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Abstract

Boreal forests are characterized by strong nutrient limitation and nitrogen is particularly scarce. Ectomycorrhizal fungi acquire nitrogen from the soil in return for carbon from their tree host and are, thus, pivotal mediators of carbon and nitrogen cycling. Anthropogenic activities during the past century have increased the amounts of reactive nitrogen supplied to boreal forest, through atmospheric deposition and fertilization. In this thesis, high-throughput sequencing of fungal communities and measurements of enzymatic activities and fungal biomass were used to assess if and how ectomycorrhizal fungi respond differently to nitrogen added by human activities than to natural variation in ecosystem fertility. The relationship between morphological characteristics of the ectomycorrhizal fungal mycelium and patterns of soil colonization was also investigated.

Ectomycorrhizal fungal community composition changed after amendments with anthropogenic nitrogen and along a gradient in natural nitrogen availability, but the effects were small and different in the natural gradient compared to the shifts induced by external N additions. The relative abundance of ectomycorrhizal fungi declined in forests influenced by anthropogenic nitrogen addition, and *Cortinarius* and *Piloderma* were particularly sensitive. In fertilized *Pinus sylvestris* forests, the loss of *Cortinarius* – presumed to be efficient decomposers – was associated with increased organic matter accumulation in the organic topsoil. Further, there were weak connections between mycelial morphology and soil foraging patterns. Instead, variation in the proliferation of extraradical mycelium was proposed to be related to differences in carbon use efficiency of ectomycorrhizal fungi.

Anthropogenic nitrogen additions are likely to induce an ectomycorrhizal tipping point, which is likely to depend on reduced host C investment. At this point, ectomycorrhizal fungal biomass rapidly decreases and trees may shift towards higher reliance on inorganic nitrogen. Possibly, the ectomycorrhizal community can initially mitigate negative effects of increased anthropogenic nitrogen supply, and maintain nitrogen limitation in the forest, by shifting towards taxa with low carbon demand and high nitrogen retention capacity.

Keywords: boreal forest, carbon cycling, ectomycorrhiza, ecosystem fertility, fertilization, forest management, fungal community, nitrogen cycling, nitrogen deposition, nutrient availability

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En jämförelse av effekter av endogent och antropogent kväve på ektomykorrhiza

Sammanfattning

Boreala skogar kännetecknas av stark näringsbegränsning och tillgången till kväve är särskilt låg. Ektomykorrhizasvampar tillgängliggör kväve från marken i utbyte mot kol från träden de lever i symbios med och blir därigenom centrala för kväve- och kolomsättning. Under det senaste århundradet har vi människor genom kvävedeposition och gödsling ökat mängden reaktivt kväve i boreala skogar. I den här avhandlingen undersöktes huruvida ektomykorrhizasvampar påverkas olika av ökad kvävetillgång beroende på om ökningen orsakas av antropogen tillförsel eller högre naturlig bördighet i marken. Utöver det undersöktes även förhållandet mellan svampmycelets morfologi och utsträckningen som ektomykorrhizasvampar koloniserar olika typer av jordsubstrat.

Artsammansättningen av ektomykorrhizasvampar förändrades efter tillförsel av antropogent kväve. Skiften i svampsamhället skedde även längst en naturlig bördighetsgradient men effekterna av ökad kvävetillgänglighet var mycket mindre än förändringarna som uppstod på grund av extern kvävetillförsel. Den relativa abundansen av ektomykorrhizasvampar minskade i skogar som utsätts för antropogen kvävetillförsel och släktena *Piloderma* och *Cortinarius* var särskilt känsliga. I gödslade tallskogar var förlusten av *Cortinarius*, som antas vara effektiva nedbrytare, kopplat till ökad ackumulering av organiskt material i markens övre skikt. Det fanns endast en svag koppling mellan mycelmorfologi och koloniseringsmönster. Istället föreslogs ektomykorrhizasvamparnas förmåga att kolonisera nya substrat vara relaterade till deras kapacitet att effektivt använda kol från värdväxten till att producera biomassa.

Antropogen kvävetillförsel gör troligtvis att en brytpunkt nås, där ektomykorrhizabiomassan drastiskt minskar på grund av att träden investerar mindre kol till sina svamppartners och istället blir träden mer beroende av oorganiskt kväve. Möjligtvis kan ektomykorrhizasvampsamhället försöka motverka de negativa effekterna av ökad antropogen kvävetillförsel genom att ändra sin artsammansättning. Genom att skifta till högre abundans av svampar med en hög förmåga omsätta kol från värdväxten till biomassa kan de binda in mycket kväve och därmed försöka upprätthålla kvävebegränsningen i boreala skogar.

Keywords: boreal skog, ekosystembördighet, ektomykorrhiza, kolomsättning, kväveomsättning, kvävedeposition, näringstillgång, svampsamhälle

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Dedication

To Stella – may you stay curious and eager to explore the world!

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. Jörgensen, K., Granath, G., Lindahl, BD., Strengbom, J. (2021). Forest management to increase carbon sequestration in boreal *Pinus sylvestris* forests. Plant and Soil, 466:165-178.

II. Jörgensen, K., Granath, G., Strengbom J., Lindahl, BD. (2021). Effects of fertilization and thinning on carbon storage in boreal pine forest soils are linked to changes in fungal communities. (submitted)

III. Jörgensen, K., Clemmensen KE., Wallander H., Lindahl BD. (2021) Mycelial foraging strategies and phenotypic plasticity of ectomycorrhizal fungi in boreal *Picea abies* forests. (manuscript)

IV. Jörgensen, K., Clemmensen KE., Wallander H., Lindahl BD. Context dependency of ectomycorrhizal fungal responses to nitrogen availability. (manuscript)

The contribution of Karolina Jörgensen to the papers included in this thesis was as follows:

I. Performed the laboratory work, participated in data analysis. Wrote the manuscript together with co-authors and was responsible for correspondence with the journal.

II. Performed the laboratory work and did the data analysis and participated in interpretation of results. Wrote the manuscript together with co-authors. Was responsible for correspondence with the journal.

III. Designed and planned the study together with co-authors. Conducted the field and laboratory work. Did the data analysis and interpreted results together with co-authors. Wrote the manuscript together with co-authors.

IV. Participated in planning together with co-authors. Conducted the field sampling and performed all lab work and data analysis. Interpreted the results and wrote the manuscript together with co-authors.

Abbreviations

aP	Acid phosphatase
BG	β-1,4-glucosidase
C	Carbon
CA	Correspondence analysis
CCA	Constrained correspondence analysis
CUE_A	Apparent carbon use efficiency
DCA	Detrended correspondence analysis
ECM	Ectomycorrhiza
HPLC	High performance liquid chromatography
ITS	Internal transcribed spacer
MnP	Manganese peroxidase
N	Nitrogen
NAG	β-1,4-*N*-acetylglucosaminidase
OM	Organic matter
P	Phosphorus
PCR	Polymerase chain reaction

1. Introduction

A central characteristic of the boreal forest is the domination of trees that form ectomycorrhizal relationships with soil fungi (Soudzilovskaia et al. 2019). Ectomycorrhiza is the symbiotic relationship in which trees supply photosynthetically derived carbon (C) to the fungus in exchange for nutrients – mainly nitrogen (N) (Smith & Read 2010). Ectomycorrhiza links aboveground and belowground C and nutrient dynamics and has implications for biogeochemical processes and productivity on both local (Kyaschenko et al. 2017b) and global scales (Soudzilovskaia et al. 2019). Due to strong N limitation, variation in endogenous N availability (i.e. made available through internal biogeochemical processes) is a strong driver that shapes the boreal forest (Högberg et al. 2017). Furthermore, anthropogenic activities during the past century have led to increased input of reactive N to boreal forests with noticeable effects on ectomycorrhizal fungal species composition and functioning (Lilleskov et al. 2002, 2019; van der Linde et al. 2018), as well as on C accumulation both above and below ground (Waldrop et al. 2004; Wang et al. 2017). Yet, we still lack understanding of whether variation in endogenous N supply and input of anthropogenic N (i.e. introduced to the ecosystem through human activities) have the same effect on ectomycorrhizal fungi. It is central to fill this knowledge gap as it is a prerequisite to understand how forest functioning is influenced by environmental changes to sustainably manage boreal forests in the future.

In this thesis, I aim to assess if and how variation in N availability caused by human activities differ in its effects on ectomycorrhizal fungi from natural variation in ecosystem fertility. Moreover, the consequent effects of variation in N supply on nutrient cycling and C dynamics in northern coniferous forests are investigated.

1.1 Characteristics of boreal forests

The boreal forest is the largest terrestrial biome and covers a substantial part of the northern hemisphere, equivalent to 11% of the terrestrial surface on earth (Bonan & Shugart 1989) (Figure 1). Soils, especially in the Fennoscandian region, are typically podzols (DeLuca & Boisvenue 2012) characterized by a purely organic mor layer (also referred to as: “organic topsoil”, “O-horizon” or “humus layer”) overlying mineral soil, boulders or bedrock. Several factors interplay to shape the boreal forest ecosystem; climate, vegetation, and soil characteristics such as low pH and slow decomposition.

Vegetation is adapted to the typically harsh climate with relatively cold, long winters and short growing periods (Bonan & Shugart 1989). Among tree genera, conifers, such as pine (*Pinus*), spruce (*Picea*) and fir (*Abies*), dominate. Other common tree species are broadleaf birch (*Betula*), alder (*Alnus*), poplar (*Populus*) and willow (*Salix*) (Larsen 1980). The understory vegetation is commonly dominated by ericaceous dwarf shrubs such as bilberry (*Vaccinium myrtillus*), lingonberry (*Vaccinium viitis-idaea*) and black crowberry (*Empetrum hermaphroditum*), as well as mosses (e.g. *Pleurozium schreberi* and *Hylocomium splendens*) and lichens (e.g. *Cladina* and *Cladonia*) (Nilsson & Wardle 2005).

