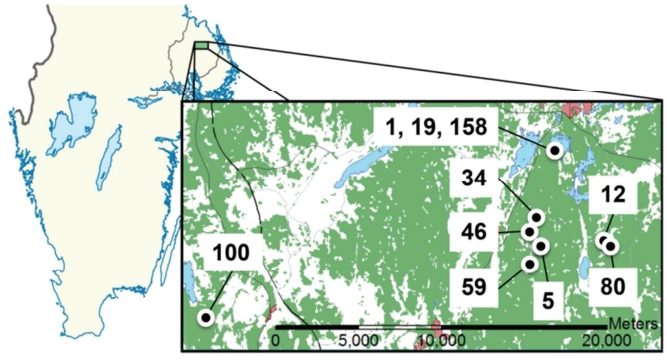

Figure 7. Map of southern Sweden, showing geographical location of Scots pine (*Pinus sylvestris*) forest stands representing the chronosequence. The age of each stand is indicated in years. The enlarged inset represents an area of 15x30 km and is 30 km north of Uppsala.

3.2 Estimating production and turnover ectomycorrhizal mycelium

The biomass of ERM in the soil is determined by the processes of mycelial production and turnover. Most previous studies have quantified the accumulation of ERM biomass in mesh bags as a proxy of production (Ekblad *et al.*, 2013; Wallander *et al.*, 2013), without compensating for turnover. Without knowing the degree of biomass turnover the actual biomass production may be underestimated in those studies. Here we address the problem by separating effects of production and turnover on the accumulation of biomass, resulting in rate estimates for the two processes. Production and turnover rates of ERM biomass can be determined by incubating ERM ingrowth mesh bags in soil over different and overlapping incubation periods and applying mathematical models (Ekblad *et al.*, 2016). The simplest way to achieve this is by using three sets of mesh bags: two sets incubated over two short consecutive periods (t_1, t_2), and one third set incubated over the entire period covered by the two first sets of bags ($t_1 + t_2$; Fig. 10a). The long-term incubated bag is expected to undergo a larger degree of turnover due to the longer incubation time.

Figure 8. Forty-six-year-old Scots pine (*Pinu sylvestris*) forest with increasing establishment of Norway spruce (*Picea abies*) and field layer vegetation dominated by *Vaccinium myrtillus* and *V. vitis-idea*. Photo: Julia Kyaschenko.

Figure 9. 100-year-old forest Scots pine (*Pinus sylvestris*) forest where Norway spruce (*Picea abies*) is more abundant in the overstory. Soil surface is covered by mosses (*i.e. Hylocomium splendens* and *Pleurozium schreberi*) and Ericaceous plants. Photo: Julia Kyaschenko.

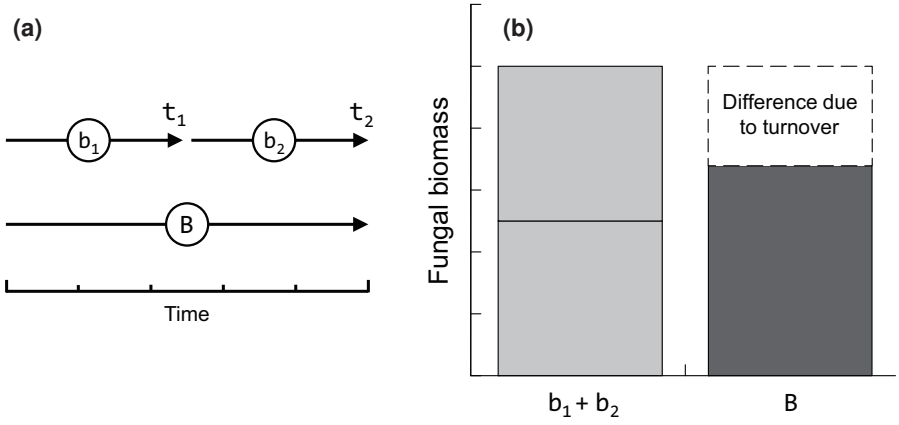

Figure 10. Principle of estimating turnover of fungal mycelium. In (a) b_1 and b_2 represent the mycelial biomass in ingrowth mesh bags with short incubation times, harvested at the time points t_1 and t_2, and B represents biomass in a mesh bags incubated throughout the whole period ($t_1 + t_2$). If turnover has occurred the combined biomass b_1 and b_2 is greater than the biomass in B (b). This difference is due to turnover, which can be calculated using an exponential decay model (Eqn 1a).

If turnover has occurred during the study period, the ERM biomass in the long-term incubated bag should be smaller than the combined accumulated ERM biomass in the two sets of bags with shorter incubations (Fig. 10b). The larger the difference is between the biomass of the long-term bag and the combined biomass of the two short-term bags, the larger the degree of turnover. This difference in ERM biomass reflects the relative degree of turnover between bags and can be used to calculate the turnover rate (μ) using an exponential decay model (Ekblad *et al.*, 2016):

$$B = b_2 + b_1 e^{-\mu(t_2 - t_1)} \qquad \text{(Eqn 1a)}$$

where B is the biomass of a mesh bag incubated over the whole period, and b_1 and b_2 is the biomass in the two sets of mesh bags incubated consecutively, at short intervals, thus one set harvested in the middle (t_1) and one set harvested at the end of the study period (t_2). The rate of ERM turnover (time^{-1}) can be determined by isolating the turnover term (μ) from the equation:

$$\mu = \frac{-ln\frac{B - b_2}{b_1}}{t_2 - t_1} \qquad \text{(Eqn 1b)}$$

When turnover is determined, the production rate of ERM can be calculated using Eqn 2b, derived from Eqn 2a (Ekblad *et al.*, 2016), which describes the temporal change in ERM biomass ($B(t)$) as a function of production (p), turnover (μ) and incubation time (t).

$$B(t) = \frac{p}{\mu}(1 - e^{-\mu t}) \quad \text{(Eqn 2a)}$$

Isolation of p gives:

$$p = \frac{B(t)\mu}{1-e^{-\mu t}} \quad \text{(Eqn 2b)}$$

Using Eqn 2b the ERM production rate can be calculated when turnover, biomass and incubation times are known. The ERM production estimate derived from these calculations differs from estimates of most previous studies as the production represents a flux - not accumulation of standing biomass in the bags. It is worth noting that production and turnover can also be estimated by fitting Eqn 2a to ERM estimates from mesh bags with increasingly longer incubation times (Ekblad *et al.*, 2016). The parametric values of production and turnover derived from the optimal fit can then be used as estimates.

3.3 Estimating carbon use efficiency ectomycorrhizal mycelium

Since CUE is an important factor representing how much C is stabilised in biomass or lost via respiration it is important in the context of mycorrhizal C storage and for predicting associated soil C fluxes. Given the considerable difficulty in quantifying C transfer from the host plants, and C investment in mycorrhizal fungal growth, there is currently no direct method of measuring the CUE of mycorrhizal ERM under field conditions. However, by quantifying the ERM biomass C production rate and the simultaneous ERM respiration, CUE can be calculated from the equation below (Manzoni *et al.*, 2012):

$$CUE = \frac{G}{G+R}. \quad \text{(Eqn 3)}$$

When the turnover rate is known, the ERM biomass production can be calculated using Eqn 2b. From the ERM biomass production, the biomass C production or growth (G) can be calculated by assuming a fungal C content of 50%. The ERM respiration (R) can be measured using a closed infra-red gas

analyser (IRGA) measuring the CO_2 efflux from the ingrowth mesh bags (Neumann & Matzner, 2014).

3.4 High-throughput sequencing of fungal communities

Recent advances in the development of DNA-based methods for analysing microbial communities have revolutionized fungal ecology (Hibbett *et al.*, 2009), and largely replaced morphologically-based taxonomical studies of fungal communities. Molecular identification of fungal species by high-throughput sequencing methods enables profiling of whole communities in environmental samples. Fungal species are identified by sequencing of the internal transcribed spacer (ITS) region within genes coding for ribosomal RNA. The ITS region is used as a universal DNA barcode for fungi in molecular techniques, since the non-coding ITS region is more variable among species than within species (Schoch *et al.*, 2012). The ITS region is flanked by more highly conserved, coding gene regions; the 18S and 28S regions, and the 5.8S region in the middle splits the ITS region into the ITS1 and ITS2 sub-regions (Fig. 11). Sequencing of the ITS2 region has proved efficient for identifying most fungal species (Lindahl *et al.*, 2013).

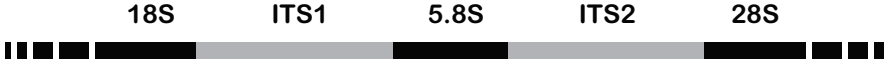

Figure 11. The internal transcribed spacer (ITS) region of ribosomal DNA in fungi is flanked by the 18S and 28S coding gene regions. The 5.8S gene region in the middle splits the ITS region into ITS1 and ITS2 sub-regions. Fungal species identification is typically done by sequencing of the ITS2 region.

To enable sequencing, the ITS2 region must first be amplified in sufficient amounts. Amplification is done by polymerase chain reaction (PCR), which involves two short, single-stranded DNA primers attaching to specific target sites in the 5.8S and 28S genes, delimiting the DNA region to be amplified, and a DNA synthesis polymerase, which copies the ITS2 sequence. The PCR process involves several cycles of heating and cooling of the samples. High temperature splits the template DNA into singled-stranded DNA, a subsequent cooling results in primers attaching to the single-stranded DNA which enables the polymerase to create a complementary strand using the primers as starting point. At the end of this process a complete double-stranded copy of the template DNA sequence has been created. After each cycle the DNA molecules representing the amplified region have been doubled. PCR

amplicons from multiple samples can be analysed simultaneously by high-throughput sequencing, therefore, each sample must be marked by an exclusive identification tags attached to the primer. Currently several high-throughput sequencing methods are available commercially, but for the thesis project only single molecule real time (SMRT) sequencing from Pacific Biosciences was used. Since SMRT sequencing is robust against sequencing output bias due to sequence length differences, the method is suitable for analysing fungal communities of ITS2 sequences which differ in length.

Large amounts of data are produced using high-throughput sequencing, and sequence reads need to be quality filtered and clustered into species level before further analyses. To do this, reads are filtered using bioinformatics tools to remove reads that are too short, have too low quality, missing primers or identification tag sequences. Reads passing the quality check are clustered into operational taxonomic units (OTUs) which represent groups of reads sharing similar DNA sequences. The threshold of similarity is typically set at 98.5% for species-level resolution when using single-linkage clustering (Kõljalg *et al*., 2013). Thus sequences which are less than 98.5% similar are separated into different species-level clusters. Based on the number of sequences in a cluster and the total numbers of clusters, the relative occurrence of OTUs can be calculated for separate samples, representing the relative abundance of fungal taxa. For the thesis project the pipeline SCATA (https://scata.mykopat.slu.se) was used to quality filter and cluster sequences.

3.5 Project descriptions

3.5.1 Paper I: Production and turnover of ectomycorrhizal mycelial biomass

In paper I we investigated how production and turnover of ectomycorrhizal fungal ERM together regulate the standing ERM biomass with increasing forest age. This was assessed across the 12-year- to 100-year-old stands of the chronosequence by determining ERM biomass, using ergosterol as a proxy, in sequentially harvested ingrowth mesh bags. Mathematical models were applied on biomass estimates to determine production and turnover rates.

In all chronosequence stands five subplots (2x2 m) were established, and from each subplot six different sets of mesh bags (giving a total of 300 bags) were incubated consecutively and in parallel from late October 2012 to mid-November 2014 (Fig. 12). In each subplot, two to four mesh bags were incubated depending on season, with a higher sampling intensity employed during summer and autumn of 2013. Harvested bags were directly replaced by new bags inserted into the same holes, to minimize disturbance.

After harvest, samples were processed and analysed for ergosterol content. Production and turnover were determined as described in section 3.2, and based on production and turnover rate estimates, a model predicting the accumulation of ERM biomass was constructed. Using the model the annual accumulation of ERM biomass was predicted for each stand of the chronosequence, and compared against empirical data. Correlations between ERM production, turnover, biomass and forest age were evaluated for statistical significance using linear regressions.

Based on the assumption that C availability for mycorrhizal fungi is correlated with forest productivity and the fact that productivity declines with forest age (Ryan *et al.*, 1997), we hypothesised (*i*) that the production rate of ERM declines with increasing forest age. We also hypothesised (*ii*) that the ERM turnover rate would decrease with the forest age, as a result of increased abundance of long-lived competitive and stress tolerant species as forests age (Cooke & Rayner, 1984).

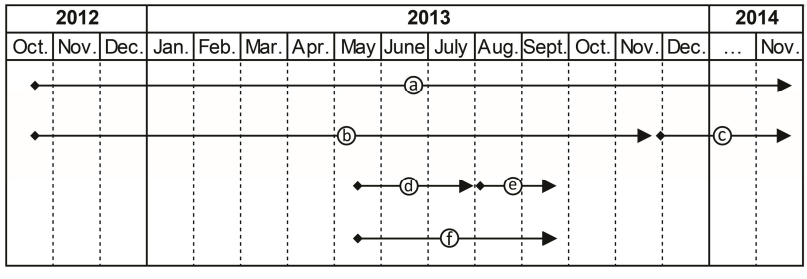

Figure 12. Incubation scheme for ingrowth mesh bags used in Paper I, incubated and harvested between October 2012 and November 2014. The beginning (♦) and end (►) of each arrow indicate the respective time points of installation and harvest of mesh bags. Letters in figure annotate mesh bags with different incubation periods.

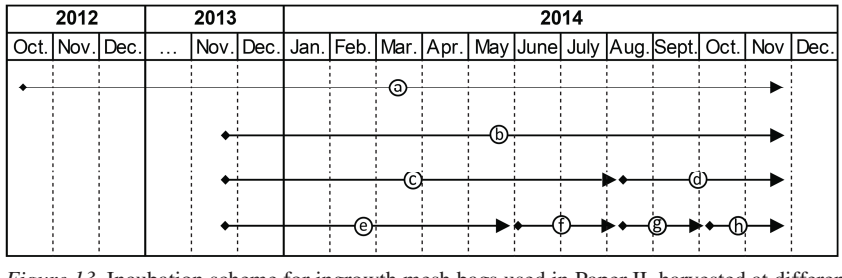

Figure 13. Incubation scheme for ingrowth mesh bags used in Paper II, harvested at different time points from October 2012 to November 2014. Beginning of arrows (♦) indicates mesh bags installation time point, and end of arrow (►) shows time point of harvests; May August, October and November. Letters in figure annotate mesh bags with different incubation periods.