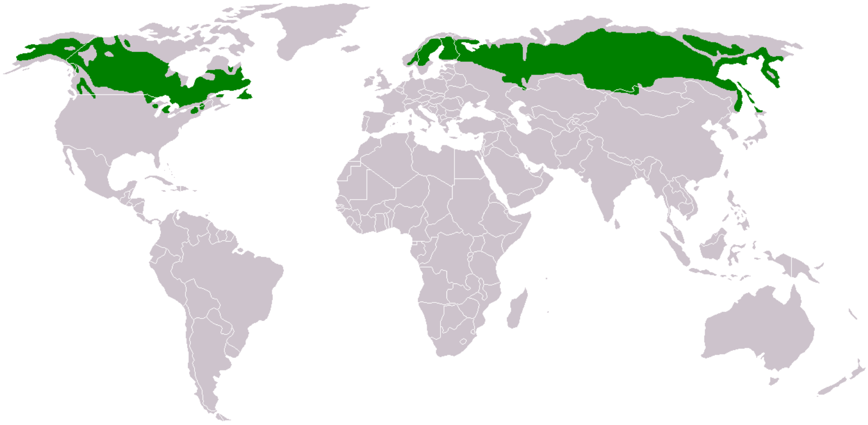

Figure 1. Global expansion of the boreal biome (highlighted in green). CC BY-SA 3.0

The slow decomposition rates restrict nutrient availability for both plants and soil organisms, and nutrient limitation is further accentuated by recalcitrant plant litter with a high C:N ratio. Together with, e.g. climatic factors that contribute to slow decomposition, this leads to the build-up of the purely organic mor layer. In Swedish coniferous forests, the mor layer constitutes around one-third of the belowground C stock (Nilsson et al. 2017). Boreal forests store around 60% of their total ecosystem C stock in the soil (Pan et al. 2011).

1.2 Nitrogen supply and cycling

Nitrogen is the strongest limiting factor for boreal forest productivity (Tamm 1991; Högberg et al. 2017). Endogenous N availability shifts along gradients of fertility, which are largely governed by hydrology and topography. Areas with relatively higher nitrogen availability are often located at low points in the landscape (Högberg 2001; Högberg et al. 2017). Usually, pH and inorganic N availability (ammonium and nitrate) are positively correlated (Sterkenburg et al. 2015) along these gradients. Moreover, high base saturation (or base-ion exchange capacity) is strongly associated with high N availability and pH (Högberg et al. 2017), highlighting the influence of geology and pedology on boreal forest ecosystem functioning.

Biological fixation of N_2 occurs in the boreal forest, albeit at low rates compared to other terrestrial ecosystems. Mosses are the primary host of N fixing bacteria, for instance, the *Stigonema* cluster (Warshan et al. 2016). Diazotrophic bacteria associated with *Pleurozium shreberi* can fix roughly 2 kg N ha^{-1} annually (DeLuca et al. 2002, 2008; Rousk et al. 2013), while the activity associated with *Sphagnum* mosses has been reported to vary between 0.4-3.2 kg ha^{-1} yr^{-1} (Granhall & Lindberg 1978).

Since the amounts of N needed to sustain plant productivity (roughly between 15-50 kg N ha^{-1} yr^{-1} (Sponseller et al. 2016) exceeds fixation rates (Korhonen et al. 2013), N has to be utilized from sources within the system. Boreal forests, therefore, rely to a high degree on re-circulation of nutrients from organic matter in the soil. Saprotrophic and particularly mycorrhizal fungi play a pivotal role in mobilizing nutrients from the soil by decomposing the organic matter and create a tight link between aboveground productivity and processes belowground.

1.3 Anthropogenic influence

1.3.1 Atmospheric nitrogen deposition

During the past century, human activities have profoundly altered the global N cycle primarily through production of mineral fertilizers from N_2 *via* the Haber-Bosh method as well as global translocation of N in i.e. agricultural products and atmospheric deposition to both terrestrial and aquatic ecosystems. Atmospheric deposition of N to terrestrial ecosystems comes in the form of reactive N in reduced (i.e. NH_4^+) or oxidized (i.e. NO_3^-) forms that can be easily assimilated and used in biological processes (Galloway et al. 2008). Although N deposition globally has increased C uptake through stimulated tree growth – corresponding to 9% of the total terrestrial C sink (Wang et al. 2017), there are adverse effects on other ecosystem properties. One possible consequence of increased input of reactive N through atmospheric deposition is ecosystem N saturation (Aber et al. 1989). In short, N saturation could occur when N additions push a forest ecosystem past a threshold where N is no longer limiting, and would result in higher rates of nitrification. Strengthened by positive feedback mechanisms, involving for instance the fungal community (Suz et al. 2021), nitrate availability may eventually exceed uptake rates and lead to N leaching (Aber et al. 1989). Atmospheric deposition is often associated with declines in soil pH (Falkengren-Grerup 1987), which may cause additional disturbance to soil biota. Additionally, N deposition may have detrimental effects on bacteria and fungi in the soil and they generally respond with decreased biomass and respiration as an effect of N deposition (Zhang et al. 2018).

Currently, the deposition rate in the boreal zone is fairly low, ranging 1-11 kg ha^{-1} yr^{-1} (Sponseller et al. 2014), compared to more southern European locations (Erisman et al. 2015). In Sweden, levels are currently low to moderate but have historically been higher, especially in the south-west (Figure 2). However, the declining trend may be broken since rates have been projected to increase in the future and reach annual rates of more than 20 kg ha^{-1} in southern Sweden, with significantly increased levels also in the middle and northern parts. The scenario is based on increased demands for reactive N to sustain food production for a growing global population as well as production of biomass to replace fossil fuels (Galloway et al. 2008).

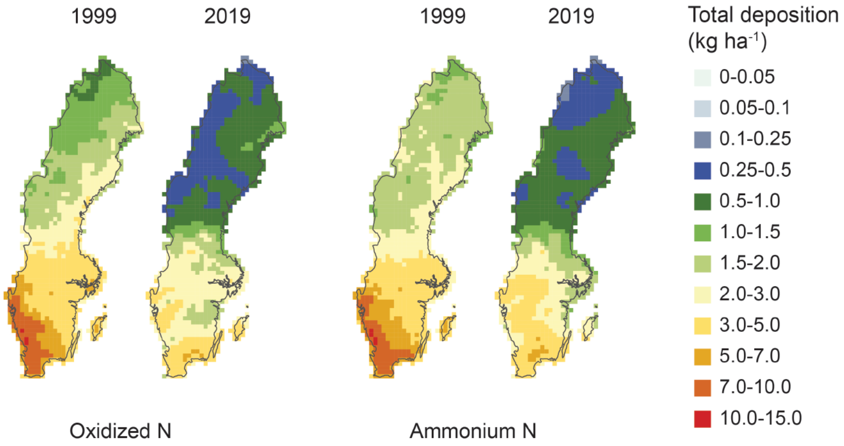

Figure 2. Total rates of annual atmospheric deposition (kg ha^{-1}) of oxidized N and ammonium N in Sweden in 1999 and 2019. Source: Swedish Meteorological and Hydrological Institute (SMHI).

1.3.2 Forest management

Globally, around two-thirds of the boreal forest is subject to forest management and is either set aside for conservation purposes, fire protection or, in line with the more general perception of management, used for timber production (Gauthier et al. 2015). In Sweden, productive forests, i.e. forests suitable for forestry, cover about 58% (23.5×10^{6} ha) of the total land area and are largely dominated by conifers (82%). Thinning, i.e. selective removal of trees to increase timber quality has been done in about 40% of the productive forest (Nilsson 2021). Nitrogen fertilization is considered a cost-effective way to increase productivity in boreal forests (Nohrstedt 2001). In Sweden, fertilization has been implemented in forestry since the 1960’s and peaked during the 1970’s. Since then, however, it has been less commonly utilized (Skogforsk, 2005). During the past decade (2009-2019), an average annual area of 50 000 ha was fertilized in Sweden, mainly in the north (Swedish forest agency, Appendix 1). The most common element to add is N, and the standard practice is to apply around 150 kg N ha^{-1} one to three times during the production cycle (of 70-150 years) (Högberg et al. 2014c).

1.4 Phosphorus in boreal forests

Although N limitation is considered “the rule” in boreal forests, the natural variation in N availability may cause other elements to become limiting at high N (Högberg et al. 2017). In temperate forests exposed to high N loads, P became the main limitation for tree growth (Braun et al. 2010). Similarly, in coniferous forests with high N availability, trees displayed signs of P limitation which was alleviated when P was added (Almeida et al. 2019). In Swedish boreal forests, P limitation of ecosystem productivity has not been reported, however N amendments may change stoichiometry in foliage (Palmqvist et al. 2020) and N use efficiency in tree biomass production (Hyvönen et al. 2008), indicating that the forests may be balancing between N and P limitation. As P becomes more limiting, N mineralization rates may increase since biota try to maintain N:P stoichiometry. This would strengthen a positive feedback on inorganic N availability that ultimately could result in N saturation (Gress et al. 2007; Vitousek et al. 2010).

1.5 Fungi in boreal forest soils

Compared to plants, fungal communities in boreal forest soils are highly diverse (Tedersoo et al. 2014). The fungal community consists of a great diversity of fungi from different phyla, but Basidiomycota and Ascomycota are the most common (Tedersoo et al. 2014). In richer conditions, the phylum Basidiomycota tends to dominate (Tedersoo et al. 2014) while fungi belonging to phylum Ascomycota (orders Helotiales and Chaetothyriales) increase in relative abundance in poorer systems (Tedersoo et al. 2014; Sterkenburg et al. 2015). The aforementioned orders generally seem to be better adapted to tolerate harsh conditions with low nutrient availability (Lindahl et al. 2007; Kyaschenko et al. 2017a) and are also highly abundant in arctic soils (Tedersoo et al. 2014).

Fungi have different trophic modes and can be divided into guilds based on their ecophysiological traits, for instance, saprotrophs and mycorrhizal fungi. Within the soil, these guilds interact with each other, shaping the nutrient dynamics of the boreal forest (Clemmensen et al. 2015; Kyaschenko et al. 2017b).

1.5.1 Saprotrophic fungi

Saprotrophic fungi are free-living decomposers that are able to produce a vast array of hydrolytic enzymes to decompose hydrolysable plant material, dead fungal tissues and other substrates in the soil (Baldrian et al. 2011). In addition, some saprotrophic white-rot basidiomycetes can also produce oxidative enzymes that are used to break down more complex structures, such as lignin (Baldrian 2008).

Saprotrophic fungi are restricted by the low N availability in the boreal forest and can cope with nutrient limitation by translocating nutrients within their, sometimes rather extensive, mycelia to sustain growth and decomposition activity in patches with higher substrate quality (Boddy 1999; Boberg et al. 2014). Further, lab studies have shown positive effects of inorganic N additions on saprotrophic decomposition activity and growth in low quality substrates (Boberg et al. 2008; Allison et al. 2009). Increased N availability in boreal forests could therefore stimulate the saprotrophic community (Kyaschenko et al. 2017b; Sterkenburg et al. 2018). However, at high anthropogenic N input, the abundance of ligninolytic saprotrophs has been reported to decrease (Entwistle et al. 2017), likely because "N mining" from recalcitrant litter becomes energetically unfavourable as inorganic N availability increases (Craine et al. 2007). Instead, the decomposing community would shift towards taxa specialized in cellulose decomposition and inorganic N uptake (Talbot & Treseder 2012).