3.5.2 Paper II: Carbon use efficiency of ectomycorrhizal mycelium

In paper II we quantified the ERM respiration and related it to total soil respiration and gross primary production (GPP), and we also estimated the carbon use efficiency (CUE) of ERM and tested whether it changed with season and forest age. Respiration of ERM was estimated by measuring the CO_2 efflux from recently harvested mesh bags placed inside in a gas-tight plastic chamber connected to an infra-red CO_2 gas analyser (IRGA). Mesh bags were incubated over different and overlapping periods in 2013, according to the harvest scheme in Fig. 13. This harvest scheme consisted of eight sets of mesh bags and was replicated across chronosequence stands aged 12- to 100-years old, in five sub-plots per stand (total of 280 bags). Soil respiration measurements were done at four different occasions when mesh bags were harvested (May, August, October and November). Respiration of ERM was related to total soil respiration to approximate the ERM contribution. Seasonal changes in total soil respiration, ERM respiration, and ERM respiration contribution to total soil respiration were evaluated for statistical significance using analysis of variance. The relationships between temperature, total soil respiration and ERM respiration were tested using linear regressions.

From ERM turnover estimates in paper I and accumulated ERM biomass in mesh bags, the production of ERM was calculated at each site, for each time interval, using Eqn 2b. Production and respiration estimates were then used to calculate CUE, using Eqn. 3. Seasonal and forest age-related changes in CUE were assessed, respectively, using analysis of variance and linear regressions. Gross primary production of the 12- and 100-year-old forest was estimated from calculations of total ecosystem respiration and eddy covariance flux measurements of net ecosystem C exchange. This approach enabled comparison between GPP, total soil respiration, and ERM respiration.

We hypothesised that (*i*) mycorrhizal fungal CUE would increase as the season progressed from early summer to late autumn, based on earlier studies demonstrating that microbial CUE typically increases with declining temperature (Manzoni *et al.*, 2012). We further hypothesized that (*ii*) CUE of ERM would decline with forest age as an effect of decreased N availability (Devêvre & Horwáth, 2000; DeLuca *et al.*, 2002; Manzoni *et al.*, 2012). We also hypothesized that (*iii*) the ERM respiration contribution to total soil respiration would increase with forest age, as relatively more C is released via respiration with declining CUE.

3.5.3 Paper III: Fungal community composition and enzyme activity along a *Pinus sylvestris* chronosequence

In paper III we examined how changes in fungal community composition were associated with enzymatic activities in soils across the chronosequence. In October 2012 nine soil cores were randomly sampled from all ten sites of the chronosequence. All green plants were removed and soil cores were divided into three parts representing the litter, fragmented litter and humus layers. Roots with a diameter more than 2 mm were removed and materials from respective soil layers were pooled within each site and homogenised. In all samples, activities of seven hydrolytic and two oxidative enzymes were measured (Saiya-Cork *et al*., 2002), DNA was extracted and fungal biomass was estimated by measurements of ergosterol content. The seven hydrolytic enzymes were cellobiohydrolase, β-1.4-glucosidase, β-1.4-xylosidase, leucine aminopeptidase, chitobiosidase, β-1.4-N-acetylglucosaminidase, acid phosphatase; and the two oxidative enzymes were Mn-peroxidase and laccase. For humus samples pH and content of C and extractable inorganic N were determined.

Community composition in the different soil layers was assessed by high-throughput SMRT-sequencing of ITS2 amplicons. Sequences were clustered into species hypotheses (Kõljalg *et al.*, 2013), and subsequently subjected to taxonomic and functional identification. Development of the fungal communities over the chronosequence, and in the different soil layers, was analysed using ordination methods. Detrended correspondence analysis (DCA) was used for graphical representation and correspondence analysis (CCA) was used to evaluate statistical significances. Correlations in community composition and enzymatic activity profiles were evaluated for significance using mantel tests (Goslee & Urban, 2007), and similarity in enzyme profiles among fungal genera/orders was visualised by principal component analysis (PCA). These approaches enabled us to establish the development of fungal communities and associated enzymatic activities with increasing forest age.

Based on the fact that ectomycorrhizal fungi are negatively affected by stand-replacing disturbances (Jones *et al.*, 2003; Grebenc *et al.*, 2009), we hypothesised (*i*) that mycorrhizal fungi will increase in abundance with increasing time since clear-cutting, whereas saprotrophs will decline as mycorrhizal species expand and take over their niche. We also hypothesized that (*ii*) early successional stage mycorrhizal species (*i.e*. Atheliaceae species) will gradually be replaced by a more diverse ectomycorrhizal fungal community with more late-successional stage species (*i.e*. *Cortinarius* species). We further hypothesised that activity of cellulose degrading enzymes should be correlated with saprotrophic fungal abundance, and activities of enzymes

involved in mobilisation of organic nutrients should correlate with abundance of *Cortinarius* species.

3.5.4 Turnover of ectomycorrhizal mycelial necromass

For the purpose of providing a more complete overview over the C fluxes associated with ERM, the production and turnover of ERM necromass (dead ERM) and total organic C were also assessed. The results are preliminary and not yet included in any manuscript. Necromass turnover determines the retention time of C in the necromass, and together with the ERM necromass size determines the C flux from the ERM necromass to the stable soil organic matter pool. The same mesh bags and corresponding biomass estimates used in Paper I (Fig. 12) were also used in this study.

The ERM necromass was estimated on the basis of the difference between fungal mass and biomass in mesh bags (Ekblad *et al*., 2016). The content of ergosterol was assumed to mainly represent the biomass and the content of chitin was assumed to represent the ERM mass, including both fungal biomass and necromass (Ekblad *et al*., 1998, 2016). Chitin was extracted as described by Ekblad and Näsholm (1996) with the modification that samples were not treated with NaOH and extracts were not subjected to derivatization (converting chitin-derived glucosamine to a fluorescent product) prior to analysis. Instead of using the conventional fluorometric detection, samples were analysed using mass spectroscopy (Olofsson & Bylund 2016). Fungal necromass was estimated from the difference between fungal mass and biomass (Ekblad *et al*., 2016). Differences in extraction efficiencies between ergosterol and chitin assays were compensated for by multiplying the extracted ergosterol and chitin amounts respectively by 1.61 and 1.41 (Montgomery *et al*., 2000; Alf Ekblad, pers. comm.). Total C content in samples was estimated by loss of ignition (1350 °C during two 5 sec burning cycles). Production and turnover of ERM necromass and total C was determined by fitting Eqn 2b to data sets representing the necromass and total organic C of ERM.

Based on Clemmensen *et al.* (2013) reporting that increased root and mycorrhizal fungal derived soil C inputs occur during boreal forest succession, we hypothesised that necromass turnover rate would decrease with increased forest age, as result of progressively increasing recalcitrance of the ERM necromass.

4 Results and Discussion

4.1 Production of ectomycorrhizal mycelium

Production of ectomycorrhizal ERM is supported by the transfer of C from their host plant, and the rate at which ERM is produced determines the rate of C input into the ERM biomass pool. While several studies have quantified the accumulation of ERM in mesh bags as a measure of production (Ekblad *et al.*, 2013), the effect of turnover on the accumulation of biomass has generally not been accounted for, potentially leading to underestimation of production (Cairney, 2012; Ekblad *et al.*, 2016; Hendricks *et al.*, 2016). In this thesis the separate contributions of production and turnover to the accumulation of ERM biomass were assessed, enabling production to be described as a flux, rather than as a pool.

4.1.1 Production of ectomycorrhizal mycelium declines with forest age

In paper I we hypothesised that the production rate of ERM biomass would decline with forest age after a mid-successional maximum, as a result of less C being allocated belowground with decreasing plant production in older forests. This hypothesis was based on the fact that belowground C transfer is correlated with plant production (Litton *et al.*, 2007), which has been shown to decrease with forest age after a mid-successional maximum (Ryan *et al.*, 1997). In agreement with our hypothesis the ERM production rate decreased with increasing forest age (Paper I and Paper II), and ranged from approximately 1.2 kg ha^{-1} day^{-1} in the 12-year-old stand to 0.5 kg ha^{-1} day^{-1} in the 100-year-old stands (Fig. 14a). The hypothesised mid-successional peak in production was shown in paper II when maximal ERM production was observed in the 34- and 46-year-old forests (Fig. 14b), which corresponds to timing of canopy closure (Croft *et al.*, 2014).

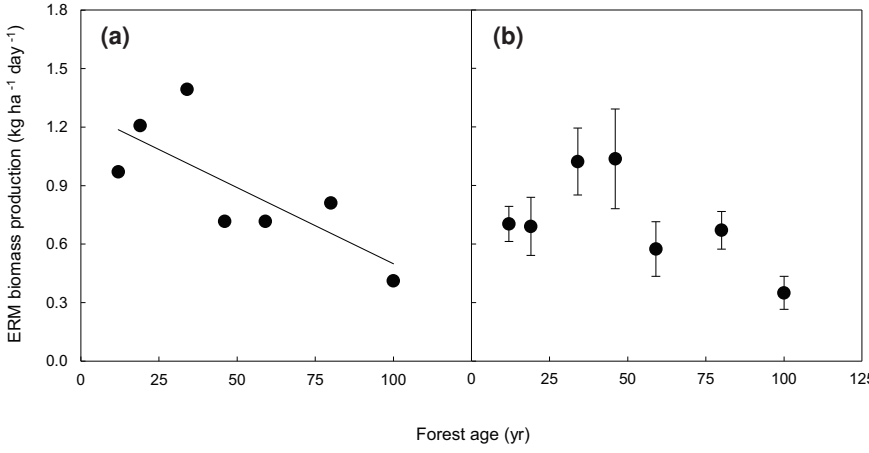

Figure 14. Estimated extraradical mycelium (ERM) biomass production as a function of forest age during the growing season of (a) 2013 and (b) 2014. The solid line in (a) represents linear regression fitted to production estimates: y = -0.008x + 1.28, r^2 = 0.57, *P* = 0.049, SE = 0.23. The error bars in (b) represents ± SE. Production estimates in (a) are based on ingrowth mesh bag set d-f (Fig. 12) and in (b) based on c-h (Fig. 13)

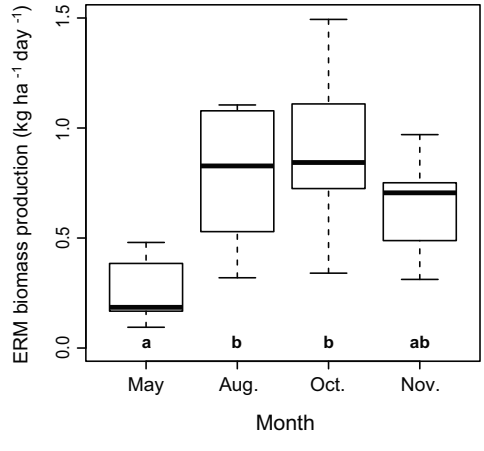

Figure 15. Production rate of extraradical mycelium (ERM) in ingrowth mesh bags consecutively harvested approximately at two months intervals in May, August, October and November. Different letters indicate significant difference between in ERM production, tested by Tukey's multiple range test at $\alpha \leq 0.05$, n = 7. Figure based on ingrowth mesh bag set e-h (Fig. 13).

Based on data from Paper II the production of ERM reached a peak during summer and early fall (Fig. 15), and seemed to be related to variation in GPP as ERM production and GPP followed a similar pattern over the season (Fig. 15 and 16). In boreal forests, fixation of C typically reaches a peak during summer (Mäkelä *et al.*, 2004, 2008; Stinziano *et al.*, 2015), thus a mid-seasonal peak in GPP is to be expected. Between years (2013 and 2014) production of ERM during the growing season was similar (Fig. 14). The fact that ERM production declined with forest age after an mid-successional maximum and reached a seasonal peak during summer when GPP was high, supports the hypothesis that C availability regulates production of ERM (Wallander, 1995; Ekblad *et al.*, 2013). However, as direct effects of C availability on ERM production were not tested, it is not possible to conclude whether C availability truly contributes to the observed variation in the ERM production across the chronosequence.

4.1.2 Does nitrogen availability explain variations in ectomycorrhizal mycelial production?

Availability of N seems to influence ERM production rate, as several studies report decreased accumulation of ERM biomass with additions of N (Nilsson & Wallander, 2003; Nilsson *et al.*, 2007; Kjøller *et al.*, 2012; Bahr *et al.*, 2013). Ekblad *et al.* (2016) reported that N fertilisation in a warm-temperate *Pinus taeda* forest halved the ERM production and standing biomass, but turnover was not affected. Therefore it seems likely that lower biomass with N addition is the result of a reduced production, rather than a change in turnover (Ekblad *et al.*, 2016). A proposed mechanism for this reduced ERM growth is that relatively more C is invested in above ground plant production, rather than allocated belowground to the mycorrhizal fungi, when N limitation is alleviated (Hobbie, 2006). In support of this, Högberg *et al.* (2010) reported that belowground C transfer in a 14-year-old *P. sylvestris* forest decreased by 60% one year after N fertilisation. Most previous studies testing the effect of N fertilization on ERM production have used rather large N additions; *i.e.* 100 kg N ha^{-1} yr^{-1} in Nilsson and Wallander (2003), but even moderate amounts (10-20 kg N ha^{-1} ya^{-1}) and natural variation in N availability, have shown a tendencies to reduce the ERM biomass (Nilsson *et al.*, 2005, 2007; Bahr *et al.*, 2013).

In boreal forests, the availability of N decreases with increasing forest age (DeLuca *et al.*, 2002; Ward *et al.*, 2014), and based on the fact that soil pH correlates with N availability (Lahti & Väisänen, 1987), it seems likely that N availability also declined across our chronosequence, since soil pH decreased with increasing forest age (Paper I and III).

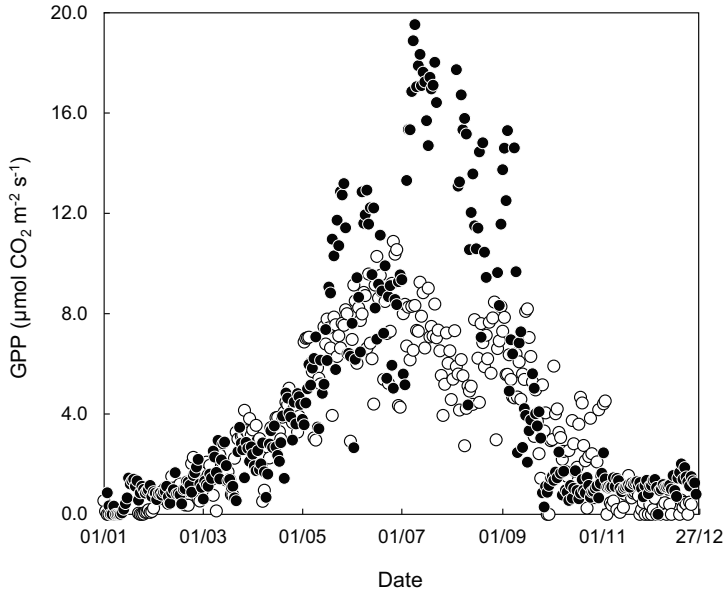

Figure 16. Gross primary production (daily averages) in 2014 of two Scots pine (*Pinus sylvestris*) forest stands aged 12- (black) and 100-years-old (white).