1.5.2 Ericoid mycorrhizal fungi

Ericoid mycorrhiza is formed between fungi belonging to Ascomycota or Basidiomycetes within the order Sebacinales and plants within the Ericaceae family (Smith & Read 2010), and common plant hosts in the Scandinavian boreal region are species within *Vaccinium*, *Calluna* and *Empetrum.* The abundance of plants potentially forming ericoid mycorrhiza is highest at high latitudes (Soudzilovskaia et al. 2019). Ericoid mycorrhizal fungi have retained a large degree of their genetic potential for decomposition when evolving from saprotrophic ancestors (Martino et al. 2018) and are therefore considered important for nutrient cycling in low productive, highly nutrient limited systems. Accordingly, they have been suggested to take part in root decomposition in coniferous forests (Kohout et al. 2021).

1.5.3 Ectomycorrhizal fungi

Ectomycorrhiza is the dominating mycorrhizal type associated with trees in boreal forest ecosystems and is characterized by a fungal mantle that encloses tree root tips. From the mantle, hyphae (the extraradical mycelium) extends out into the soil matrix (Smith & Read 2010). Ectomycorrhizal fungi provide the associated plant with nutrients acquired either by uptake from the soil solution or by accessing nutrients from solid organic matter by exudation of different enzymes (Read & Perez-Moreno 2003; Read et al. 2005; Lindahl & Tunlid 2015; Lindahl & Clemmensen 2016). The extraradical mycelium significantly increases the soil volume that can be explored for nutrients and constitutes 75% of the total surface area of the ectomycorrhiza (Leake et al. 2004). Another proposed plant benefit of ectomycorrhiza is that the fungi may serve as protectors against root pathogens (Marx 1972), and facilitate establishment of conspecific plant seedlings (Bennett et al. 2017).

Ectomycorrhizal fungi have evolved independently from several lineages of saprotrophic ancestors (Tedersoo & Smith 2013; Strullu-Derrien et al. 2018). In general, this involved a loss of the ability to break down plant cell walls to gain C (Kohler et al. 2015). Instead, ectomycorrhizal fungi rely on metabolic C provided by their host to sustain growth and support N uptake from the soil. Some N is taken up in mineralized form from the soil solution. However, capacity for enzymatic breakdown of organic matter to access organically bound N is also present in ectomycorrhizal fungi and some taxa have retained a capacity for decomposition of recalcitrant organic matter, either through the ability to produce manganese peroxidases (*Cortinarius*, *Lactarius* and *Russula* species) (Bödeker et al. 2009, 2014) or Fenton-mechanisms (*Paxillus*) (Shah et al. 2016). These oxidative mechanisms are energy demanding and rely on high input of host C (Lindahl & Tunlid 2015; Baskaran et al. 2017).

Morphology and physiology of ectomycorrhizal fungi

Ectomycorrhizal fungi use their extraradical mycelium to explore the soil, both to access nutrients and to colonize new roots. The concept of “exploration types” was invented by Agerer (2001) as a way to classify ectomycorrhizal fungi based on the morphology of the extraradical mycelium on ectomycorrhizal root tips. The concept also encompasses generalizations about physiology, e.g. whether the mycelium is hydrophobic or hydrophilic.

In short, the commonly used exploration types are described as follows: "Contact type" mycorrhizae form dense, smooth, hydrophilic mantles around the roots without extensive formation of extramatrical mycelium. "Short" and "medium smooth" types are also hydrophilic, but form emanating, non-aggregated hyphae that expand through a large volume of the soil, maximising the hyphal surface area exposed to the soil solution. "Medium fringe", "medium mat" and "long" types of ectomycorrhizae form extensive mycelia characterised by aggregated, hydrophobic rhizomorphs (Figure 3).

The exploration types could be used to denote ecological function since there are some common traits within each type. Short distance and medium distance smooth types, which maximize the area of hydrophilic hyphae that extend into the soil may be adapted for rapid uptake of labile N forms (ammonium, nitrate, amino acids). Types with hydrophobic cords may, instead, display more directed growth toward patches of immobile, organic resources (Finlay & Read 1986; Nygren et al. 2008; Hobbie & Agerer 2010).

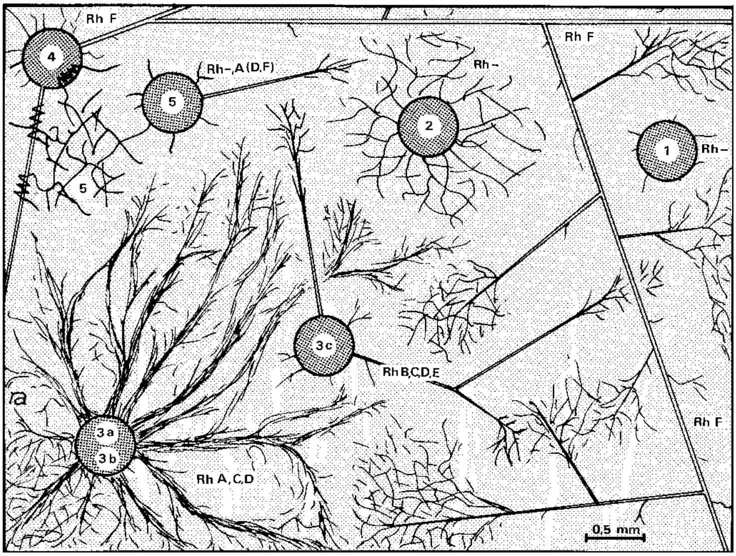

Figure 3 Schematic of ectomycorrhizal exploration types as proposed by Agerer (2001). Numbers correspond to the different exploration types: (1) contact type, (2) short-distance, (3a) medium distance fringe, (3b) medium distance mat, (3c) medium distance smooth, (4) long-distance and (5) pick-a-back exploration types. Reprinted by permission from Springer Nature: Mycorrhiza, *Exploration types of ectomycorrhizae*, Agerer, R. 2001.

1.5.4 Interactions among soil fungi in boreal forests

In boreal forests, saprotrophic fungi are usually confined to the fresh litter layer on top of the organic topsoil while ectomycorrhizal fungi inhabit the more decomposed humus layers (Lindahl et al. 2007; Clemmensen et al. 2013; Kyaschenko et al. 2017b). The spatial separation is proposed to arise due to competitive exclusion of saprotrophic litter fungi by ectomycorrhizal fungi in the more decomposed litter (Lindahl et al. 2010; Bödeker et al. 2016) and has been suggested to slow down litter decomposition rates and contribute to the build-up of organic matter in boreal forests – a mechanism commonly known as the "Gadgil effect" (Gadgil & Gadgil 1971, 1975). A universal existence of a Gadgil effect in boreal forests can, however, be questioned since exclusion of roots in a girdling experiment did not promote exploitation of deeper layers by basidiomycete decomposers (Sterkenburg et al. 2018). It is likely that it is mainly relevant in the fresh litter layer rather than in the deeper humus layers of boreal soils.

Ectomycorrhizal and ericoid mycorrhizal fungi co-exist in the deeper layers of the organic topsoil (Clemmensen et al. 2021) but understanding of how they interact and contribute to both C translocation and decomposition in deeper organic layers is still lacking. A recent study showed that presence of ericoid mycorrhizal plants strengthened the positive relationship between ectomycorrhizal tree dominance and soil C/N ratio (Ward et al. 2021). This suggests that ericoid mycorrhizal species further constrict ecosystem nutrient availability and ectomycorrhizal symbiosis could be a strategy that is necessary for nutrient mobilization (leading to increasing soil C/N) in systems with high ericoid abundance. In that scenario, boreal forests could be considered as a transitional state between tundra and temperate forest (Lindahl & Clemmensen 2016).

1.6 Fungi and C stocks

Root-associated fungi have been suggested to contribute to the belowground C stock through inputs of root-derived C and fungal necromass (Clemmensen et al. 2013, 2015; Kyaschenko et al. 2019). Fungal necromass may interact with complex root-derived organic molecules and contribute to a long-term stable organic matter sink in boreal forest soils (Adamczyk et al. 2019). Among the root-associated fungi, dark septate endophytes from the phylum Ascomycota (e.g. within the orders Pezizales and Leotiales) produce particularly persistent mycelia that contain melanin and decomposes slowly (Mandyam & Jumpponen 2005; Fernandez & Kennedy 2018).

In addition to contributing to the soil organic matter stock, fungi also take part in organic matter decomposition. The net effect on C storage in soil is determined by the balance of input and output, and processes mediated by fungi have previously been shown to correlate strongly with C stocks (Clemmensen et al. 2013, 2021; Kyaschenko et al. 2017b; Lindahl et al. 2021). Ectomycorrhizal fungi link N and C cycles in boreal forest soils through their selective mining for N (Lindahl & Tunlid 2015). Particularly, oxidative decomposition using manganese peroxidases has been identified as a crucial regulator of organic matter stocks in boreal forest soils (Kyaschenko et al. 2017b; Stendahl et al. 2017), and ectomycorrhizal fungi are pivotal drivers of this process (Sterkenburg et al., 2018). While all boreal forest are sinks for organic matter (Pan et al. 2011), the strength of the belowground sink is influenced by the composition of the ectomycorrhizal community. Notably, the presence of manganese peroxidase producing *Cortinarius* species decreases the amount of organic matter in the mor layer by, on average, 33% in Swedish coniferous forests (Lindahl et al. 2021).

1.7 Ectomycorrhizal fungi and nitrogen availability

While the ectomycorrhizal symbiosis is generally considered mutualistic, i.e. beneficial for both partners, it can restrict plant productivity under certain conditions. In environments with low N availability, ectomycorrhizal fungi may not deliver enough N to their host relative to the C that the tree invests. Instead, N limitation may be enhanced since nutrients are retained in the fungal extraradical mycelium (Näsholm et al. 2013; Kyaschenko et al. 2019). As fertility increases, ectomycorrhizal fungi may deliver a higher ratio of their acquired N to their host (Näsholm et al. 2013). However, at high inorganic N availability, it may be more beneficial for the trees to invest in non-mycorrhizal roots to avoid the C drain incurred by the fungus (Franklin et al. 2014). As a response to decreased C flow, ectomycorrhizal fungi would have to adapt, initially, by displaying phenotypical plasticity and reduce their mycelial production to persevere. Eventually, as N availability increases, the ectomycorrhizal fungal community composition would likely shift towards a higher abundance of nitrophilic species while nitrophobic species would decline (van der Linde et al. 2018; Suz et al. 2021).

At a certain point, the system could reach a tipping-point where it rapidly transitions into P limitation (Aber et al. 1989; Suz et al. 2021). It is plausible that some ectomycorrhizal species, among them *Imleria badia* (Almeida et al. 2019), which are specialized in P acquisition would benefit under this N saturated scenario. However, ultimately, N saturation could induce a shift towards a system dominated by arbuscular mycorrhiza under very high N conditions (Averill et al. 2018).

1.7.1 Nitrogen effects on ectomycorrhizal biomass

Due to their pivotal role in N cycling in boreal forests, numerous studies have investigated the effects of variation in N availability on ectomycorrhizal fungi. The responses of ectomycorrhiza have, however, been inconsistent. Across natural gradients of N availability, mycelial biomass has correlated both positively (Kalliokoski et al. 2010; Sterkenburg et al. 2015) and negatively (Nilsson et al. 2005) with soil fertility. Across gradients of atmospheric deposition, ectomycorrhizal mycelial production decreased with increased N input (Kjøller et al. 2012; Bahr et al. 2013). Similar declines are often observed in fertilization studies (Nilsson & Wallander 2003; Demoling et al. 2008; Högberg et al. 2014a). In arctic tundra, fertilization increased the overall fungal biomass and ectomycorrhizal mycelial production (Clemmensen et al. 2006). The opposing responses to external inputs of N could relate to the initial nutrient availability at the sites and depend on whether the increased N availability alleviated N limitation on productivity or pushed the system beyond an optimum at which N availability would exceed uptake.

1.7.2 Nitrogen effects on community composition

In systems with changes in ectomycorrhizal biomass, co-occurring community shifts are also often observed. Ectomycorrhizal fungal communities change along natural fertility gradients (Toljander et al. 2006; Kranabetter et al. 2009; Kyaschenko et al. 2017b) and following anthropogenic N input (Arnolds 1991; Lilleskov et al. 2002, 2019; Cox et al. 2010; Kjøller et al. 2012; Hasselquist & Högberg 2014). Even though ectomycorrhizal biomass may return to pre-fertilization levels after termination of N input, there is considerable uncertainty whether the communities fully recover (Strengbom et al. 2001; Hasselquist & Högberg 2014; Högberg et al. 2014b; Choma et al. 2017).

N-responses of taxa within the ectomycorrhizal fungal guild can be related to functional traits such as exploration type (Figure 3) and hydrophobicity of the extraradical mycelium (Agerer 2001). A common feature of some ectomycorrhizal fungal genera that consistently respond negatively to N deposition (*Cortinarius*, *Tricholoma* and *Suillus*) is the formation of hydrophobic, medium- or long-distance exploration mycelia (Lilleskov et al. 2011; Suz et al. 2014; van der Linde et al. 2018). On the contrary, *Cenococcum*, *Inocybe*, *Lactarius* and *Tylospora* seem to be favoured in nutrient-rich conditions (Cox et al. 2010; Lilleskov et al. 2011; Sterkenburg et al. 2015; van der Linde et al. 2018). Other genera (*Tomentella*, *Russula* and *Piloderma*) display mixed responses to increased N availability (Toljander et al. 2006; Cox et al. 2010; Lilleskov et al. 2011; Sterkenburg et al. 2015; van der Linde et al. 2018; Kyaschenko et al. 2019).

Reports of varying direction of responses both between and within genera call for efforts to understand the mechanisms that shape fungal communities in relation to N availability. One possible explanation for the within-genus mixed responses is that there is large intrageneric variation in the adaptability or plasticity to increased N supply. Another possibility is that the mixed response of genera could depend on the endogenous N availability status of the study system. Accordingly, genus responses would depend on if the additional N pushes the system towards or away from an N optimum which is likely conditioned by the C flow from the host (van der Linde et al. 2018; Suz et al. 2021).

1.8 Ecosystem-level responses to anthropogenic nitrogen

Cycling of C and N is tightly linked in boreal forests and to a large extent mediated by ectomycorrhizal fungi. Consequently, anthropogenically induced shifts in N availability may have concomitant effects on C storage through effects on the activity and composition of ectomycorrhizal fungal communities. Increased belowground C stocks are often observed in boreal forests after external N additions (Hyvönen et al. 2008; Janssens et al. 2010; Maaroufi et al. 2015; Entwistle et al. 2017). The stimulated C accumulation is commonly attributed to decreased rates of soil respiration (Olsson et al. 2005; Janssens et al. 2010; Maaroufi et al. 2015) which could be caused by hampered respiration from both roots (Forsmark et al. 2021), ectomycorrhizal hyphae (Vallack et al. 2012) and saprotrophs (Olsson et al. 2005). However, similarly as the effects of anthropogenic N on e.g. ectomycorrhizal biomass, respiratory responses may differ depending on N load. In northern latitude *Pinus sylvestris* forests, moderate N addition rates increased respiration from ectomycorrhizal hyphae while high rates had a negative effect (Hasselquist et al. 2012).

Other functional shifts that may occur in N amended systems are related to the enzymatic capacity of the fungal community. In a temperate forest, taxa with ligninolytic capacity decreased after long-term N addition (Entwistle et al. 2017). Down-regulated decomposition, particularly the suppression of oxidative decomposition mechanisms (Carreiro et al. 2000; Entwistle et al. 2018; Zak et al. 2019) may contribute to build up of larger C stocks. These examples are from temperate ecosystem types, and it remains to be elucidated whether the same responses to N addition occur also in boreal forests and which fungi are involved.

1.9 The central question – is all nitrogen the same?

It is clear that both endogenous and anthropogenic N affect the composition and functioning of ectomycorrhizal communities in boreal forests. However, the varying direction of responses along natural fertility gradients and in N amended systems highlights the need to better understand the context dependency of the relationships.

In this thesis, two alternative hypotheses that could explain how “ectomycorrhizal performance” changes in response to increased N availability and also explain discrepancies among previous studies were be tested (Figure 4). Ectomycorrhizal domination in forests is maintained by positive feedbacks on plant species composition (Bennet et al. 2017). By facilitating organic nutrient cycling (Phillips et al. 2013), the ectomycorrhizal symbiosis maintains N limitation in the system and thereby supress non-ectomycorrhizal vegetation (Northup et al. 1995; Franklin et al. 2014). The concept of ectomycorrhizal performance, thus, relates to the capacity to maintain the boreal forest in an N limited state while enabling exploitation of organic nutrient pools and thereby provide a positive plant-soil feedback.

In this thesis, I have used a combination of ectomycorrhizal biomass measurements, characterization of community composition and assays of related enzymatic activity to make an aggregated estimate of ectomycorrhizal performance. Particularly enzymatic activities of manganese peroxidase, β-1,4-*N*-acetylglucosaminidase and acid phosphatase are tightly linked to the functional performance of the ectomycorrhizal community in dominant low-fertility boreal forests (Kyaschenko et al. 2017a; Sterkenburg et al. 2018; Pérez-Izquierdo et al. 2021). Members of the ectomycorrhizal fungal genus *Cortinarius* (Lindahl et al. 2021) as well as other genera with medium- or long-distance exploration type mycelium (Clemmensen et al. 2015) have been highlighted as playing a central role as facilitators of organic matter turnover.

The alternative hypotheses are:

(a) Increased N availability, regardless of source, has the same effect on ectomycorrhizal performance. Increased N availability would have positive effects in nutrient poor ecosystems. However, there is a threshold in N availability above which additional increases in N supply have negative effects on ectomycorrhizal performance.

(b) Endogenous N supply is always positively correlated with ectomycorrhizal performance. Anthropogenic N, on the other hand, has a negative effect.

The rationale for hypothesis (a) is that ectomycorrhizal performance would depend on the balance between ectomycorrhizal demand for N and C. The optimum for ectomycorrhizal performance would be reached at fungal N-C co-limitation where the increased N supply is perfectly matched by host C investment. In this scenario, studies that have observed positive correlations between ectomycorrhizal fungal activity and N availability, mostly in natural fertility gradients would be located below the optimum. In contrast, in studies where negative effects of external N addition were observes, increased N anthropogenic supply would have pushed the system past the threshold for N-C co-limitation and the fungi are negatively affected by decreased allocation of C to ectomycorrhizal symbiosis.

Meanwhile, the rationale for hypothesis (b) is that the effects of endogenous and anthropogenic N on ectomycorrhizal performance are fundamentally different. In natural systems, plant growth, ectomycorrhizal performance and N cycling would be positively correlated. However, the positive relations among these factors would be disrupted by external N addition.

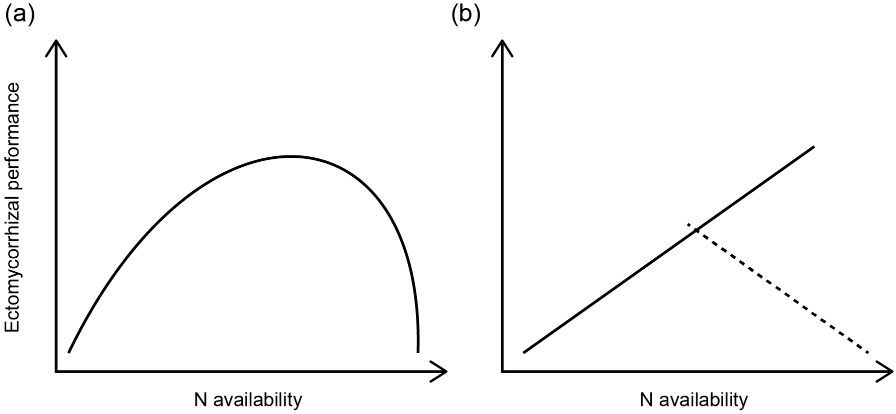

Figure 4. Alternative hypotheses for the response of ectomycorrhizal fungi to N availability. (a) A consistent unimodal response of the performance (biomass, community composition, enzymes) in response to N, regardless of N source. (b) Positive relationship between ectomycorrhizal performance and endogenic N availability (solid line) and negative relationship with exogenic N supply (dashed line).