Soluble ammonium pools were also generally larger in young forests compared to old forests (Paper I and III). Despite this probable decline in N availability, the ERM production was negatively correlated with forest age. Our results are thus inconsistent with previous observations of N availability reducing the ERM growth. Reduced ERM production despite the decline in N availability could be due to variation in C availability have a stronger effect on the ERM production, than variations in N availability, at least under the current conditions. However, the inconsistency could also be the result of ERM production not being linearly related to N availability. For example, Treseder and Allen (2002) proposed a parabolic relationship between mycorrhizal fungal growth and soil nutrient availability (Fig. 17), with maximal mycorrhizal growth when nutrient availability limits plant growth but not that of the mycorrhizal fungi, and diminished growth under low and high N availabilities, when either N or C limit the growth of the mycorrhizal fungi. Since most previous studies testing effects of N on ERM accumulation have involved larger N additions, studying ERM production rate over short natural nutrient gradients is required to fully establish the relationship between ERM production and nutrient availability, and to test the hypothesis of Treseder and Allen (2002).

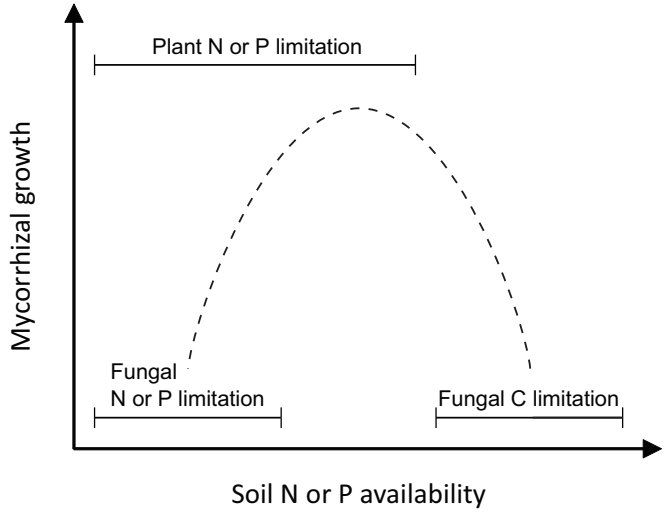

Figure 17. Conceptual model showing mycorrhizal growth and its relationship to nutrient availability, adapted from Treseder & Allen (2002). At the lowest nutrient levels both plant and mycorrhizal fungus are nutrient limited, resulting in a low growth. At low nutrient levels the plant allocates large amounts of C to the mycorrhiza fungi, resulting in a rapid growth. At the highest nutrient levels the plant will allocate little C belowground, resulting in slow mycorrhizal growth.

4.1.3 Ectomycorrhizal mycelial production estimates compared to other studies

As the production rate of ERM has only been estimated recently in two other studies (Ekblad *et al.*, 2016 and Hendricks *et al.*, 2016), there is little data to compare the production rate estimates of Paper I and II with. In control plots of a sub-tropical 25-year-old *Pinus palustris* forest Hendricks *et al.* (2015) reported the production to be 0.8 kg ha^{-1} day^{-1}, and in control plots of a warm-temperate 27-year-old *P. taeda* forest Ekblad *et al.* (2016) found the production to be 1.3 kg ha^{-1} day^{-1}. Despite the difference in climate, production estimates in Paper I and II are similar to those of Hendricks *et al.* (2015) and Ekblad *et al* (2016). Although none of the forests in the chronosequence was exactly 25-years-old the regression in Fig. 14a suggests that the ERM production would be 1.1 kg ha^{-1} day^{-1} in a 25-year-old forest, which is between the estimates of Hendrick *et al.* (2015) and Ekblad *et al.* (2016). This indicates that the daily ERM production is about 1 kg ha^{-1} in young *Pinus* forests despite differences in climate and tree species. However, the extent to which ERM production rates of varies between forest types and conditions should be investigated further, since the available data is currently very limited.

4.2 Respiration and carbon use efficiency of ectomycorrhizal mycelium

Since ectomycorrhizal fungal ERM contribute at least one-third of microbial biomass in boreal forest soils (Högberg & Högberg, 2002), their contribution to the total soil respiration may be substantial. Although ERM respiration contribution has been estimated previously in some temperate European forests (Heinemeyer *et al.*, 2007; Moyano *et al.*, 2008; Fenn *et al.*, 2010; Heinemeyer *et al.*, 2012; Neumann & Matzner, 2014), no study has yet investigated the contribution of ERM to the total soil respiration in boreal forests ecosystems, although efforts have been made to estimate the combined respiration contribution of roots and root associated microorganisms (Högberg & Högberg, 2002).

In paper II we addressed this knowledge gap by investigating the ERM respiration contribution to total soil respiration over the season and across the chronosequence. Furthermore, we combined measurements of ERM respiration with estimates of ERM production to calculate the CUE of ERM over the season and across the chronosequence. We hypothesized that CUE of ERM would increase as the season progresses from early summer to late autumn, since earlier studies have demonstrated that microbial CUE increases with declining temperature (Manzoni *et al.*, 2012). We also hypothesised that the CUE of ERM would decline with increasing forest age, as an effect of intensified N limitation. This hypothesis was based on the fact that microbial CUE has been shown to decline with decreasing N availability (Sinsabaugh *et al.*, 2013), which is a typical characteristic of aging boreal forests (DeLuca *et al.*, 2002; Ward *et al.*, 2014). We further hypothesised that respiration contribution of ERM to total soil respiration would increase with forest age, as an effect of a forest age-related decrease in CUE

4.2.1 Carbon use efficiency of ectomycorrhizal mycelium vary over the season

In agreement with our first hypothesis (Paper II), CUE of ERM increased over the season from 0.022 ± 0.004 in May to 0.232 ± 0.018 in November (Fig. 18). This ten-fold increase in CUE over the season highlights a physiological plasticity of mycorrhizal fungal communities to adapt to changes in environmental conditions. Across different forests and agricultural sites CUE of soil microbes have values ranging from 0.022 to 0.563 (Sinsabaugh *et al.*, 2015, 2016), and the CUE of conifer litter decomposers has been found to vary between 0.1 and 0.4 (Manzoni *et al.*, 2008).

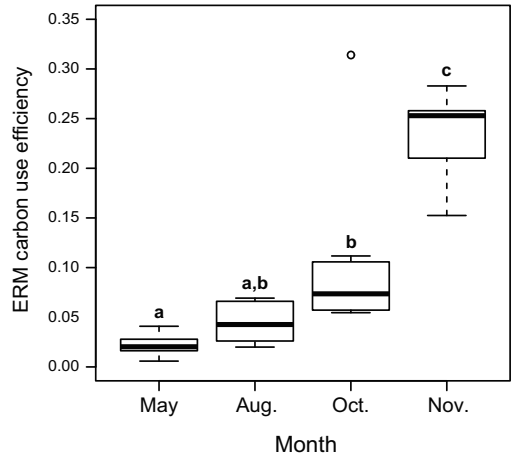

Figure 18. Carbon use efficiency of extraradical mycelium (ERM) increased over the growing season ($P < 0.001$). Time points with the same letter do not differ significantly in Tukey's multiple range test at $\alpha \leq 0.05$, n = 7.

Most of our CUE estimates on ERM fall within these ranges, but they are generally lower than values typical for soil microbial saprotrophs (Manzoni *et al.*, 2012; Sinsabaugh *et al.*, 2015). This could be explained by mycorrhizal fungi (unlike saprotrophic microorganism) having direct access to a large C supply via their host plant's photoassimilate production (Heinemeyer *et al.*, 2006; Heinemeyer *et al.*, 2012). The low CUE values during summer and early fall seem to be related to high C availability as GPP in the 12- and 100-year-old stands reached a maximum during June-August (Fig. 16). Photoassimilate production has been shown to strongly influence the respiration rate of mycorrhizal fungal ERM (Heinemeyer *et al.*, 2006), and Heinemeyer *et al.* (2012) hypothesised that respiration of ERM may function as a C "overflow tap", in which surplus photoassimilates are released. The considerably low CUE values observed during summer support this "overflow tap" hypothesis and suggest that most of the C allocated to the mycorrhizal ERM is released via respiration rather than used for growth.

4.2.2 Carbon use efficiency of ectomycorrhizal mycelium declines with increasing forest age

In line with our second hypothesis (paper II) the CUE of EMR declines with increasing forest age (Fig. 19) in May, August and November, and ranged from 0.032-0.006, 0.066-0.022 and 0.278-0.164, respectively (Fig. 19). However, for unknown reasons the CUE of ERM in October did not decline with age (Fig. 19c). As mentioned previously availability of N typically declines with increasing forest age in boreal forests, and it seems to be a trend for our chronosequence as well since pH decreased with age and ammonium levels were highest in the youngest forests (Paper I and III). This decline in N availability could explain the observed decrease in CUE of mycorrhizal fungal ERM over the chronosequence.

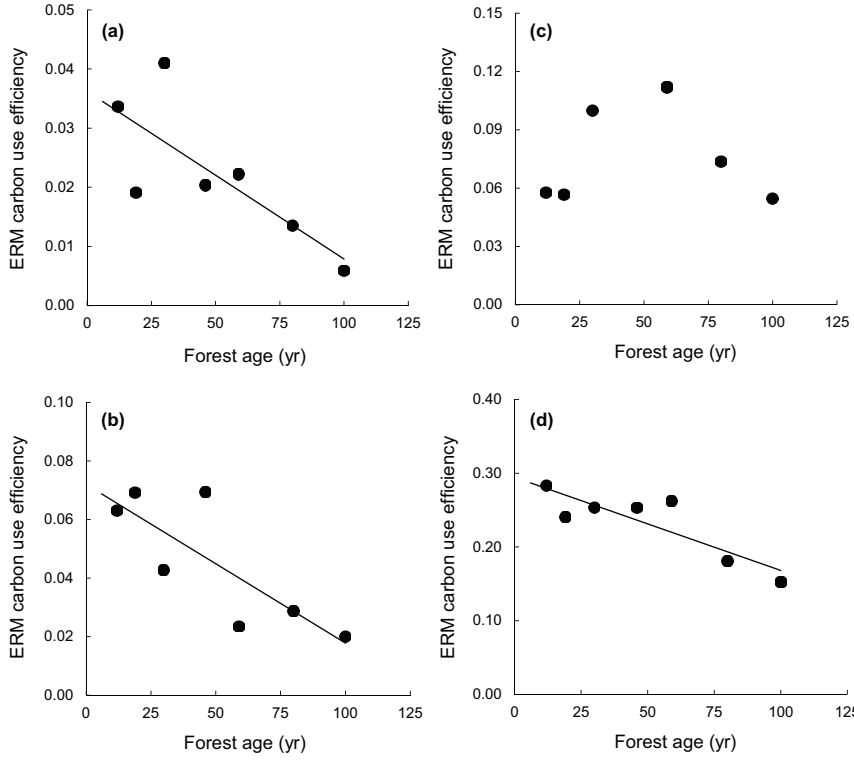

Figure 19. Carbon use efficiency of extraradical mycelium (ERM) in (a) May, (b) August, (c) October and (d) November, and its relationship to forest age. Lines represent linear regressions fitted to the data. May: y = 0.036 - 0.0003x, r^2 = 0.60, P = 0.040, Aug: y = 0.072 - 0.0005x, r^2 = 0.65, P = 0.029, Nov: y = 0.29 - 0.0013x, r^2 = 0.74, P = 0.012. One outlier in (c) is not shown. See Fig. 18 for value of outlier.

Hypothetically, when N is limited and transfer of C is large, high CUE may be detrimental for the mycorrhizal symbiosis as a large proportion of N taken up would be incorporated into the ERM biomass to fulfil the stoichiometric balance between C and N (Manzoni *et al.*, 2012). Immobilization of N in the ERM biomass would lead to less N being available for host-transfer. To maintain N allocation to its host spite of N limitation, the mycorrhizal fungi would need to reduce its own CUE to prevent large amounts of N being immobilised in its biomass. The mycorrhizal fungi could also increase its turnover rate to decrease the N immobilisation in its biomass, however this seems like a poor strategy since large N amounts could potentially still remain in fungal cell-walls, bound in chitin. The apparent decrease in N availability across the chronosequence could thus explain the decline in CUE as being a mechanism to maintain host N allocation despite increased N limitation. Interestingly there is evidence of mycorrhizal fungi immobilising large amounts of N when N is severely limited and the belowground C transfer is high (Näsholm *et al.*, 2013). Whether this is an effect of a CUE that is too high should be investigated further.

The theory outlined above is based on the fact that the relationship between C:N in the ERM biomass is relatively constrained; that the mycorrhizal fungus cannot alter its C:N composition sufficiently to adapt to the natural variations in N and C availability. Previous studies demonstrate that the C:N composition of the ERM biomass varies over different forest types and conditions; between 14 to 29, with a mean value of ca. 20 (Wallander *et al.*, 2003; Clemmensen *et al.*, 2006; Mikusinska *et al.*, 2013; Ekblad *et al.*, 2016), however this variation is small in comparison to terrestrial plants in which the foliage C:N composition varies between 5 to >100 (Elser *et al.*, 2000).

Decreased mycelial CUE with forest age can explain patterns of an increased root and ectomycorrhizal fungal respiration during boreal forest succession, as observed by Goulden *et al.* (2011). However, this increase in respiration may also be the result of an increased C transfer to roots and mycorrhiza (Goulden *et al.*, 2011), as increased belowground C transfer with increased boreal forest age has been reported previously (Baret *et al.*, 2015). On the other hand, others have suggested that belowground C transfers decreases with age (Ryan *et al.*, 1997; Tang *et al.*, 2014), thus forest age related variations in belowground C transfer are still uncertain.