2. Objectives

The main objective of this thesis is to better understand how fungal communities, especially ectomycorrhizal fungi, respond to differences in N availability in boreal forests. It aims to investigate whether effects of increased N availability are the same along natural fertility gradients (Paper IV) as in forests where N availability is increased through fertilization (Paper II) or atmospheric deposition (Paper IV). Further, effects of N on ecosystem functioning, either direct or mediated by the fungal community, are investigated in the context of C storage and forest management (Paper I & II). In addition, morphological differences among ectomycorrhizal fungal genera and phenotypic responses to variation in N availability are investigated (Paper III).

The specific objectives were to:

I. Quantify ecosystem carbon stocks after long-term thinning and fertilization with nitrogen and phosphorus (Paper I).

II. Evaluate whether forest management-induced changes in soil carbon storage were related to fungus-mediated mechanisms (Paper II).

III. Find out whether previously proposed ectomycorrhizal exploration types correspond to observed soil colonization patterns, and whether such colonization patterns vary across a nutrient availability gradient (Paper III).

IV. Investigate whether effects of increased N availability are the same along natural fertility gradients as in forests amended with N through fertilization or atmospheric deposition (Papers II and IV).

3. Methods

3.1 Soil sampling

The work in this thesis relies largely on correlations between and among biotic and abiotic factors and aims to test hypotheses and make postulations on an ecosystem scale. Since boreal forest soils display a high degree of spatial heterogeneity, this aim requires a sampling strategy that represents the ecosystem scale. To achieve this, 25 soil cores (3 cm in diameter) were sampled in a grid pattern across a 20×20 m plot and pooled into one composite sample used for all subsequent analyses (Paper I, II and IV). In Paper I and II, only the organic topsoil was sampled. This strategy allowed for quantification of the C stock in this horizon.

Since soil types were more varied in Paper IV, the organic topsoil plus 7 cm of mineral soil was sampled. This less quantitative sampling method was chosen to acquire an equal representation of the fungal communities at the sites.

3.2 Metabarcoding of fungal communities

Early studies of fungi relied on fruit body observations or morphological characteristics of isolated fungi growing in pure culture or of ectomycorrhizal root tips. In addition to being time-consuming, these methods overlooked a substantial part of fungal diversity since not all fungi grow fruit bodies or are culturable. New methods, based on molecular identification of genetic markers, have enabled us to explore the diversity of fungi in soils more deeply.

In this thesis, I used metabarcoding of the internal transcribed spacer (ITS) region on the high-throughput sequencing PacBio Sequel platform (Pacific Biosciences, California Inc.) to characterize the fungal communities. The internal transcribed spacer ITS region is a universal fungal barcode (Schoch et al. 2012) that consists of two regions with high interspecific variation (ITS1 and ITS2). ITS regions are flanked by highly conserved regions suitable for designing universal primers that can be used for polymerase chain reaction (PCR). In this thesis, the primer combinations gITS7-ITS4+ITS4a (Paper II & IV) and fITS7-ITS4 (Paper III) with unique identification tags in both primer ends were used (Ihrmark et al. 2012; Clemmensen et al. 2016). They amplify relatively short fragments (250-400 bp) that span the ITS2 region (Schoch et al. 2012).

The PacBio Sequel sequencing platform was chosen because of its rather low discrimination against long marker fragments compared to other available methods (Castaño et al. 2020). However, the sequencing depth is lower than for other platforms but it was not a problem for this work, which focuses on larger-scale trends that are more likely to be observable in the more dominant taxa than in the rare ones.

Primer selection is pivotal in metabarcoding studies, since primers may be more or less specific and differ in their ability to amplify the fungal community in a representative way. The inclusion of ITS4a also captures the recently described class *Archaeorhizomyces* (Rosling et al. 2011)*,* which can make up quite a substantial part of fungal reads from soil samples. In Paper III, the objective was to focus on the ectomycorrhizal community composition. Therefore, the more specific fITS7 was used together with ITS4 to obtain a more targeted amplification of relevant taxa.

After sequencing, the raw sequence reads were analysed using the bioinformatics pipeline SCATA (Sequence Clustering and Analysis of Tagged Amplicons, http://scata.mykopat.slu.se). After an initial quality check, sequences were clustered into Species Hypotheses (Kõljalg et al. 2013) (hereafter "species") based on single linkage clustering with a threshold of 98.5% similarity to enter a cluster. All unique sequences were removed before clustering to minimize the impact of PCR and sequencing errors. The species hypotheses were identified by matching them to the UNITE database (Nilsson et al. 2019).

3.3 Fungal biomass measurements – ergosterol

In this thesis, I used ergosterol amount (Nylund & Wallander 1992; Kyaschenko et al. 2017a) to estimate fungal biomass in bulk soil. Concentrations were determined using high-performance liquid chromatography (HPLC) as described in Hagenbo et al. (2017).

Ergosterol is a compound in fungal cell membranes and is a widely used marker to estimate the amount of living fungal biomass (Nylund & Wallander 1992; Ekblad et al. 1998). Although some fungi contain no or low amounts of ergosterol, among them arbuscular mycorrhizal fungi, ergosterol extraction and quantification is considered to be a rather robust method for estimation of biomass of the major groups of fungi present in boreal forests (Baldrian et al. 2013). There are other methods to quantify fungal biomass, for instance, real-time PCR (qPCR) of ITS copies and concentrations of phospholipid-derived fatty acids (PLFAs) or chitin. Concentrations of ergosterol and the fungal PLFA 18:2ω6,9 generally correlate well (Frostegård & Bååth 1996). Real-time PCR may, however, generate more uncertain estimates since copy numbers of targeted genes may vary among species in the sample.

Ergosterol concentrations were used together with sequencing data to quantify the biomass of ectomycorrhizal fungi. These estimates were based on the assumption that the biomass of ectomycorrhizal fungi is proportional to their relative abundance in the fungal community.

3.4 Enzyme assays

Organic matter in boreal forest soils consists of compounds of varying composition and complexity. Fungi can utilize C and nutrients from it by producing extracellular enzymes that degrade larger organic molecules into smaller ones that can be taken up through the fungal cell wall. Assays of extracellular enzyme activities are a way to obtain a snapshot of potential decomposition activity at the site, and by correlating enzyme data with e.g. C stocks and fungal community composition, inference about ecosystem processes may be made. It is worth noting, however, that many enzymes can be produced by a wide range of decomposing organisms, including bacteria, and that activity estimates from bulk soil therefore represent the whole decomposer community.

Assays of extracellular hydrolytic (acid phosphatase, β-glucosidase and β-1,4-*N*-acetylglucosaminidase) and oxidative enzymes (manganese peroxidase) were based on the protocols by Daniel et al. (1994), Saiya-Cork *et al.* (2002) and Sinsabaugh *et al.* (2005) that were further developed by Kyaschenko *et al.* (2017a). In short, assays of both hydrolytic and oxidative enzyme activity were done in well homogenized slurries of soil in 50 mM sodium acetate buffer with a pH similar to soil. Soil suspensions were then incubated with enzyme specific substrates that are processed into fluorescent or coloured products if the targeted enzyme is present in the soil slurry. After incubation, concentrations of the reaction product was measured and converted to enzyme concentrations based on standard curves.

3.4.1 Hydrolytic enzymes

Hydrolytic enzymes catalyse hydrolysis of specific chemical bonds. In this thesis, I assayed hydrolytic enzymes used for decomposition of cellulose and chitin as well as P containing esters.

Decomposition of cellulose requires an array of cellulolytic enzymes that contribute to a stepwise degradation of cellulose to glucose. The last decomposition step, cleaving cellobiose into two glucose molecules, is catalysed by β-glucosidase. β-1,4-*N*-acetylglucosaminidase is involved in the final steps of degradation of chitin in fungal cell walls, and thus, plays an important role in mycelial turnover. Acid phosphatase is exuded by plants, bacteria and fungi to mobilise organically bound phosphorus in soils.

Assays of hydrolytic enzyme activities were done by adding enzyme-specific fluorogenic 4-methylumbelliferone substrates to soil slurries. After 30-90 min of incubation in the dark, fluorescence was measured on a fluorescence spectrophotometer (LS 50B, PerkinElmer Ink. Hopkinton, MA, USA) with 365 nm excitation and 450 emission wavelengths. For each sample, a negative control was included to account for quenching by adding the fluorogenic substrate to the soil slurry and measure fluorescence immediately.

3.4.2 Oxidative enzymes

Oxidative enzymes are less specific than hydrolytic enzymes and can decompose non-hydrolysable compounds in low quality organic matter. I measured the activities of manganese peroxidase, which is a Class II peroxidase unique to agaricomycete fungi (Floudas et al. 2012). Although being predominantly produced by saprotrophic fungi, it can also be produced by some ectomycorrhizal genera such as *Cortinarius* (Bödeker et al. 2014). The enzyme oxidizes Mn^{2+} to Mn^{3+}, which in turn oxidizes organic matter. The enzyme requires H_2O_2 as an electron acceptor, which is also produced and exuded by the fungus, in an energy demanding process.

In the assay, 3-dimethylaminobenzoic acid (DMAB) and 3-methyl-2-benzothiazoline hydrazine hydrochloride (MBTH) was added to the soil slurry together with different combinations of ethylenediaminetetraacetic acid (EDTA), $MnSO_4$ and hydrogen peroxide (H_2O_2) (Table 1). An inhibition control with commercial horseradish peroxidase (Paper II) (Pérez-Izquierdo et al. 2021) or recombinant manganese peroxidase from *Phanerochaete chrysosporium* (CAS number 114995-15-2, Sigma-Aldrich, Darmstadt, Germany) (Paper IV) was also included.