4.2.3 Constant respiration contribution by ectomycorrhizal mycelium irrespective of forest age

Despite the decrease in CUE across the chronosequence, the hypothesised increase in contribution by ERM to total soil respiration with forest age was not observed. Apart from the ERM respiration reaching a peak in the 34-year-old forest, ERM contributed to a relatively constant proportion of the total soil respiration across the chronosequence (Paper II), with the ERM respiration contribution ranging from 13 to 26%, and with an average of 19 ± 2% (Fig. 20). A parallel increase in heterotrophic respiration across our chronosequence could have counteracted a potential increase in ERM respiration contribution (Saiz *et al*., 2006), but this question requires further investigation. The result of a constant ERM respiration contribution across the chronosequence is consistent with the results of Bond-Lamberty (2004) and Saiz *et al.* (2006) who also found that roots and root-associated mycorrhizal fungi contributed with a relatively constant proportion (c. 20% and 54%, respectively) to the total soil respiration across chronosequences of *Picea mariana* and *Picea sitchensis* forest stands. Moreover, the relative ERM respiration contributions reported in Paper II are similar to values reported from coniferous forest in other studies. For example Heinemeyer *et al.* (2007) reported a 25-35% ERM respiration contribution in a 15-year *Pinus contorta* forest, and Neumann and Matzner (2014) reported a 18-44% of ERM respiration contribution in a young (~10 years) *P. abies* forest. Our values differ from the ERM respiration contribution measurements made in temperate deciduous forests; Moyano *et al.* (2008) reported mycorrhizal mycelium contributing to be by 3% of total soil respiration in a *Fagus sylvatica* forest, and Fenn *et al.* (2010) reported a contribution of 8 ± 3% in a forest dominated by *Acer pseudoplatanus* and *Fraxinus excelsior.*

The difference between coniferous and deciduous forests in ERM respiration contribution could be due to a proportionally larger ERM biomass in coniferous forests compared to deciduous forests. The ERM biomass in deciduous forest appears to be smaller compared to coniferous forest, based on comparison in ERM biomass between Swedish *Quercus robur* forest (Nilsson *et al.*, 2007) and *P. abies* forests in the same region (reviewed by Ekblad *et al.*, 2013). However, since ERM biomass and respiration was shown not to correlate in Paper II and in the study by Neumann and Matzner (2014), it seems likely that there is an alternative explanation behind the difference in ERM respiration contribution between forest types. Potentially, a larger transfer of C to belowground could explain the greater ERM respiration contribution in coniferous forests.

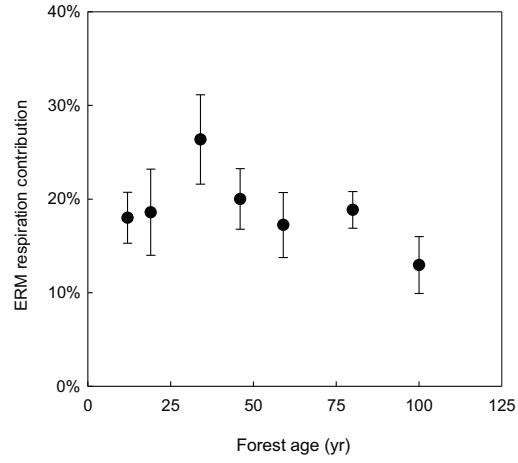

Figure 20. Contribution of extraradical mycelium (ERM) to total soil respiration and its relationship to forest age. Error bars represents ± SE.

However, this seems not to be the case as the proportion of GPP allocated belowground appears to be similar in temperate coniferous and deciduous forests (Gill & Finzi, 2016). A more likely explanation behind the greater ERM respiration contribution of coniferous forests is that the CUE of ERM is lower. If the CUE of ERM is related to the soil C:N, as observed in saprotrophic microorganisms (Sinsabaugh *et al.*, 2013, 2016), a lower CUE of ERM in coniferous forests than in deciduous forest is likely, since the C:N ratio in coniferous forests soils is typically larger than the C:N ratio of deciduous forests soils (Cools *et al.*, 2014). Thus, even if the belowground C transfer is similar between temperate deciduous and coniferous forest (Gill & Finzi, 2016), a lower CUE of ERM in coniferous forests would translate to a greater ERM respiration contribution.

4.2.4 Relationship between ectomycorrhizal mycelial respiration and gross primary production

A parabolic relationship between ERM respiration and GPP was observed (Fig. 21a), with the mean contribution of ERM respiration to GPP varying by 19, 19, 50, and 15% in May, August, October and November, respectively (Fig. 21b). These are high estimates, as belowground transfer represents 25-63% of GPP in forest ecosystem (Litton *et al.*, 2007). However, the values are likely to be overestimates since a large part (10-20%) of the respiration may be derived from stored starch reserves rather than recent photoassimilates (Högberg *et al.*, 2001). The parabolic relationship between ERM respiration and GPP indicates

that C is being invested in plant components rather than allocated to the mycorrhiza, when GPP is high (*i.e.* during summer). Transfer of C belowground has also been shown to vary with the season in boreal forest; being low in early summer and high in late summer (Högberg *et al.*, 2001). Thus the maximum contribution of ERM respiration to GPP in October can be explained by a seasonal maximum belowground C transfer. However, it must be acknowledged that the contribution of ERM respiration to GPP is uncertain and needs to be investigated further, since the degree of uncertainty and size of errors are not known due to the small data set.

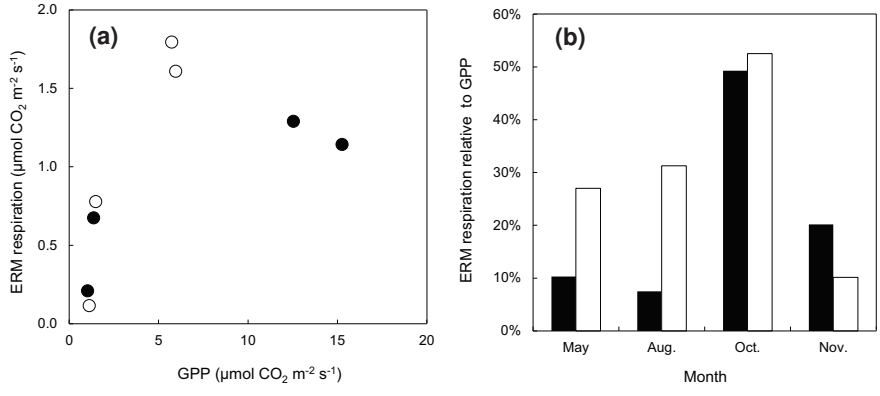

Figure 21. (a) Respiration of extraradical mycelium (ERM) plotted against gross primary production (GPP) in *Pinus sylvestris* forests aged 12- (black) and 100-year-old (white), and (b) seasonal change in the contribution of ERM to GPP.

4.3 Turnover of ectomycorrhizal mycelial biomass

Ever since Wallander *et al.* (2001) pioneered the ingrowth mesh bags technique to measure ERM production, a large number of studies have emerged providing estimates for ERM production for different forest types and conditions (Ekblad *et al.*, 2013). Mesh bags are typically incubated over a single period, ranging from several months to a year, whereupon the fungal biomass content is quantified and used as a production estimate. One limitation of the technique is that the resulting production estimate represents the accumulation of ERM biomass, which depends on the process of production and turnover. Therefore, the production estimate derived from mesh bags incubated over a single period represents a biomass net-increase rather than a production rate. To quantify the ERM production *rate*, the effect of turnover on the accumulated biomass needs to be accounted for. In Paper I we addressed

this inaccuracy by estimating the rate of ERM biomass turnover across the chronosequence.

4.3.1 Turnover of ectomycorrhizal mycelium declines with forest age and explains variation in standing mycelial biomass

In paper I we hypothesised that the rate of ERM biomass turnover would decrease with increased forest age, as a result of increased abundance of long-lived competitive and stress-tolerant species as forests mature (Cooke & Rayner, 1984). In agreement with our hypothesis, results from Paper I demonstrate that the turnover rate of ERM decreases with increased forest age, from 7 to 1 times yr^{-1} (Fig. 22). This corresponds to an increasing longevity from 25 days in the youngest forests up to a year in the oldest forests. Despite the decrease in production with increasing forest age, the ERM biomass was found to increase across the chronosequence, from 50 to 112 kg ha^{-1} (Fig. 23). This apparent contradiction of high biomass in spite of low production was explained by the drastic decline in turnover, thus turnover rather than the production regulated the standing ERM biomass across the chronosequence. A large biomass has previously been assumed to be the result of a rapid mycelial production, but the current result clearly illustrated that a high standing biomass can be a result of low turnover, rather than a rapid production.

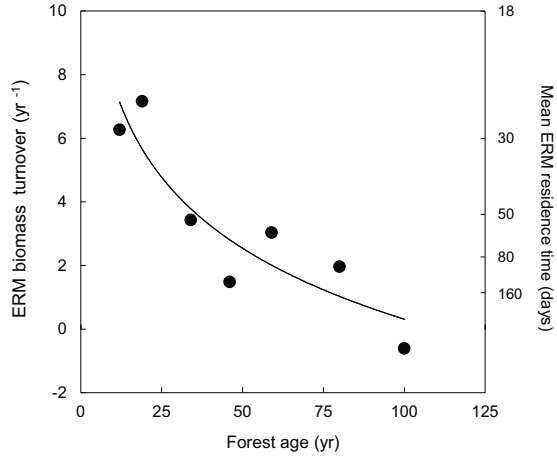

Figure 22. Estimated extraradical mycelial (ERM) biomass turnover and mean residence time as a function of forest age. Solid line in represents logarithmic regression function fitted to turnover estimates: $y_1 = -3.22\text{Log}_e(x) + 15.14$ $r^2 = 0.83$, $P = 0.005$, SE = 1.24. Value below zero is due to no observed turnover for the 100-year-old forest stand. Function describing the mean residence time of ERM (in days): $y_2 = 180/(-3.22\text{Log}_e(x) + 15.14)$.

We did not find support for turnover varying much over the growing season. Using the production and turnover estimates of Paper I we constructed a model to predict the accumulation of ERM biomass over a growing season. This model prediction compared well with empirical estimates (r^2 = 0.84, P = 0.004), which suggested that variation in turnover during the growing season is small. However, despite the strong correlation between predicted and measured biomass, the predicted biomass was consistently somewhat lower than the measured biomass. This could result from ERM production and turnover occurring outside the growing season, in line with observations by Coutts and Nicholl (1990).

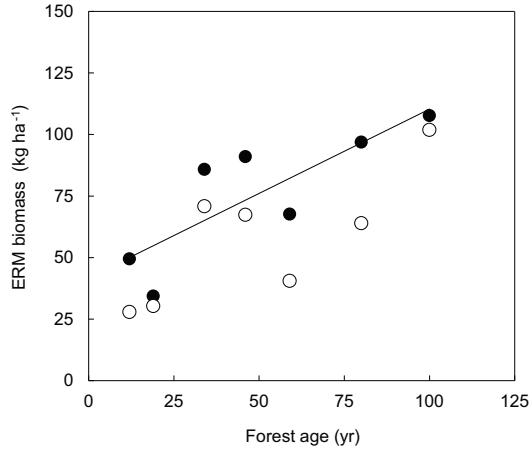

Figure 23. Averaged extraradical mycelium (ERM) biomass in mesh bags incubated for 12 and 13 months (●) and predicted biomass after one growing season (180 days) (○), and its relationship to forest age. The line represents linear regression fitted to the mesh bag data: y = 0.7x + 42, r^2 = 0.68, P = 0.02.

4.3.2 Comparison of ectomycorrhizal mycelium and fine root turnover

Yuan and Chen (2012) demonstrated that the rate of fine root turnover across a boreal post-fire chronosequence decreases with increasing forest age, similar to our observations on ERM turnover in. However, unlike ERM turnover which declined from 7 to 1 times yr^{-1} in forest aged 12 to 100 years, turnover of fine root declined from 1.2 to 0.4 times yr^{-1} in forests aged 11 to 94 years. Because of the close linkage between fine roots and ERM it is possible that the factors regulating the rate of fine root turnover are also regulating ERM turnover.

Yuan and Chen (2012) proposed that tree species composition may contribute to variations in turnover. Although the occurrence of *P. abies* increased across our chronosequence, all stands were heavily dominated by *P. sylvestris*, thus changes in tree species composition do not seem to drive variation in ERM turnover in our case. Yuan and Chen (2012) also suggested that the ratio of N and phosphorus (P) in soil is coupled to fine root turnover; that slow turnover and growth is a way to reduce P requirement with increasing soil N:P ratio with increased forest age. Fast ERM growth and turnover appears to be coupled with a high P demand as relatively more P rich RNA is required to support fast protein synthesis (Yuan *et al.*, 2011). Furthermore, along a fertility gradient in the Hawaiian island, availability of P contributed more to variations in fine root turnover than availability of N, and low P availability was related to low turnover (Ostertag, 2001). Availability of P could be an important factor regulating ERM dynamics, since Wallander and Nylund (1992) as well as Ekblad *et al.* (1995) demonstrated that P starvation of *P. sylvestris* seedlings resulted in a large accumulation of ERM biomass. This increase in biomass could be an effect of decreased ERM turnover, rather than an increase in production (Paper I). The extent to which P availability plays a role in regulating the ERM turnover should be investigated further (Ekblad *et al.*, 2013), as it seems to be an important factor regulating both fine root and ERM dynamics.

4.3.3 Ectomycorrhizal mycelial turnover estimates compared to other studies

Turnover of ERM has been estimated in two other recent studies; Hendricks *et al.* (2016) and Ekblad *et al.* (2016). Hendricks *et al.* (2016) found turnover to be 10 ± 3 times yr^{-1} in a 25-year-old subtropical *P. palustris* plantation and Ekblad *et al.* (2016) found turnover to be 13 times yr^{-1} in a 27-year-old warm temperate *P. taeda* forest. Similar to Ekblad *et al.* (2016) and Hendricks *et al.* (2016) when using the regression in Fig. 14a we obtain a turnover of 4.8 times yr^{-1} for 25-year-old forests which corresponds to a turnover of 9.3 times yr^{-1} when compensating for differences in growing season length between the different climates. This suggests that the ERM turnover is similar in forests of a similar age despite differences in climate. Since it is unclear what regulates ERM turnover it is difficult to say why turnover would be similar across different forest types and conditions. However, forest growth and plant biomass accumulation decline after a mid-successional maximum in nearly all even aged forest types (Ryan *et al.*, 1997). A potential cause behind the similarity in turnover between studies could be this universal pattern, since aboveground plant production and belowground C transfer is tightly associated

(Litton *et al.*, 2007). However, the similarity between studies could be purely coincidental or an effect of something else as the age-related pattern of forest production, described by Ryan *et al.* (1997) differs in the way it progresses between forest types (He *et al.*, 2012).

4.3.4 Turnover of ectomycorrhizal mycelial necromass

Production of ERM necromass is determined by the rate of ERM biomass turnover and the ERM biomass size, and it represents a C flux from the ERM biomass to the ERM necromass pool. Biomass turnover determines the retention time of C in the biomass, and necromass turnover determines the retention time of C in the necromass. Necromass turnover was hypothesised to decrease with increased forest age, as a result of progressively increasing recalcitrance of the ERM necromass. This hypothesis was based on observations of Clemmensen *et al.* (2013) reporting increased roots and mycorrhizal fungal derived soil C inputs during boreal forest succession.

There was no significant correlation between ERM necromass turnover and forest age, instead the overall rate of necromass turnover was 2.0 ± 0.6 times yr^{-1} (mean ± SE) which represents a mean residence time of 178 days (Fig. 24a, Table 1). Since necromass turnover did not vary across the chronosequence, there was no correlation between turnover of necromass and turnover of biomass.

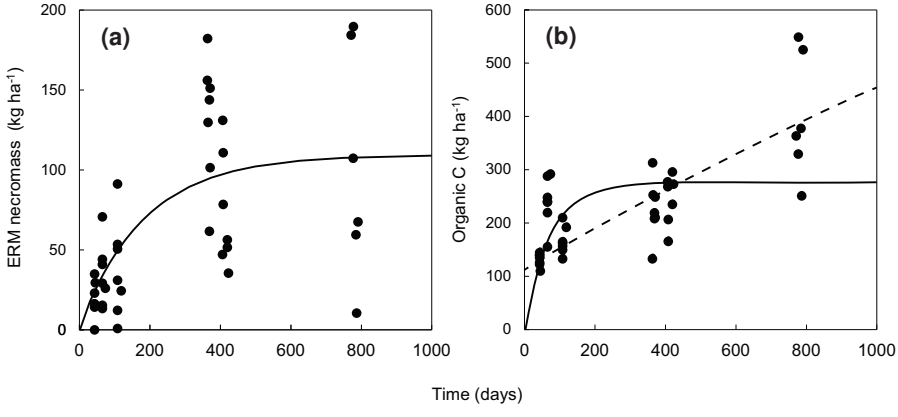

Figure 24. Estimated extraradical mycelial (ERM) necromass (a), and organic C (b) in mesh bags as a function of incubation time. Lines represents Eqn 2a fitted to the respective data sets. Dashed line in (b) represents Eqn 2a fitted to data when the approximate C contribution from dissolve organic matter has been accounted for. Parameter estimates from the model fit on production and turnover are presented in table 1.

Table 1. Production and turnover rates of extraradical mycelial (ERM) necromass and organic C

	Production rate ($kg\ ha^{-1}\ d^{-1}$)	Turnover rate (yr^{-1})	Mean residence time (d)
ERM necromass	0.6 (0.2)*	2.0 (0.8)	185
Organic C (including DOC)	4.3 (0.9)	5.5 (1.3)	67
Organic C (excluding DOC)	0.4 (0.1)	0.1 (0.3)	3160

*Values in parentheses indicates standard errors

Thus there seems to be no direct relationship between necromass and biomass turnover, which can be explained by biomass and necromass turnover being regulated by different factors and processes. To the best of my knowledge, the turnover of ERM necromass has only been estimated on one previous occasion by Ekblad *et al.* (2016). In their study they reported an ERM necromass turnover of 1.5 times yr^{-1} with 95% CI from (1.0 – 3.0), in a warm temperate *P. taeda* forest. Although Ekblad *el al.* (2016) determined a faster necromass turnover it must be highlighted that our estimates lies well within the 95% confidence limit of Ekblad *et al.* (2016). Thus it is uncertain if the rate of necromass turnover across our chronosequence is different from the necromass turnover in the *P. taeda* forest in the Ekblad *et al.* (2016) study. Moreover, with a large standard error (0.6) the estimated necromass turnover across our chronosequence is uncertain, and could be due to the difficulty in accurately quantifying the ERM necromass. In the absence of techniques directly measuring the ERM necromass, the necromass needs to be estimated from the difference between fungal mass and biomass, derived from quantification of chitin and ergosterol, respectively. Upscaling content of chitin and ergosterol to a fungal mass or biomass also depends on the use of conversion factors, which contain individual degrees of inaccuracy (Joergensen & Wichern, 2008; Wallander *et al.*, 2013). Thus the reliability and accuracy of estimating necromass may be low as the errors in converting chitin to mass, and ergosterol to biomass, are uncertain.

Total C turnover in mesh bags was greater than the necromass turnover (Fig. 24b, Table 1); 4.3 ± 0.9 times yr^{-1} (mean ± SE) this is probably due to dissolved organic matter diffusing into the bag from the surrounding soil. Based on the difference in amounts of total C and fungal mass (chitin content) in mesh bags after only 43 days of incubation, the typical amount of dissolved organic matter in a mesh bag seems to be about 5 mg which corresponds to 110 kg when scaled up over an hectare. When the contribution of dissolved organic

C was compensated for in the analysis, the turnover of organic C was 0.1 ± 0.3 times yr^{-1} which corresponds to a mean residence time of 3160 days (Fig. 24b, Table 1). However, given the large standard error (0.3) this estimate is highly uncertain. The time scale of the study is too short for an accurate estimation of ERM organic C turnover.

4.4 Fungal community succession related to forest development

Since forest development relates to differences in tree age structure, soil properties and vegetation, the fungal community composition changes during succession in relation to forest development (Twieg *et al.*, 2007; Wallander *et al.*, 2010; Sun *et al.*, 2015). This may in turn feedback on variations in soil C cycling and storage (Clemmensen *et al.*, 2015). However, the coupling between processes regulating soil C cycling and storage and changes in species composition of fungal communities is poorly established. In paper III we addressed this knowledge gap by relating fungal community composition changes to enzymatic profile and activities, across the chronosequence. Based on the fact that species in the taxonomical group Atheliaceae (*i.e. Tylospora* and *Piloderma*) have been shown to be abundant in young forest (Twieg *et al.*, 2007; Wallander *et al.*, 2010; Sun *et al.*, 2015), we hypothesised that the ectomycorrhizal community in younger forest would be dominated by Atheliaceae species which would progressively be replaced by a more diverse ectomycorrhizal community. We further hypothesised that fungal community changes would be related to changes in enzymatic activities in the soil, particularly that enzyme activities involved in organic nutrient mobilisation would correlate with the abundance of *Cortinarius* species.

4.4.1 Shift in ectomycorrhizal fungal community composition with increasing forest age

In accordance with our first hypothesis, the abundance and diversity of ectomycorrhizal fungi increased with increasing forest age, whereas abundance of saprotrophs declined (Fig. 25). In young forests the ectomycorrhizal fungal community was dominated by Atheliaceae species, and in mature forests the community was dominated by *Cortinarius* and *Russula* species (Fig. 26, 27). The high abundance of Atheliaceae species in young forests is in agreement with previous observations (Twieg *et al.*, 2007; Wallander *et al.*, 2010; Sun *et al.*, 2015). Moreover, as abundance of Atheliacea species reached a peak in the 34-year-old forest (Paper III) it seems likely that they contributed to the peak in ERM respiration and production (Paper II).

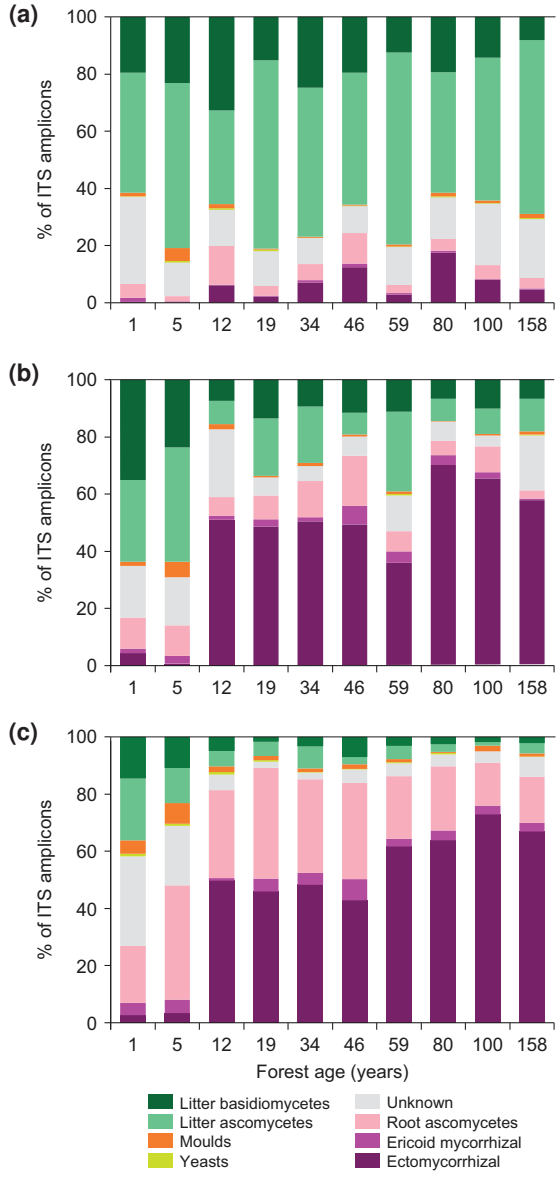

Figure 25. Distribution of fungal functional groups in different organic soil layers: (a) litter (b) fragmented litter, and (c) humus, of 10 Scots pine (*Pinus sylvestris*) forest stands of different ages (1–158 years), as estimated by PacBio sequencing of amplified ITS2 markers. Abundances are given as percent of the identified amplicon sequences (accounting for 92% of total sequences).

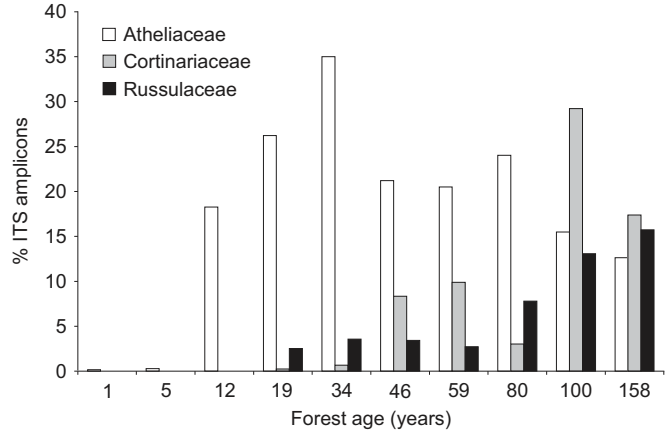

Figure 26. Distribution of the most dominant ectomycorrhizal fungal families in the humus layer of 10 Scots pine (*Pinus sylvestris*) forest stands of different ages (1–158 years), as indicated by PacBios equencing of amplified ITS2 markers.

Differences in community composition could be a contributing factor to the observed differences in ERM turnover across the chronosequence (see section 4.3). For example young stands were characterised by a high abundance of *Tylopspora* species which are hypothesised to have a high turnover as they have been characteristics of being fast growing and favoured by high C and N availabilities availability (Parrent & Vilgalys, 2007; Wallander *et al.*, 2010; Sterkenburg *et al.*, 2015), but this remain to be further tested. One may expect that community changers in mycorrhizal exploration types are coupled with changes in ERM turnover, (*i.e.* fast turnover for exploration types forming a large ERM network) but no clear shift in exploration types was seen across the chronosequence (Paper III). For example in young stands short- and medium-distance exploration types (*Tylospora* and *Piloderma* species) dominated the mycorrhizal community, and in mature stands contact- and medium-distance exploration types dominated (*Russula* and *Cortinarius* species). Since no significant differences in exploration type was found across the chronosequence a shift in exploration types with increasing forest age do not appear to be a driver behind variations in turnover, but this needs to be further investigated. Laboratory experiments suggest that the ectomycorrhizal fungus *Cenococcum geophilum* is associated with slow necromass turnover (Koide & Malcom, 2009; Fernandez & Koide, 2014). However abundance of *Cenococcum geophilum* was similar in all forests across the chronosequence (Fig. 27), which could contribute to the explanation why necromass turnover did not vary significantly with forest age.

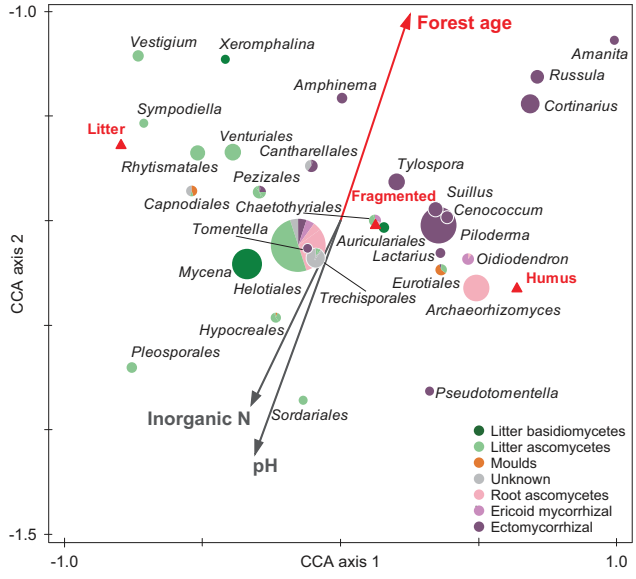

Figure 27. Distribution of fungal genera/orders in organic soil layers of 10 Scots pine (*Pinus sylvestris*) forests stands of different ages (1–158 years), as visualised by a species plot of a canonical correspondence analysis (CCA) based on PacBio sequencing of amplified ITS2 markers. The CCA included 66 fungal genera/orders, but only the 30 most abundant genera/orders are shown. Circles are colour coded according to functional groups with area indicating relative abundance. Triangles and the red vector represent constraining environmental variables, while grey vectors represent additional supplementary variables plotted in ordination space. Axes 1 and 2 respectively explained 19.1 and 8.9% of the total inertia of 1.3.

4.4.2 Enzyme activates in litter and fragmented litter soil layers

As hypothesised, fungal community composition in the litter and a fragmented litter layers, *i.e*. the upper organic soil layers, correlated with enzyme profiles (Fig. 28), at genus and order levels (Paper III). Enzymatic activities differed over the soil profile (Fig. 28). In litter, saprotrophic fungi dominated and Cantharellales and saprotrophic fungi within the genera of Pleosporales, Hypocreales and Trechisporales correlated positively with hydrolytic enzymes acting on polysaccharides, phenols and N-containing substrates (Fig. 28a). In fragmented litter, the fungal community composition was dominated by ectomycorrhizal species and the enzyme profile was largely different from the litter layer. For example, ectomycorrhizal species within the genera of *Cortinarius*, *Cenococcum* and *Russula*, were positively correlated with activities of enzymes used in mobilising N and P from organic matter (Fig. 28b); (*i*) β-1.4-nacetylglucosaminidase, (*ii*) acid phosphatase, and (*iii*) manganese-peroxidase. The correlation between *Cortinarius* abundance and activities of manganese-peroxidase is in line with previous observations

(Bödeker *et al.*, 2014), and further strengthens the support for *Cortinarius* species being important for mobilising nutrients bound in organic matter complexes (Lindahl & Tunlid, 2015). Given the fact that the enzyme profile was related to the fungal community composition, the difference in enzyme profile between the litter and the fragmented litter soil layers is to be expected, as the fungal community composition changes with soil depth. With soil depth, the abundance of saprotrophic fungi decreases and abundance of mycorrhizal fungi increases (Lindahl *et al.*, 2007; Clemmensen *et al.*, 2015). This gradual change in fungal community composition was observed in soil from all forests aged more than 12-years-old (Fig. 25). Thus it seems like the stratification of soil fungal communities in nutrient limited boreal forest soils follows a universal pattern which is unrelated to forest age.