Oxidation of DMAB and MBTH results in an indamine dye, and absorbance at 590 nm was measured at 3 min intervals during 45-60 min in a SpectraMax Plus 384 microplate reader (Molecular Devices, Sunnyvale, CA, USA). Absorbance was converted to activities of enzyme (µU gOM^{-1}) by using standard curves based on the commercial peroxidases. The difference in concentration between the different combinations (Table 1) of EDTA, $MnSO_4$ and H_2O_2 were used to calculate specific oxidative mechanisms:

3-2 = Manganese peroxidase activity
2-1 = Manganese-independent peroxidase activity
3-1 = Total peroxidase activity

Table 1. *Combinations of substrates and chemicals in the manganese peroxidase assay.*

1	**2**	**3**	**4**
Soil slurry	Soil slurry	Soil slurry	Soil slurry
DMAB MBTH	DMAB MBTH	DMAB MBTH	DMAB MBTH
EDTA	EDTA H_2O_2	$MnSO_4$ H_2O_2	$MnSO_4$ H_2O_2 Commercial peroxidase

3.4.3 Methodological considerations – enzyme assay

In this thesis, I used composite samples from 25 soil cores collected across 20×20 m plots to measure enzyme activities and relate them to fungal community composition, site fertility, or ecosystem C stocks that were scaled up from the same composite sample. This approach generates estimates of average activities at a plot scale at the time of sampling. However, forests soils are highly patchy, and decomposers are heterogeneously distributed. Consequently, activities of extracellular enzymes would, too, be expected to be patchy across a forest plot. In a temperate forest, enzyme activities varied substantially between single soil cores (4.5 cm in diameter) collected within a 36 m^2 plot (Stursova et al. 2016). Enzyme activities also vary vertically across different horizons in temperate (Šnajdr et al. 2008) and boreal (Kyaschenko et al. 2017b; Sterkenburg et al. 2018) forests. In addition, there may also be differences between seasons (Voříšková et al. 2014). Given this, a composite sample may give a more representative measure of enzymatic activity at a stand scale but it entails a risk of obscuring enzymatic heterogeneity.

Small scale heterogeneity (perhaps even within mm of soil) may cause problems for ecosystem-scale measurements since very high enzymatic activity in a small fraction of soil could lead to overestimations of the average activity at a site. To avoid this risk, proper sample subsampling is needed when doing the enzyme assays.

To investigate the importance of appropriate subsampling for measurements of enzyme activities, I did a small scale trial using two different humus soils that had been homogenized in the same way as the soils in Paper II. Previous work has often been based on soil slurries made from a small soil subsample (around 1 g). The same method was used in Paper II.

Slurries were prepared from four subsamples from each soil at an initial concentration of 2 g of organic matter in 50 ml acetate buffer and diluted to a final concentration of 0.001 gOM ml^{-1}. I measured activities of manganese peroxidase and β-glucosidase and assessed the variation between subsamples taken from the same composite soil sample. Activities of manganese peroxidase varied considerably between replicates (Figure 5a) while β-glucosidase activities were more consistent (Figure 5b). From this, I concluded that a 2 g subsample was not enough to even out the influence of "enzymatic hotspots". Consequently, the approach for Paper IV was to increase the amount of soil to make the soil slurry to 5 gOM in 500 ml acetate buffer before diluting it to a final concentration suitable for the assay.

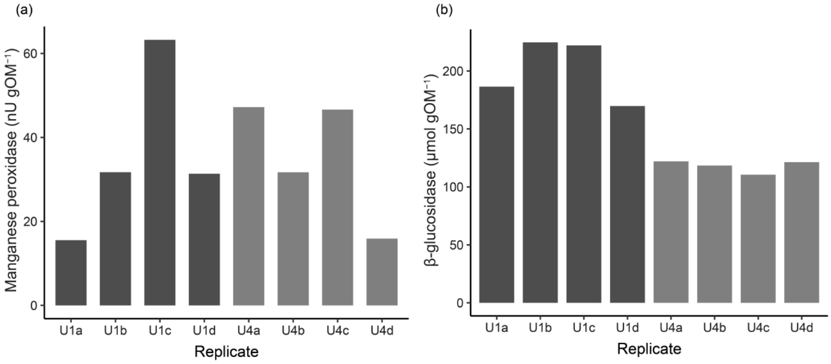

Figure 5. Activities of (a) manganese peroxidase (nU gOM^{-1}) and (b) β-glucosidase (μmol gOM^{-1}) from a resampling test with four biological replicates from the organic topsoil of two *Picea abies* forests in central Sweden. Activites of enzymes were measured from 2 g of freshly frozen soil suspended in 50 ml acetate buffer (pH = 5) and diluted to a final concentration of 0.001 gOM ml^{-1}. Measurements of absorbance (manganese peroxidase) and fluorescence (β-glucosidase) were done in four technical replicates per sample.

3.5 Statistical methods

In Paper I, II and III, linear mixed effect models were used. Linear mixed effect models are appropriate when evaluating data from experiments with nested designs, i.e. where samples are not independent. Examples of non-independence are when multiple samples are taken from the same location or if a site is repeatedly sampled over time. The models can evaluate “fixed effects” of explanatory variables, such as treatments or environmental factors, while accounting for “random factors” related to non-independence. Another advantage is that linear mixed effect models are good at handling missing data and unbalanced experimental designs. In this thesis, the R (R Core Team 2020) package lme4 (Bates et al. 2015) was used to fit linear mixed effect models.

When performing a large number of statistical tests, the risk of detecting false positives (Type I errors) increases. This risk can be decreased by correcting for multiple testing (Noble 2009). In this thesis, the Benjamini-

Hochberg method (Benjamini & Hochberg 1995) was used. In short, the procedure divides the P-values into percentiles by sorting them in ascending order and the adjustment is made by dividing the P-value by its percentile rank (Noble 2009). This method is less conservative than the widely used Bonferroni correction, which is performed by multiplying P-values by the number of tests performed. The Bonferroni method may, due to its strictness, increase the rates of false negatives (Type II errors) (Perneger 1998). P-value corrections were performed using the emmeans package (Lenth et al. 2021) in R.

In Paper IV, the experimental design allowed for statistical testing without inclusion of a random factor. I used the mvabund package (Wang et al. 2012) in R to fit individual generalized linear models with negative binomial linking functions to all fungal species in the fungal community dataset.

Linear modelling is appropriate to use when the response of a single factor is investigated, but to analyse more complex data, multivariate methods may be more useful. In this thesis, Canoco 5 was used for multivariate statistical analyses. Correspondence analysis (CA) is an ordination method that relies on reciprocal, weighted averages of species and sample scores and is appropriate for analyses of species abundance data, since it can handle unimodal responses in community gradients and scattered occurrence of species (Ramette 2007). Canonical correspondence analysis (CCA), an extension of CA, constrains the ordination to be linearly dependent on explanatory variables, e.g. environmental parameters. Monte Carlo permutations were used to test whether the constrained ordination accounted for a statistically significant share of community variation. Additionally, the permutation parameters can also be adapted to a hierarchical experimental setup, making CCA versatile for many ecological experiments (Smilauer & Leps 2014).

Relationships between sampled communities can be visualized using detrended correspondence analysis (DCA). When plotting CA scores in ordination space, an artificial arch effect (horse shoe pattern) often emerges, particularly if one of the underlying gradients is much stronger than the others. By detrending the data, this arch can be removed, while keeping relations among species and samples, and better visualisation of the community in relation to explanatory variables can be obtained (Ramette 2007).

4. Project descriptions

4.1 Paper I and II: Impact of forest management on carbon stocks and fungal communities

The aims of these papers were to evaluate the effects of different forest management practices on C storage (Paper I) and fungal community composition and functioning (Paper II). This was done in a long term field experiment in 29 *Pinus sylvestris* forests distributed along a 1300 km gradient in Sweden (56.4-67.5°N). At each site, different combinations of thinning and fertilization, without within-site replication of treatments, had been implemented in 0.1 ha plots since the establishment of the experiment (1966-1980) (Nilsson et al. 2010; Bergh et al. 2014). At all 29 sites, four treatments were selected: "No thinning", "Thinning", "Thinning + N" and "Thinning + NP", and in 9 of the 29 sites, an additional treatment of "No thinning + N" was included in the study, generating a total plot number of 125. In summer 2016, 25 soil cores from the organic topsoil were sampled and pooled into one composite sample in all 125 plots. Living parts of mosses and roots coarser than 2 mm were removed before pooling. The soil samples were used to quantify C stocks, measure the C/N ratio and pH, extract ammonium and nitrate (Paper I). In addition, they were used to characterize the fungal community, analyse enzyme activities and measure ergosterol content to determine fungal biomass (Paper II).

4.1.1 Paper I: Ecosystem carbon stocks

The aim of Paper I was to quantify the effects of different forest management scenarios on C stocks in trees and organic topsoils. Throughout the experimental period, the Swedish Infrastructure for Ecosystem Science (SITES) has monitored the growth of trees in the plots (Figure 5). This data was used to estimate C stocks in tree biomass both in standing trees and stems that were removed in thinning operations by applying allometric functions (Laasasenaho 1982; Marklund 1988; Petersson & Ståhl 2006). Carbon stocks in the organic topsoil were determined from the composite sample collected in 2016. Concentrations of extractable inorganic N (ammonium and nitrate) were measured to evaluate signs of N saturation and, thus, risk of leaching of N. To investigate potential mechanisms for changes in belowground C stocks, soil CO_2 efflux was estimated in summer 2017 by measuring soil respiration at ten positions in all plots except the ones subjected to the "Thinning + NP" treatment. Changes in C stock were evaluated over an average experimental period of 40 years, and effects of the different management practices were evaluated with linear mixed-effect models.

Figure 6. Pinus sylvestris stand in the fertilization study. Individual trees in a plot are marked, and growth has been monitored regularly during the experimental period. Photo: Joachim Strengbom

4.1.2 Paper II: Fungal mediated regulation of soil carbon stocks

Paper II investigated the potential role of fungi in regulating C stocks in the organic topsoil under different forest management scenarios. The study was based on metabarcoding of the fungal community using PacBio Sequel sequencing of ITS2 markers. Bioinformatics was performed using the SCATA pipeline, and sequences were clustered into species hypotheses and taxonomically assigned by matching with the UNITE database. Management effects were evaluated on species, functional guild and community levels. To assess the impact of forest management on the functioning of the fungal community, fungal biomass was estimated by ergosterol analysis, and assays of activities of β-1,4-*N*-acetylglucosaminidase, β-glucosidase, acid phosphatase and manganese peroxidase were performed. In addition, correlations between potential regulators (standing tree biomass, fungal biomass, relative abundances of fungal guilds or *Cortinarius* species) and C stocks in the organic topsoil were evaluated. The overall effects of the different management practices on fungal community composition were tested with CCA and graphically presented with DCA. The impact of forest management on the relative abundance of individual species and guilds was evaluated using linear mixed-effect models. The interaction with latitude was included in the models to investigate whether treatment effects were similar across the whole latitudinal transect. Correlations between C stocks and potential regulatory factors were also evaluated with linear mixed-effect models.