Despite the significant relationship between fungal community composition and forest age, and between fungal community composition and enzyme activities, a direct effect of forest age on enzymatic activities did not receive statistical support. It is possible that the sampling was insufficient to capture the indirect effect of forest age on enzyme activities, as estimating enzymatic activities is notoriously difficult due to high variability in spatial distribution of different soil enzymes (Baldrian, 2014). Increased enzymatic activity with increasing forest age may alleviate the forest age-related decline in N availability, as the mycorrhizal fungi provide plants with access to organic nutrient pools, and decrease the retention of nutrients. Thus, a forest-age-related increase in enzymatic activities may play an important role in maintaining the long-term forest productivity by stimulating plant photosynthesis via improved nutrient mineralisation. This mechanism is supported by the correlations between enzymatic activities, fungal community composition and forest age, although the present results are insufficient to fully verify its existence. Increased resource investment into enzyme production with increasing forest age could contribute to the decline in production and CUE with forest age (Paper II). However, this is uncertain as the release of C via enzymes was not studied in this thesis. The relative investment of C into biomass enzymes should be investigated further.

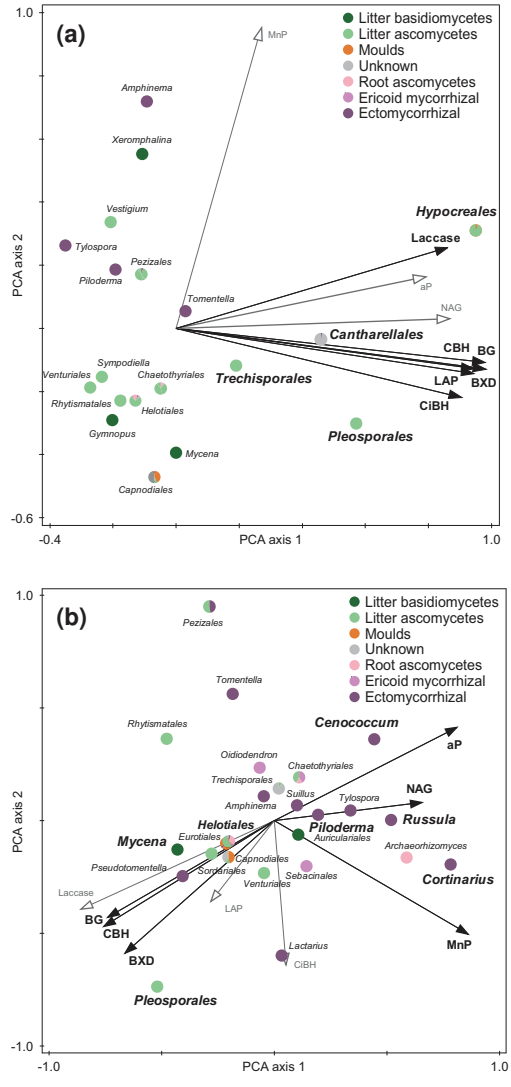

Figure 28. Similarity in enzyme profiles among fungal genera/orders, based on principal component analysis (PCA) of their specific enzyme activities, calculated as abundance weighted averages. The figures are based on enzyme activity analyses and PacBio sequencing of amplified ITS2 markers from the (a) litter and (b) fragmented litter soil layers of 10 Scots pine (*Pinus sylvestris*) forests 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.

4.5 Methodological considerations

Ingrowth mesh bags are suitable for studying ERM dynamics, and to enable incorporation of mycorrhizal C fluxes into ecosystem models, extending the application of mesh bags has been called for (Wallander *et al.*, 2013). However, the technique also presents a range of possible limitations, which are discussed below.

4.5.1 Sand as a growing substrate in mesh bags

Mesh bags are typically filled with sand to select for mycorrhizal fungal ingrowth and to enable standardised comparisons between treatments (Wallander *et al.*, 2001; Wallander *et al.*, 2013). However, sand also has limitations, since it does not reflect the surrounding chemical and physical conditions of natural soil (Hendricks *et al.*, 2006). For example, Hendricks *et al.* (2006) reported that mycorrhizal colonisation in 10 cm wide ingrowth cores was higher when natural soil was used instead of sand, which suggests that colonisation in sand does not fully reflect the colonisation rates in natural soil. Although sand may lead to underestimates it is possible than natural soil may lead to overestimates. Removing natural soil from its original environment and sieving severs roots and mycelium, resulting in an enrichment of substrates. Since some ectomycorrhizal fungi are known to exploit organic matter for acquisition of N (Lindahl & Tunlid, 2015) this may potentially lead to increased fungal production. Furthermore, selecting natural soil as a growth substrate may also impair subsequent analyses, as backgrounds of soil fungal biomass may be large and variable (Wallander *et al.*, 2013). A potential way to lessen the importance of substrate choice is to minimise the size of bags (Mikusinska *et al.* 2013). With 2-cm-wide bags at least 75% of the total bag volume is within 0.5 cm from the surface, thus the surrounding soil should have a relatively large influence on conditions inside the mesh bags. In the present studies the maximum width of the mesh bags was only 2 cm. However, when the aim is to compare relative differences between treatments, the choice of substrate may be less important.

4.5.2 Colonisation by non-mycorrhizal fungi in mesh bags

Although mycorrhizal fungi dominate the ingrowth in mesh bags (Wallander *et al.*, 2001; Kjøller, 2006), saprotrophic fungi may also colonise to some extent (Parrent & Vilgalys, 2007; Wallander *et al.*, 2010). The production of ERM may also vary among ectomycorrhizal species (Agerer, 2001), thus the community colonising mesh bags may be skewed in relation to the surrounding

soil communities which may affect production and turnover estimates (Wallander *et al*., 2013). The extent to which this is so is highly uncertain, since knowledge of how ERM dynamics vary among fungal species is still very limited.

4.5.3 Uncertainty of biomarker conversion factors

There may be errors associated with the conversion factors used to recalculate ergosterol to fungal biomass and chitin to fungal mass. In all studies fungal biomass was calculated by assuming an ergosterol content of 3 μg ergosterol mg^{-1} dry matter. This conversion factor was determined from ectomycorrhizal fungi in pure cultures (Salmanowicz & Nylund, 1988). Furthermore, Ekblad *et al.* (1998) found a similar ergosterol conversion factor (3.2 ±1.3 μg mg^{-1}) for ERM of *Paxillus involutus* symbiotically associated with *P. sylvestris* grown in microcosm systems. In the same study Ekblad *et al.* (1998) also determined a conversion factor for glucosamine derived from chitin, representing 45.0 μg mg^{-1} dry matter fungal mass. Ekblad *et al.* (2016) derived a very similar value (44.0 μg mg^{-1}) for ERM collected from the field. Although natural variation in ergosterol in ectomycorrhizal ERM appear to be small (Hagerberg *et al.*, 2003), the extent of the variation is still not yet fully established. When a larger number of fungal taxa colonize mesh bags it is possible that natural variations in ergosterol or chitin are evened out, but this question requires further investigation.

4.5.4 Estimating respiration of ectomycorrhizal mycelium from mesh bags

A benefit of estimating the ERM respiration from harvested mesh bags is that potential lateral diffusion of CO_2 from the surrounding soil into the mesh bags is avoided, which may otherwise be a problem when directly measuring the CO_2 from permanently installed mesh bags or mesh collars. The major disadvantage of this method is that it may not be suitable for studying small variations in ERM respiration, *i.e.* over short time scales, as disturbance of mesh bags may influence the CO_2 efflux of bags. Thus I can only recommend the outlined method to study large variations in ERM respiration, when the relative contribution from measurement errors is small. Upon measurement a linear increase in CO_2 must be confirmed for each measurement, as a non-linear increase would be the result of a large part of the CO_2 efflux not being derived from respiration. The degree of disturbance must be kept to a minimum prior to measurements, and during the measurement the plastic chamber containing the mesh bags should be buried in the soil to keep the mesh bag at

ambient soil temperature. Free-living microorganisms associated with the ERM may also contribute to the CO_2 efflux from the mesh bag. However, since their biomass is small in comparison to that of ERM, their contribution to CO_2 measurement is likely to be negligible.

4.6 Conclusions

The different studies described in this thesis concerned quantification of C fluxes associated with ectomycorrhizal ERM and assessment of how they vary with increasing forest age and with season. The main results underline the significance of the ERM in regulating and contributing to soil C fluxes and pools.

In papers I and II we found that the rate of biomass production declines with increasing forest age, and that the ERM production seemed to reach a mid-successional maximum, similar to the universal pattern of forest growth (Ryan *et al.*, 1997). Since aboveground plant production is related to belowground C transfer (Litton *et al.*, 2007), it is probable that the transfer of C to the ERM also reaches a mid-successional maximum and thereafter declines. Variations in C allocation through belowground C transfer can provide a mechanistic explanation for the decrease in ERM production, but since we only establish correlations between forest age and ERM production it is not possible to conclude what the causalities are. Thus the factors regulating ERM production need to be investigated further.

Despite the decrease in ERM production we found that the standing biomass of ERM increased with forest age. This apparent contradiction was explained by the drastic decline in turnover across the chronosequence, from seven times to one time per year. Thus more long-lived ERM in older forest enabled more biomass to accumulate. We cannot conclude which factors were involved in regulating the variation in turnover. However, the decline in ERM turnover followed a similar pattern to the decline in fine root turnover, and this might be because factors regulating fine root turnover also regulate turnover of ERM. Thus, forest age-related variations in N and P requirements could play a part in regulating the ERM turnover.

In paper II we found that most of the C allocated to the ERM is released via respiration due to low CUE. Moreover, we found that CUE increased over the season, and we proposed that this is regulated by a seasonal variation in C availability. A peak in gross primary production during summer further supports the likelihood of a declining C availability from summer to autumn. We also found that the mycelial CUE declines with increasing forest age. Although we cannot establish the factors driving the variations in CUE across

the chronosequence, we believe that it is related to reduction in N availability with increasing forest age. We propose that when N becomes progressively more limited mycorrhizal fungi decrease their CUE to reduce the amount of N immobilised in its biomass, enabling the fungi to allocate N to their host plants despite low availability.

In paper III we reported a shift in soil fungal communities and associated enzyme activities along the chronosequence. Abundance of ectomycorrhizal taxa increased with forest age, and the ectomycorrhizal community was dominated by Atheliaceae species in young forests, and by *Cortinarius* and *Russula* species in mature forests. Enzyme activities involved in mobilising N and P from organic matter were positively correlated with abundance of ectomycorrhizal species within the genera of *Cortinarius*, *Cenococcum* and *Russula*. Despite significant correlation between fungal community composition and forest age, and fungal community composition and enzyme activities, a significant relationship between forest age and enzymatic activities was not found. It is possible that our sampling was insufficient to capture the indirect effects of forest age on enzyme activities. We propose that compositional shifts in fungal species and enzymes play an important role in maintaining forest productivity by facilitating organic nutrient mineralisation.

Taken together, the results presented in this thesis provide a quantitative insight into the C fluxes associated with ectomycorrhizal ERM and highlight some potential drivers which may explain the observed variation over seasonal and decadal scales. At this early stage it is difficult to determine the extent to which C fluxes associated with ectomycorrhizal ERM regulate soil C storage. However, the result of Paper I clearly underlines the importance of turnover in regulating the ERM biomass, which likely affect the retention time of C and N in the biomass, which may feedback on other ecosystem processes. For example a large ERM biomass due to low turnover may enhance the mobilisation of nutrients from recalcitrant soil organic matter which may in turn affect C storage negatively. On the other hand, a large biomass may immobilise large amounts of N which may be an efficient strategy for preventing competitors from acquiring N. If that is the case it could explain why saprotrophic fungi decreased with increasing forest age. With suppression of saprotrophs, it is possible that the C storage would increase due to reduced decomposition rates, but this needs to be investigated further.

It is my hope that the estimates presented in this thesis can be used as a starting point to include ectomycorrhizal fungi into ecosystem C models, and that the estimates improve models' capacity to predict soil C dynamics in response to environmental change and management. I also hope that the work in this thesis can serve as a motivator for similar studies in other ecosystem type, as understanding of C fluxes associated with ERM in other forest ecosystems would us provide with a fundamental insight into the role of mycorrhizal fungi in the global C and N cycles.

5 Future Prospects

Although the studies in this thesis addressed some of the previous uncertainties concerning the role ectomycorrhizal ERM plays in regulating and contributing to soil C cycling, there are still several questions that remain unanswered.

One of the largest questions remaining is what factors regulate the turnover of ERM biomass. The large and steady decline in turnover across the chronosequence indicates that turnover is regulated on a fundamental level, even more so when results from this thesis, together with other studies, show that turnover is similar in similarly aged forest, irrespective of the climate (Ekblad *et al.*, 2016; Hendricks *et al.*, 2016). To determine the mechanism(s) regulating turnover, a first step would be to estimate ERM turnover rates in more forest ecosystems. Experiments testing whether N and P additions have an effect on turnover could also contribute to understanding the factors regulating turnover. Furthermore, the extent to which fungal community composition regulates turnover should also be further explored, as we could not establish whether fungal community composition was responsible for variation in turnover. Also the extent of which the mycorrhizal community in mesh bags differs from that of the surrounding soil should be investigated further. Mesh bags are thought to select for fast-growing fungi (Wallander *et al.*, 2013), and it is possible that a skewed abundance of mycorrhizal fungal species in bags relative to the soil may affect the production and turnover estimates.

The fact that we did not observe any variation in ERM necromass turnover could be due methodological uncertainties. The decomposition of ERM necromass should therefore also be studied more carefully under field conditions. It would be useful to study the biochemical change in ERM necromass with decomposition to better understand the mechanisms regulating necromass turnover. Identification of the main ERM necromass decomposers would also be an important step in better understanding necromass turnover

and provide a theoretical base for further hypothesis testing. The release of C via exudates and enzymes was not studied in this thesis, but relating the release rate of exudate to belowground C transfer or to ERM production would provide additional information on the partitioning of C within the ERM network. Currently, the quantitative importance of ERM exudation remains largely unknown.