Paper II specifically tested the hypotheses that increased C stock following fertilization would be linked to (1) higher mycelial biomass, an effect that (2) would be reduced by thinning. Alternatively, fertilization and increased C stocks would be linked to (3) decreased abundance of ectomycorrhizal fungi, particularly *Cortinarius* species as well as (4) decreased activity of decomposer enzymes, especially fungal peroxidases. Hypotheses (1) and (2) assume that increased C stock is an effect of increased input of persistent litter (here mycelial necromass), whereas hypotheses (3) and (4) assume that increased C stock primarily is due to down-regulated decomposition linked to reduced ectomycorrhizal N-mining.

4.2 Paper III: Ectomycorrhizal traits and nutrient foraging

The main objective of Paper III was to test whether pre-assigned ectomycorrhizal exploration types (Agerer 2001) could be used to predict patterns of substrate colonization. Further, signs of morphological variation within ectomycorrhizal genera were evaluated across different levels of nutrient availability in the soil.

During the growing season of 2019 (May-November), ingrowth-bags (Wallander et al. 2001) made from 8×10 cm nylon with a mesh size of 50 µm (Sintab AB, Oxie, Sweden) were incubated in ten mature *Picea abies* forests with different pH and levels of N availability in central Sweden (59.3-60.6°N). The small mesh size allowed ingrowth of fungal mycelium but excluded roots, enabling specific sampling of extra-radical mycelium of ectomycorrhizal fungi. The bags were filled with sand or forest soil. Soil bags were filled with four different soil types with different C and N concentrations, inorganic N availability and pH, and some sand bags were amended with apatite as a source of P. The bags were buried in five replicated "cafeterias" at each of the ten forest sites. The cafeterias were one metre across and contained all bag types. At the end of the incubation period, two soil cores were taken from the middle of each cafeteria, one for measuring pH and inorganic N concentration and one from which ectomycorrhizal roots were isolated. Characterization of the ectomycorrhizal community in bags and on roots was conducted by metabarcoding of ITS2 markers on the PacBio Sequel platform. Sequences were clustered and assigned to species hypotheses using the SCATA pipeline in combination with the UNITE database. Preference for roots *versus* bags, soil bags *versus* sand bags and the different soil substrates were evaluated on a genus level and tested using linear mixed-effects models with relative abundance of an ectomycorrhizal fungal genus as the response variable, substrate type as an explanatory variable and cafeteria nested within site as a random factor. The effect of differences in inorganic N availability and pH on colonisation patterns were evaluated by including an interaction term between pH or N availability and substrate type in the models.

Specifically, Paper III tested the hypotheses that:

- Contact exploration types would not extensively colonise bags.
- Short and medium smooth exploration types would be prolific in all substrates.
- Medium fringe, medium mat and long exploration types would selectively colonize soil-filled bags and proliferate more extensively in soil than other exploration types.

Figure 7. Ingrowth mesh bags filled with different substrates used in the cafeteria experiment (Paper III). The bags were made from 8×10 cm nylon with a 50 µm mesh size. Photo: Karolina Jörgensen

Figure 8. Ingrowth bags after six months of field incubation in mature *Picea abies* forests in central Sweden. Mycelial ingrowth is clearly visible in the bags. Photos: Karolina Jörgensen

4.3 Paper IV: Fertility gradients in regions with low and high nitrogen deposition

In Paper IV, I selected mature *Picea abies* forests along fertility gradients (Figure 9) in two regions in Sweden, in the southern part of the boreal region (60°N) and the northern part of the nemoral region (56-57°N). The regions were selected since N deposition rates have historically been, and still are, higher in the nemoral region than in the boreal region of Sweden (Figure 2). Thus, the experimental setup allowed for a discussion of the effects of different nitrogen sources (endogenous vs. anthropogenic) on the variation in ectomycorrhizal communities, fungal biomass and enzyme activities. While differences between the regions may be conditional on several confounding factors, such as climate and land-use history, N deposition is likely one of the major drivers of differences between the selected forests. Nitrogen deposition has been identified as one of the main controls on ectomycorrhizal fungi, across systems where tree host is the same (van der Linde et al. 2018).

In autumn 2018, 29 forests were sampled (14 in the boreal region and 15 in the nemoral region) by collecting 25 soil cores with a diameter of 3 cm in a grid pattern across 20x20 m plots and the cores were pooled into one composite sample per site. Sampling depth spanned the organic topsoil plus 7 cm of mineral soil (or down to rock). Samples were used to characterize ectomycorrhizal fungal biomass and community composition by metabarcoding of ITS2 markers using PacBio Sequel sequencing, as well as for estimates of inorganic N availability, total C and N concentrations, and analyses of enzyme activities.

The gradients spanned largely overlapping ranges in N availability (N/C ratio and inorganic N), and host species and developmental stage were the same in both regions. Soil pH in the boreal region correlated positively with N availability and was on average 4.7 (min 4.0, max 6.1). In the nemoral region, pH was less variable (average, 4.2, max 4.5, min 4.0) and did not correlate with N availability. Likely, atmospheric deposition in the nemoral region has disrupted the otherwise naturally occurring positive correlation between N availability and pH (Sterkenburg et al. 2015). Since the gradients are un-replicated, the results will have to be confirmed across more regions with varying N deposition.

The colonization patterns of fungal genera in the cafeteria experiment (Paper III) were used to classify ectomycorrhizal genera in the fungal communities as either fast or slow growing (referring to the formation of extraradical mycelium relative to root colonisation). Effects of N/C-ratio and inorganic N availability in the soil on the relative abundance of these "subguilds" was evaluated. Responses to N availability were evaluated with linear models with relative abundance of ectomycorrhizal fungi and fast *vs.* slow growing taxa as response variables and N/C-ratio, inorganic N and their interaction with "Region" as explanatory variables. In addition, I tested the responses to N availability of a "key ectomycorrhizal fungal community". The key community consisted of 25 genera that were present at 10 or more sites. In total, they made up on average 96% of the mycorrhizal sequence reads (70-100%, median 98%). The responses of these genera were tested by fitting individual generalized mixed linear models to them using the manyglm function in mvabund (Wang et al. 2012). Models were fitted using a negative binomial linking function and assessed by ANOVA with 1000 bootstraps. P values were adjusted for multiple testing and were considered to be significant if $P \leq 0.05$.

In Paper IV, the hypothesis that responses of ectomycorrhizal fungal biomass, community composition and associated enzyme activities would differ between the two regional fertility gradients was tested. Ectomycorrhizal fungi were expected to be negatively related to N availability in the region with high N deposition but positively or weakly related in the low N deposition area.

Figure 9. Examples of study sites in the gradient study in Uppland (top row) and Skåne/Halland (bottom row). Nitrogen availability increases towards the right. Photos: Karolina Jörgensen

5. Results and Discussion

5.1 Ectomycorrhizal soil foraging patterns

The aim of Paper III was to test the assumption that ectomycorrhizal exploration types (Agerer 2001) generally correspond to patterns of soil foraging under field conditions. Exploration types and ectomycorrhizal fungal genera showed different preferences for roots *vs.* ingrowth bags and sand *vs.* soil in the ingrowth bags (Figure 10).

The contact type ascomycete *Hyaloscypha* displayed colonization patterns in accordance with the hypothesis *i.e.* with little extra-radical growth. In contrast, *Lactarius* and *Russula* were equally abundant in bags and on roots, although with a strong preference for soil-filled over sand-filled bags. Thus, the production of fine emanating hyphae may be extensive, which implies that contact type ectomycorrhizae may be more active in nutrient uptake than previously assumed.

The results were generally well aligned with the hypothesis that ectomycorrhizal fungi with short distance and medium distance smooth exploration type mycelium would be prolific soil colonizers and therefore abundant in ingrowth bags. The same colonization patterns were also hypothesized for medium distance fringe types, with the addition that they would preferentially colonize soil-filled bags over sand-filled bags because of their presumed role in mining N from organic matter. Interestingly, genera with medium distance fringe exploration type mycelia did not behave as hypothesized, nor did they display similar colonization patterns to each other. *Amphinema* behaved similarly to the short and medium distance smooth types and were highly abundant in bags, with a preference for sand-filled bags. In contrast, *Cortinarius* and *Piloderma* were poor colonizers of bags, although, *Piloderma* displayed some preference towards soil-filled bags.

While the formation of hydrophobic rhizomorphs could be a strategy to efficiently direct nutrient acquisition towards patches of organic matter, it is not known to what extent genera with this trait actually mine for organically bound N. *Cortinarius* species do possess oxidative capacity (Bödeker et al. 2009) and are associated with organic matter turnover (Lindahl et al. 2021). However, the ability of *Piloderma* to mine for organically bound N is unknown, although they do produce some class II peroxidase-like enzymes (Shah et al. 2016). The generality of N mining capacity of medium distance fringe exploration types would have to be further studied.

One way for ectomycorrhizal fungi to cope with changes in the environment is by displaying phenotypic plasticity, e.g. make adaptations in morphology. Ectomycorrhizal species have been shown to be able to alter hyphal growth, including the formation of rhizomorphs, in response to abiotic variables such as pH or N deposition loads (van der Linde et al. 2018). In Paper III, *Cortinarius* colonization of roots was higher as N availability and pH increased (Figure 11a-b) while proliferation in soil did not change. Similar patterns were observed for *Lactarius* in response to increased pH (Figure 11c). *Cenococcum* and *Hyaloscypha* seemingly colonized roots to a lesser extent as pH increased, but the latter was more prolific soil colonizers at high pH (Figure 11d-e). Further, *Pseudotomentella* increased in abundance in sand bags, but not in soil bags, at higher pH. These observations could indicate phenotypic plasticity. Alternatively, since the analyzes were done on a genus level, shifts along the pH or inorganic N gradients could also be due to species turnover within the genera and rather point towards shifts in community composition at the species/genotype level.

Based on Paper III, the usefulness of ectomycorrhizal exploration types as traits that denote soil foraging patterns can be questioned as many of the patterns observed in the experiment were not general across exploration types. Possibly, other traits are more important for soil exploration and nutrient foraging.