Finally, and perhaps most importantly, the estimates of ERM biomass production, turnover, respiration, carbon use efficiency and necromass turnover should be used in ecosystem models to predict soil C cycling and dynamics. Since mycorrhizal fungi link aboveground and belowground C fluxes, as well as micro- and macroscale processes, inclusion of ERM-associated C fluxes in the next generation of ecosystem models may significantly improve their capacity to predict responses of mycorrhiza-mediated processes to forest management and environmental changes. Although care should be taken when extrapolating the results of this thesis to other studies of forest ecosystem, or applying them to predict ERM C dynamics over long time scale, the result presented here are promising for our ability to go on step further in predicting and understanding soil C cycling.

References

Averill, C., Turner, B.L. & Finzi, A.C. (2014). Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*, 505(7484), pp 543–545.

Axelsson, E. & Axelsson, B. (1986). Changes in carbon allocation patterns in spruce and pine trees following irrigation and fertilization. *Tree Physiology*, 2(1–3), pp 189–204.

Bahr, A., Ellström, M., Akselsson, C., Ekblad, A., Mikusinska, A. & Wallander, H. (2013). Growth of ectomycorrhizal fungal mycelium along a Norway spruce forest nitrogen deposition gradient and its effect on nitrogen leakage. *Soil Biology and Biochemistry*, 59, pp 38–48.

Baldrian, P. (2014). Distribution of extracellular enzymes in soils: spatial heterogeneity and determining factors at various scales. *Soil Science Society of America Journal*, 78(1), pp 11–18.

Baldrian, P., Větrovský, T., Cajthaml, T., Dobiášová, P., Petránková, M., Šnajdr, J. & Eichlerová, I. (2013). Estimation of fungal biomass in forest litter and soil. *Fungal Ecology*, 6(1), pp 1–11.

Baret, M., Pepin, S., Ward, C. & Pothier, D. (2015). Long-term changes in belowground and aboveground resource allocation of boreal forest stands. *Forest Ecology and Management*, 350, pp 62–69.

Baskaran, P., Hyvönen, R., Berglund, S.L., Clemmensen, K.E., Ågren, G.I., Lindahl, B.D. & Manzoni, S. (2016). Modelling the influence of ectomycorrhizal decomposition on plant nutrition and soil carbon sequestration in boreal forest ecosystems. *New Phytologist*, DOI: 10.1111/nph.14213.

Batjes, N.H. (1996). Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science*, 47(2), pp 151–163.

Bauhus, J., Paré, D. & Côté, L. (1998). Effects of tree species, stand age and soil type on soil microbial biomass and its activity in a southern boreal forest. *Soil Biology and Biochemistry*, 30(8–9), pp 1077–1089.

Beare, M.H., Hu, S., Coleman, D.C. & Hendrix, P.F. (1997). Influences of mycelial fungi on soil aggregation and organic matter storage in conventional and no-tillage soils. *Applied Soil Ecology*, 5(3), pp 211–219.

Blaško, R., Holm Bach, L., Yarwood, S.A., Trumbore, S.E., Högberg, P. & Högberg, M.N. (2015). Shifts in soil microbial community structure, nitrogen cycling and the concomitant

declining N availability in ageing primary boreal forest ecosystems. *Soil Biology and Biochemistry*, 91, pp 200–211.

Bonan, G.B. & Shugart, H.H. (1989). Environmental-factors and ecological processes in boreal forests. *Annual Review of Ecology and Systematics*, 20, pp 1–28.

Bond-Lamberty, B., Wang, C. & Gower, S.T. (2004). Contribution of root respiration to soil surface CO_2 flux in a boreal black spruce chronosequence. *Tree Physiology*, 24(12), pp 1387–1395.

Bödeker, I.T.M., Clemmensen, K.E., de Boer, W., Martin, F., Olson, Å. & Lindahl, B.D. (2014). Ectomycorrhizal *Cortinarius* species participate in enzymatic oxidation of humus in northern forest ecosystems. *New Phytologist*, 203(1), pp 245–256.

Cairney, J.W.G. (2012). Extramatrical mycelia of ectomycorrhizal fungi as moderators of carbon dynamics in forest soil. *Soil Biology and Biochemistry*, 47, pp 198–208.

Chapin, F.S., McFarland, J., McGurie, A.D., Euskirchen, E.S., Ruess, R.W. & Kielland, K. (2009). The changing global carbon cycle: linking plant–soil carbon dynamics to global consequences. *Journal of Ecology*, 97(5), pp 840–850.

Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R.D., Wardle, D.A. & Lindahl, B.D. (2013). Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*, 339(6127), pp 1615–1618.

Clemmensen, K.E., Finlay, R.D., Dahlberg, A., Stenlid, J., Wardle, D.A. & Lindahl, B.D. (2015). Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytologist*, 205(4), pp 1525–1536.

Clemmensen, K.E., Michelsen, A., Jonasson, S. & Shaver, G.R. (2006). Increased ectomycorrhizal fungal abundance after long-term fertilization and warming of two arctic tundra ecosystems. *New Phytologist*, 171(2), pp 391–404.

Cooke, R.C. & Rayner, A.D.M. (1984). *Ecology of Saprotrophic Fungi*. London and New York: Longman.

Cools, N., Vesterdal, L., De Vos, B., Vanguelova, E. & Hansen, K. (2014). Tree species is the major factor explaining C:N ratios in European forest soils. *Forest Ecology and Management*, 311, pp 3–16.

Coutts, M.P. & Nicoll, B.C. (1990). Growth and survival of shoots, roots, and mycorrhizal mycelium in clonal Sitka spruce during the first growing season after planting. *Canadian Journal of Forest Research*, 20(7), pp 861–868.

Croft, H., Chen, J.M. & Noland, T.L. (2014). Stand age effects on Boreal forest physiology using a long time-series of satellite data. *Forest Ecology and Management*, 328, pp 202–208.

DeLuca, T., Nilsson, M.-C. & Zackrisson, O. (2002). Nitrogen mineralization and phenol accumulation along a fire chronosequence in northern Sweden. *Oecologia*, 133(2), pp 206–214.

Devêvre, O.C. & Horwáth, W.R. (2000). Decomposition of rice straw and microbial carbon use efficiency under different soil temperatures and moistures. *Soil Biology and Biochemistry*, 32(11–12), pp 1773–1785.

Ekblad, A. & Näsholm, T. (1996). Determination of chitin in fungi and mycorrhizal roots by an improved HPLC analysis of glucosamine. *Plant and Soil*, 178(1), pp 29–35.

Ekblad, A., Mikusinska, A., Ågren, G.I., Menichetti, L., Wallander, H., Vilgalys, R., Bahr, A. & Eriksson, U. (2016). Production and turnover of ectomycorrhizal extramatrical mycelial biomass and necromass under elevated CO_2 and nitrogen fertilization. *New Phytologist*, 211(3), pp 874–885.

Ekblad, A., Wallander, H., Godbold, D.L., Cruz, C., Johnson, D., Baldrian, P., Björk, R.G., Epron, D., Kieliszewska-Rokicka, B., Kjøller, R., Kraigher, H., Matzner, E., Neumann, J. & Plassard, C. (2013). The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. *Plant and Soil*, 366(1–2), pp 1–27.

Ekblad, A., Wallander, H. & Nasholm, T. (1998). Chitin and ergosterol combined to measure total and living fungal biomass in ectomycorrhizas. *New Phytologist*, 138(1), pp 143–149.

Elser, J.J., Fagan, W.F., Denno, R.F., Dobberfuhl, D.R., Folarin, A., Huberty, A., Interlandi, S., Kilham, S.S., McCauley, E., Schulz, K.L., Siemann, E.H. & Sterner, R.W. (2000). Nutritional constraints in terrestrial and freshwater food webs. *Nature*, 408(6812), pp 578–580.

FAO (1998). *World reference base for soil resources*. Rome: Food and agriculture organization of the United Nations. 84.

Fenn, K.M., Malhi, Y. & Morecroft, M.D. (2010). Soil CO_2 efflux in a temperate deciduous forest: environmental drivers and component contributions. *Soil Biology and Biochemistry*, 42(10), pp 1685–1693.

Fernandez, C.W. & Koide, R.T. (2014). Initial melanin and nitrogen concentrations control the decomposition of ectomycorrhizal fungal litter. *Soil Biology and Biochemistry*, 77, pp 150–157.

Fransson, P.M.A., Anderson, I.C. & Alexander, I.J. (2007). Ectomycorrhizal fungi in culture respond differently to increased carbon availability. *FEMS microbiology ecology*, 61(2), pp 246–257.

Gill, A.L. & Finzi, A.C. (2016). Belowground carbon flux links biogeochemical cycles and resource-use efficiency at the global scale. *Ecology Letters*, 19(12), pp 1419–1428.

Godbold, D.L., Hoosbeek, M.R., Lukac, M., Cotrufo, M.F., Janssens, I.A., Ceulemans, R., Polle, A., Velthorst, E.J., Scarascia-Mugnozza, G., Angelis, P.D., Miglietta, F. & Peressotti, A. (2006). Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter. *Plant and Soil*, 281(1–2), pp 15–24.

Goslee, S.C. & Urban, D.L. (2007). The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*, 22, pp 1–19.

Goulden, M.L., Mcmillan, A.M.S., Winston, G.C., Rocha, A.V., Manies, K.L., Harden, J.W. & Bond-Lamberty, B.P. (2011). Patterns of NPP, GPP, respiration, and NEP during boreal forest succession. *Global Change Biology*, 17(2), pp 855–871.

Grace, J. & Rayment, M. (2000). Respiration in the balance. *Nature*, 404(6780), pp 819–820.

Grebenc, T., Christensen, M., Vilhar, U., Čater, M., Martín, M.P., Simončič, P. & Kraigher, H. (2009). Response of ectomycorrhizal community structure to gap opening in natural and managed temperate beech-dominated forests. *Canadian Journal of Forest Research*, 39(7), pp 1375–1386.

del Giorgio, P.A. & Cole, J.J. (1998). Bacterial growth efficiency in natural aquatic systems. *Annual Review of Ecology and Systematics*, 29(1), pp 503–541.

Hagerberg, D., Thelin, G. & Wallander, H. (2003). The production of ectomycorrhizal mycelium in forests: relation between forest nutrient status and local mineral sources. *Plant and Soil*, 252(2), pp 279–290.

He, L., Chen, J.M., Pan, Y., Birdsey, R. & Kattge, J. (2012). Relationships between net primary productivity and forest stand age in U.S. forests. *Global Biogeochemical Cycles*, 26(3), pp 1–19.

van Hees, P.A.W., Jones, D.L., Finlay, R., Godbold, D.L. & Lundström, U.S. (2005). The carbon we do not see - the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. *Soil Biology and Biochemistry*, 37(1), pp 1–13.

Heinemeyer, A., Hartley, I.P., Evans, S.P., De la Fuente, J.A.C. & Ineson, P. (2007). Forest soil CO_2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. *Global Change Biology*, 13(8), pp 1786–1797.

Heinemeyer, A., Ineson, P., Ostle, N. & Fitter, A.H. (2006). Respiration of the external mycelium in the arbuscular mycorrhizal symbiosis shows strong dependence on recent photosynthates and acclimation to temperature. *New Phytologist*, 171(1), pp 159–170.

Heinemeyer, A., Wilkinson, M., Vargas, R., Subke, J.A., Casella, E., Morison, J.I.L. & Ineson, P. (2012). Exploring the ”overflow tap” theory: linking forest soil CO_2 fluxes and individual mycorrhizosphere components to photosynthesis. *Biogeosciences*, 9(1), pp 79–95.

Hendricks, J.J., Mitchell, R.J., Kuehn, K.A. & Pecot, S.D. (2016). Ectomycorrhizal fungal mycelia turnover in a longleaf pine forest. *New Phytologist*, 209(4), pp 1693–1704.

Hibbett, D.S., Ohman, A. & Kirk, P.M. (2009). Fungal ecology catches fire. *New Phytologist*, 184(2), pp 279–282.

Hobbie, E.A. (2006). Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. *Ecology*, 87(3), pp 563–569.

Högberg, M.N., Briones, M.J.I., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Thornton, B., Hurry, V., Linder, S., Nasholm, T. & Högberg, P. (2010). Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. *New Phytologist*, 187(2), pp 485–493.

Högberg, M.N., Bååth, E., Nordgren, A., Arnebrant, K. & Högberg, P. (2003). Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs – a hypothesis based on field observations in boreal forest. *New Phytologist*, 160(1), pp 225–238.

Högberg, M.N. & Högberg, P. (2002). Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist*, 154(3), pp 791–795.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M. & Read, D. J. (2001). Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, 411(6839), pp 789–792.

Hynes, H.M. & Germida, J.J. (2012). A chronsequential approach to investigating microbial community shifts following clearcutting in boreal plain forest soils. *Canadian Journal of Forest Research*, 42(12), pp 2078–2089.

Joergensen, R.G. & Wichern, F. (2008). Quantitative assessment of the fungal contribution to microbial tissue in soil. *Soil Biology and Biochemistry*, 40(12), pp 2977–2991.

Jones, M.D., Durall, D.M. & Cairney, J.W.G. (2003). Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. *New Phytologist*, 157(3), pp 399–422.

Kjøller, R. (2006). Disproportionate abundance between ectomycorrhizal root tips and their associated mycelia. *FEMS Microbiology Ecology*, 58(2), pp 214–224.

Kjøller, R., Nilsson, L.-O., Hansen, K., Schmidt, I.K., Vesterdal, L. & Gundersen, P. (2012). Dramatic changes in ectomycorrhizal community composition, root tip abundance and mycelial production along a stand-scale nitrogen deposition gradient. *New Phytologist*, 194(1), pp 278–286.

Koide, R.T., Fernandez, C. & Malcolm, G. (2014). Determining place and process: functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. *New Phytologist*, 201(2), pp 433–439.

Koide, R.T. & Malcolm, G.M. (2009). N concentration controls decomposition rates of different strains of ectomycorrhizal fungi. *Fungal Ecology*, 2(4), pp 197–202.

Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S. T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M. & Larsson, K.-H. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*, 22(21), pp 5271–5277.

Lahti, T. & Väisänen, R.A. (1987). Ecological gradients of boreal forests in South Finland: an ordination test of Cajander's forest site type theory. *Vegetatio*, 68(3), pp 145–156.

Lezica, R.F. & Quesada-Allué, L. (1990). Chitin. *Methods in plant biochemistry*, (2), pp 443–481.

Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högberg, P., Stenlid, J. & Finlay, R.D. (2007). Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytologist*, 173(3), pp 611–620.

Lindahl, B.D. & Tunlid, A. (2015). Ectomycorrhizal fungi - potential organic matter decomposers, yet not saprotrophs. *New Phytologist*, 205(4), pp 1443–1447.

Lindroth, A., Grelle, A. & Morén, A.-S. (1998). Long-term measurements of boreal forest carbon balance reveal large temperature sensitivity. *Global Change Biology*, 4(4), pp 443–450.

Lindroth, A., Lagergren, F., Aurela, M., Bjarnadottir, B., Christensen, T., Dellwik, E., Grelle, A., Ibrom, A., Johansson, T., Lankreijer, H., Launiainen, S., Laurila, T., Mölder, M., Nikinmaa, E., Pilegaard, K., Sigurdsson, B.D. & Vesala, T. (2008). Leaf area index is the principal scaling parameter for both gross photosynthesis and ecosystem respiration of northern deciduous and coniferous forests. *Tellus B*, 60(2), pp 129–142.

Litton, C.M., Raich, J.W. & Ryan, M.G. (2007). Carbon allocation in forest ecosystems. *Global Change Biology*, 13(10), pp 2089–2109.

Mäkelä, A., Hari, P., Berninger, F., Hänninen, H. & Nikinmaa, E. (2004). Acclimation of photosynthetic capacity in Scots pine to the annual cycle of temperature. *Tree Physiology*, 24(4), pp 369–376.

Mäkelä, A., Pulkkinen, M., Kolari, P., Lagergren, F., Berbigier, P., Lindroth, A., Loustau, D., Nikinmaa, E., Vesala, T. & Hari, P. (2008). Developing an empirical model of stand GPP with

the LUE approach: analysis of eddy covariance data at five contrasting conifer sites in Europe. *Global Change Biology*, 14(1), pp 92–108.

Manzoni, S., Jackson, R.B., Trofymow, J.A. & Porporato, A. (2008). The global stoichiometry of litter nitrogen mineralization. *Science*, 321(5889), pp 684–686.

Manzoni, S., Taylor, P., Richter, A., Porporato, A. & Ågren, G.I. (2012). Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist*, 196(1), pp 79–91.

Mikusinska, A., Persson, T., Taylor, A.F.S. & Ekblad, A. (2013). Response of ectomycorrhizal extramatrical mycelium production and isotopic composition to in-growth bag size and soil fauna. *Soil Biology and Biochemistry*, 66, pp 154–162.

Montgomery, H.J., Monreal, C.M., Young, J.C. & Seifert, K.A. (2000). Determinination of soil fungal biomass from soil ergosterol analyses. *Soil Biology and Biochemistry*, 32(8–9), pp 1207–1217.

Moorhead, D.L. & Sinsabaugh, R.L. (2006). A theoretical model of litter decay and microbial interaction. *Ecological Monographs*, 76(2), pp 151–174.

Moyano, F.E., Kutsch, W.L. & Rebmann, C. (2008). Soil respiration fluxes in relation to photosynthetic activity in broad-leaf and needle-leaf forest stands. *Agricultural and Forest Meteorology*, 148(1), pp 135–143.

Näsholm, T., Högberg, P., Franklin, O., Metcalfe, D., Keel, S.G., Campbell, C., Hurry, V., Linder, S. & Högberg, M.N. (2013). Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? *New Phytologist*, 198(1), pp 214–221.

Neumann, J. & Matzner, E. (2014). Contribution of newly grown extramatrical ectomycorrhizal mycelium and fine roots to soil respiration in a young Norway spruce site. *Plant and Soil*, 378(1–2), pp 73–82.

Nilsson, L.O., Bååth, E., Falkengren-Grerup, U. & Wallander, H. (2007). Growth of ectomycorrhizal mycelia and composition of soil microbial communities in oak forest soils along a nitrogen deposition gradient. *Oecologia*, 153(2), pp 375–384.

Nilsson, L.O., Giesler, R., Baath, E. & Wallander, H. (2005). Growth and biomass of mycorrhizal mycelia in coniferous forests along short natural nutrient gradients. *New Phytologist*, 165(2), pp 613–622.

Nilsson, L.O. & Wallander, H. (2003). Production of external mycelium by ectomycorrhizal fungi in a Norway spruce forest was reduced in response to nitrogen fertilization. *New Phytologist*, 158(2), pp 409–416.

Nylund, J. & Wallander, H. (1992). Ergosterol analysis as a means of quantifying mycorrhizal biomass. *Methods in Microbiology*, 24, pp 77–88.

Olofsson, M.A. & Bylund, D. (2016). Liquid chromatography with electrospray ionization and tandem mass spectrometry applied in the quantitative analysis of chitin-derived glucosamine for a rapid estimation of fungal biomass in soil. *International Journal of Analytical Chemistry*.

Olsson, P.A., Larsson, L., Bago, B., Wallander, H. & Van Aarle, I.M. (2003). Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi. *New Phytologist*, 159(1), pp 7–10.

Ostertag, R. (2001). Effects of nitrogen and phosphorus availability on fine-root dynamics in Hawaiian montane forests. *Ecology*, 82(2), pp 485–499.

Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire, A.D., Piao, S., Rautiainen, A., Sitch, S. & Hayes, D. (2011). A large and persistent carbon sink in the world's forests. *Science*, 333(6045), pp 988–993.

Parrent, J.L. & Vilgalys, R. (2007). Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO_2 and nitrogen fertilization. *New Phytologist*, 176(1), pp 164–174.

Pregitzer, K.S. & Euskirchen, E.S. (2004). Carbon cycling and storage in world forests: biome patterns related to forest age. *Global Change Biology*, 10(12), pp 2052–2077.

Pumpanen, J., Ilvesniemi, H., Kulmala, L., Siivola, E., Laakso, H., Kolari, P., Helenelund, C., Laakso, M., Uusimaa, M. & Hari, P. (2008). Respiration in boreal forest soil as determined from carbon dioxide concentration profile. *Soil Science Society of America journal*, 72(5), pp 1187–1196.

Raich, J.W. & Potter, C.S. (1995). Global patterns of carbon dioxide emissions from soils. *Global Biogeochemical Cycles*, 9(1), pp 23–36.

Read, D.J. & Perez-Moreno, J. (2003). Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist*, 157(3), pp 475–492.

Ryan, M.G., Binkley, D. & Fownes, J.H. (1997). Age-related decline in forest productivity: pattern and process. In: Begon, M. & Fitter, A.H. (Eds) *Advances in Ecological Research*. pp 213–262. Academic Press.

Rygiewicz, P.T. & Andersen, C.P. (1994). Mycorrhizae alter quality and quantity of carbon allocated below ground. *Nature*, 369(6475), pp 58–60.

Saiya-Cork, K.R., Sinsabaugh, R.L. & Zak, D.R. (2002). The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biology and Biochemistry*, 34(9), pp 1309–1315.

Saiz, G., Byrne, K.A., Butterbach-Bahl, K., Kiese, R., Blujdeas, V. & Farrell, E.P. (2006). Stand age-related effects on soil respiration in a first rotation Sitka spruce chronosequence in central Ireland. *Global Change Biology*, 12(6), pp 1007–1020.

Salmanowicz, B. & Nylund, J.E. (1988). High-performance liquid-chromatography determination of ergosterol as a measure of ectomycorrhiza infection in Scots pine. *European journal of forest pathology*, 18(5), pp 291–298.

Schlesinger, W.H. & Andrews, J.A. (2000). Soil respiration and the global carbon cycle. *Biogeochemistry*, 48(1), pp 7–20.

Simard, S.W., Jones, M.D. & Durall, D.M. (2003). Carbon and nutrient fluxes within and between mycorrhizal plants. In: van der Heijden, M.G.A. & Sanders, I.R. (Eds) *Mycorrhizal Ecology*. pp 33–74. Berlin: Springer Berlin Heidelberg.

Simpson, R.T., Frey, S.D., Six, J. & Thiet, R.K. (2004). Preferential accumulation of microbial carbon in aggregate structures of no-tillage soils. *Soil Science Society of America journal*, 68(4), pp 1249-1255.

Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L. & Richter, A. (2013). Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecology Letters*, 16(7), pp 930–939.

Sinsabaugh, R.L., Shah, J.J.F., Findlay, S.G., Kuehn, K.A. & Moorhead, D.L. (2015). Scaling microbial biomass, metabolism and resource supply. *Biogeochemistry*, 122(2–3), pp 175–190.

Sinsabaugh, R.L., Turner, B.L., Talbot, J.M., Waring, B.G., Powers, J.S., Kuske, C.R., Moorhead, D.L. & Follstad Shah, J.J. (2016). Stoichiometry of microbial carbon use efficiency in soils. *Ecological Monographs*, 86(2), pp 172–189.

Smith, E. S. & Read, D. J. (2008). *Mycorrhizal symbiosis*. 3rd. ed London: Academic Press.

Sterkenburg, E., Bahr, A., Durling, M.B., Clemmensen, K.E. & Lindahl, B.D. (2015). Changes in fungal communities along a boreal forest soil fertility gradient. *New Phytologist*, 207(4), pp 1145–1158.

Stinziano, J.R., Hüner, N.P.A. & Way, D.A. (2015). Warming delays autumn declines in photosynthetic capacity in a boreal conifer, Norway spruce (*Picea abies*). *Tree Physiology*, 35(12), pp 1303–1313.

Sun, H., Santalahti, M., Pumpanen, J., Köster, K., Berninger, F., Raffaello, T., Jumpponen, A., Asiegbu, F.O. & Heinonsalo, J. (2015). Fungal community shifts in structure and function across a boreal forest fire chronosequence. *Applied and Environmental Microbiology*, 81(22), pp 7869–7880.

Swift, M., Heal, O. & Anderson, J. (1979). *Decomposition in terrestrial ecosystems*. Oxford UK: Blackwell.

Tang, J., Luyssaert, S., Richardson, A.D., Kutsch, W. & Janssens, I.A. (2014). Steeper declines in forest photosynthesis than respiration explain age-driven decreases in forest growth. *Proceedings of the National Academy of Sciences*, 111(24), pp 8856–8860.

Taylor, A.F.S., Martin, F. & Read, D.J. (2000). Fungal diversity in ectomycorrhizal communities of Norway spruce [*Picea abies* (L.) Karst.] and beech (*Fagus sylvatica* L.) along north-south transects in Europe. In: Schulze, E.-D. (Ed) *Carbon and Nitrogen Cycling in European Forest Ecosystems*. pp 343–365. Berlin: Springer.

Treseder, K.K. & Allen, M.F. (2002). Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. *New Phytologist*, 155(3), pp 507–515.

Twieg, B.D., Durall, D.M. & Simard, S.W. (2007). Ectomycorrhizal fungal succession in mixed temperate forests. *New Phytologist*, 176(2), pp 437–447.

Wallander, H. (1995). A new hypothesis to explain allocation of dry matter between mycorrhizal fungi and pine seedlings in relation to nutrient supply. *Plant and Soil*, 168–169(1), pp 243–248.

Wallander, H., Ekblad, A., Godbold, D.L., Johnson, D., Bahr, A., Baldrian, P., Björk, R.G., Kieliszewska-Rokicka, B., Kjøller, R., Kraigher, H., Plassard, C. & Rudawska, M. (2013). Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forests soils - a review. *Soil Biology and Biochemistry*, 57, pp 1034–1047.

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

Wallander, H., Nilsson, L.O., Hagerberg, D. & Bååth, E. (2001). Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist*, 151(3), pp 753–760.

Wallander, H., Nilsson, L.O., Hagerberg, D. & Rosengren, U. (2003). Direct estimates of C:N ratios of ectomycorrhizal mycelia collected from Norway spruce forest soils. *Soil Biology and Biochemistry*, 35(7), pp 997–999.

Wang, B. & Qiu, Y.-L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*, 16(5), pp 299–363.

Ward, C., Pothier, D. & Paré, D. (2014). Do boreal forests need fire disturbance to maintain productivity? *Ecosystems*, 17(6), pp 1053–1067.

Wilkinson, A., Alexander, I.J. & Johnson, D. (2011). Species richness of ectomycorrhizal hyphal necromass increases soil CO_2 efflux under laboratory conditions. *Soil Biology and Biochemistry*, 43(6), pp 1350–1355.

Yuan, Z.Y. & Chen, H.Y.H. (2012). Fine root dynamics with stand development in the boreal forest. *Functional Ecology*, 26(4), pp 991–998.

Yuan, Z.Y., Chen, H.Y.H. & Reich, P.B. (2011). Global-scale latitudinal patterns of plant fine-root nitrogen and phosphorus. *Nature Communications*, 2, pp 1–6.

Acknowledgements

Making 1600 mesh bags, grinding sand from mesh bags (by hand) over several months, spending many long and cold days in the field (installing and harvesting mesh bags, of course), and finally stressing with data analysis and manuscripts, *may* not sound like a particularly fun experience, but in my case it has been an very enjoyable ride, since I have been lucky to be surrounded by a bunch of very nice people. Thank you all!

First I want to express my gratitude to my main supervisor Petra Fransson for giving me this opportunity to do this PhD, and for her continuous support and patience, and for always believing in me. Petra has always been open for discussions and questions, and for that I am very forever grateful as it has helped me tremendously. I also want to express my sincere thanks to my awesome co-supervisors, Karina Clemmensen, Alf Ekblad and Roger Finlay.

Karina, your optimism, enthusiasm, creativity and kindness has been an incredible source of inspiration. Also thank you for giving me the opportunity to teach students - I really enjoyed that experience.

Alf, your enthusiastic engagement and considerably deep knowledge on forest ecology has been of a great help. Thank you for speaking your mind out in times when it was needed, and thank you for your careful feedback on experimental designs and on manuscripts. It improved projects and manuscripts substantially.

Roger, thank you for always being very supportive and friendly, and also for your very helpful linguistic support. Whenever I had a lot of things on my mind it very nice to take a lunch break with you to listen to your awesome anecdotes and discuss interesting things with you (*i.e.* fungi, bacteria, recent events, politics, technology, the P = NP problem, etc..). I must say that I really enjoyed that.

A special thank goes to Björn Lindahl for being my honorary co-supervisor. Your patience, encouragement and very constructive comments and inputs

have been very supportive and helpful. You have been always available for discussions, which I am very thankful for.

Special thanks to my two closest PhD colleagues; Julia Kyaschenko and Preeti Baskaran. It was very nice to not take the PhD journey alone and having you around, working concurrent projects have been both fun and inspiring. I wish you both the best of luck in all your future endeavours.

I also want to thank my colleague Shahid Mahmood for all the interesting and inspiring discussions on mycorrhizal mycelium. The many late talks with Shahid in the field lab during my second year helped me improve and develop my projects. A very special thanks goes to all the Mykopat family members, as I felt that the very friendly environment at the department of Forest Mycology and Plant Pathology was an optimal setting to do science.

Last but definitively not least, I want to thank my better half; Ala, for her continuous loving support during all these years. Thank you for always believing in me, and being there during times when I needed you the most. Also thank you for helping me during several very hectic periods of field work, since managing time was only possible with your assistance and dedication to help. Moreover, I am very grateful in you helping me making mesh bags, from morning to evening, at several occasions. Your assistance and encouragement have been a tremendous support. Thank you my love.