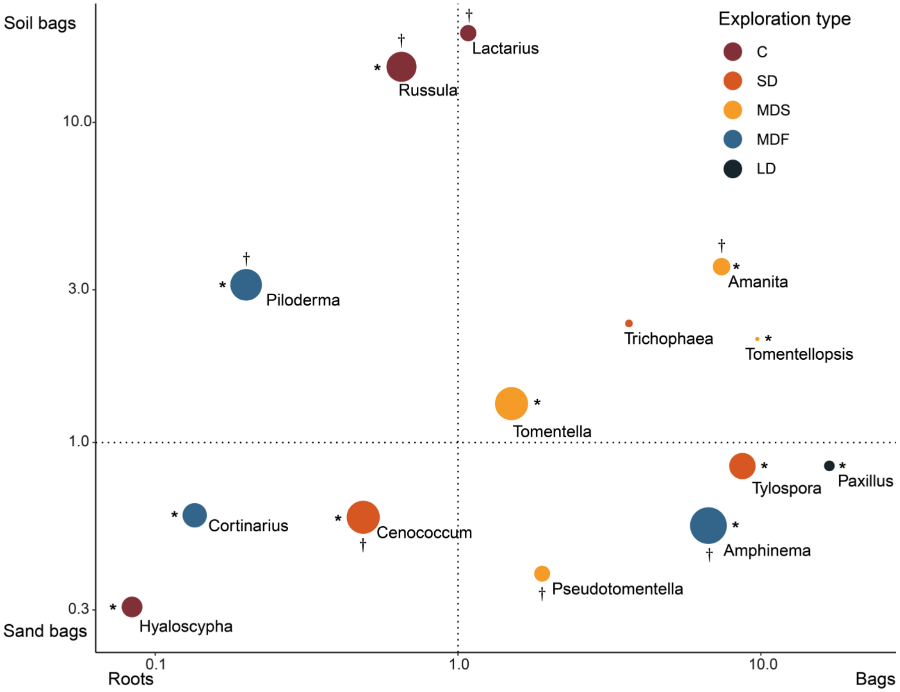

Figure 10. Modelled abundance ratios of ectomycorrhizal genera in roots *vs* bags (x-axis) and in soil bags *vs.* sand bags (y-axis). To normalize the scales of the two axes, relative preference was calculated by dividing the model estimate by the intercept of the mixed effect linear models for comparisons of non-transformed relative abundance on roots and in ingrowth bags and in soil- and sand bags. Point size corresponds to adjusted relative abundance of the ectomycorrhizal genera. Abbreviations stand for: C = contact, SD = short distance, MDS = medium distance smooth, MDF = medium distance fringe and LD = long distance. Symbols (* for root *versus* ingrowth meshbag comparison, † for soil- *versus* sand bag comparison) next to points denote significant (Benjamini-Hochberg adjusted p-value≤0.05) from mixed effect linear models. Position of symbols relative to points show direction of the effect. Dashed vertical and horizontal lines denote equal abundances in bags and on roots, and in soil bags and sand bags, respectively. Axis scales are log-transformed.

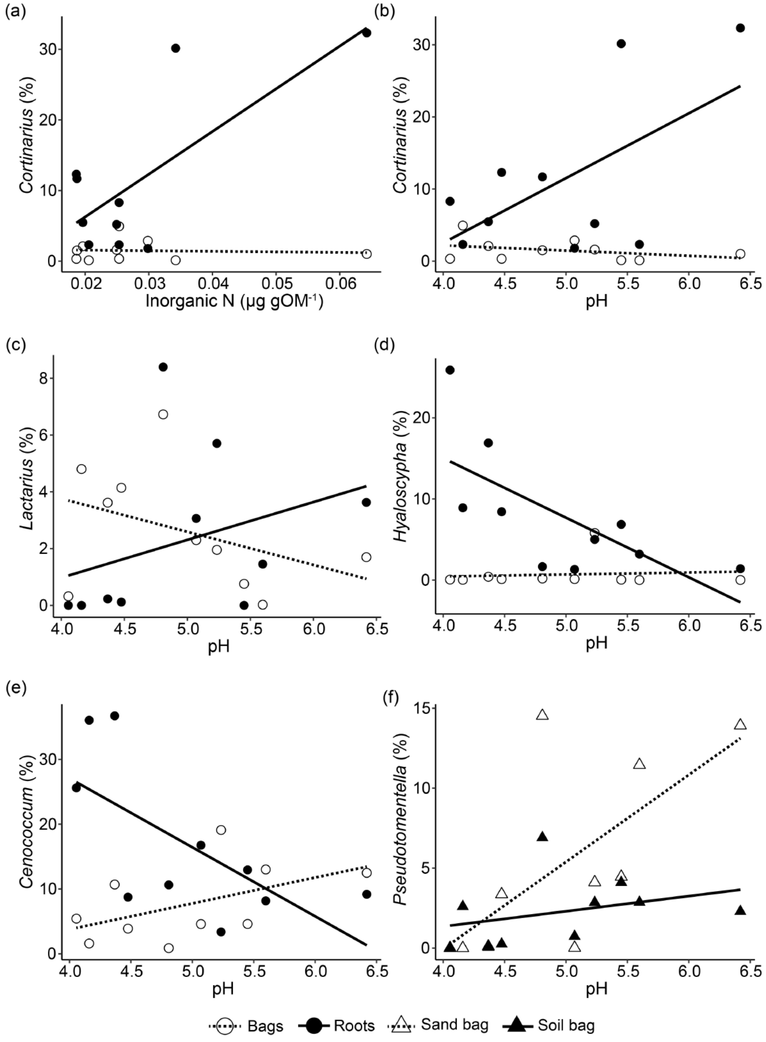

Figure 11. Phenotypic plasticity of ectomycorrhizal genera in response to inorganic N and pH. Abundance (percent of ectomycorrhizal reads from PacBio Sequel sequencing of ITS2 markers) of *Cortinarius* in bags and on roots across gradients of inorganic N availability (a) and organic topsoil pH (b), abundance of *Lactarius* (c), *Hyaloscypha* (d) and *Cenococcum* (e) in bags and on roots plotted against pH and abundance of *Pseudotomentella* in sandbags and soil bags across organic topsoil pH (f). These genera displayed phenotypic plasticity ($P \leq 0.05$) in linear mixed-effect models that included an interaction between substrate type and site fertility indicators. Lines are fitted regression lines.

5.1.1 Could ectomycorrhizal colonization patterns relate to carbon use efficiency?

Across ecosystems, ectomycorrhizal symbiosis has been proposed to be a strategy that maintains N limitation in boreal forests and, thus, favour trees that form ectomycorrhiza relative to non-ectomycorrhizal species (Näsholm et al. 2013; Franklin et al. 2014; Henriksson et al. 2021). The C that trees invest in their fungal partners is used for fungal biomass production and to fuel nutrient foraging and transport. The C cost for maintaining the symbiosis may vary, since ectomycorrhizal fungi possess different morphological and physiological traits and thereby differ in their ability to deliver nutrients back to their host.

Ectomycorrhizal fungi could therefore be considered more or less expensive for trees to associate with, depending on how much C is invested in them and what the C is used for. The concept of "apparent CUE (CUE_A)" could be helpful to disentangle under which circumstances some ectomycorrhizal species would be more beneficial than others to associate with. From a fungal oriented view, CUE_A would be defined as the proportion of assimilated C that remains as mycelial biomass, without explicitly taking into account the processes that regulate outputs of C (Manzoni et al. 2018). Thus, it does not encompass biomass turnover, which could be central in the functioning of ectomycorrhiza, but is instead based on standing biomass. In boreal forests, ectomycorrhizal mycelia can turn over between one and seven times per year (Hagenbo et al. 2017), and turnover rates are likely related to the composition of the fungal community (Kyaschenko et al. 2017a).

In Paper III, colonization and proliferation of ectomycorrhizal mycelia in different substrates was not clearly linked with ectomycorrhizal exploration types. Potentially, the results could, instead, be related to the CUE_A of genera that colonized the ingrowth bags. On one hand, ectomycorrhizal fungi that form more extensive mycelia could be expected to be more costly for the host since they need to sustain mycelial production while accessing nutrients from the soil (Finlay & Read 1986; Leake et al. 2001; Rosling et al. 2004; Lilleskov et al. 2011). However, the rapid production of extraradical mycelium of *Amanita*, *Amphinema*, *Paxillus* and *Tylospora*, despite the relatively low abundance on ectomycorrhizal root tips, implies that growth can be maintained at low C cost. Hence, these genera could have high CUE_A. While the large mycelial biomass would likely cause substantial N retention and aggravate nutrient limitation in trees (Näsholm et al. 2013), this may still be a beneficial strategy for trees in the long run, since N is kept within the ectomycorrhizal system and may be utilized later. This would, for instance, be beneficial in systems with moderate rates of inorganic N input (Bahr et al. 2015).

Cortinarius and *Piloderma*, which had low standing biomass despite a presumably high C flow from their host, would have a low CUE_A. Likely, these rhizomorph-forming genera explore the soil for patches of organic matter by extending individual hyphae into the soil matrix. When a resource patch is found, they may be able to redirect resources to it by turning over "old" biomass and form a cord that can redistribute acquired nutrients (Finlay & Read 1986), similarly to the strategy of saprotrophic cord forming fungi (Boddy 1999). I think that particularly *Cortinarius* benefits from such a strategy since their oxidative decomposition is energetically demanding and therefore would only be favourable if used at nutrient containing microsites in the heterogeneous soil environment. Consequently, that would cause the oxidation to be strongly patchy and restricted only to these hotspots for decomposition activity. This tentative strategy would, however, need to be tested in future research.

5.2 Effects of nitrogen on fungal communities

5.2.1 Fungal biomass

In Paper IV, total fungal biomass tended to correlate negatively (albeit non-significantly) with N/C (Figure 12a, P=0.08) and inorganic N (Figure 12b, P=0.08) across *Picea abies* stands in both regions. However, it correlated negatively with inorganic N in the nemoral region (P=0.016) when the regions were tested separately. The opposite response of fungal biomass to increased N availability was observed in *Pinus sylvestris* forests in Paper II. Repeated N fertilization, totalling an average of 881 kg N ha^{-1} during 40 years, instead stimulated fungal biomass, and the greatest increase occurred in non-thinned N fertilized stands. Additions of P together with N did not have any additional effect on fungal biomass. The stimulatory effect of N fertilization was most pronounced at higher latitudes (Figure 13a), where the N/C ratio was the lowest (Figure 14), suggesting N limitation on ectomycorrhizal growth in low fertile soils (Högberg et al. 2021).

5.2.2 Ectomycorrhizal abundance and biomass

In Paper IV, ectomycorrhizal abundance correlated negatively with the N/C ratio and inorganic N availability in the nemoral *Picea abies* gradient but it was not affected by N supply in the boreal region (Figure 12c-d). The different responses between the regions were further accentuated when accounting only for the ectomycorrhizal share of fungal biomass. Then, ectomycorrhizal biomass declined dramatically with increased N availability in the nemoral region while no relationship with N availability could be detected in the boreal region (Figure 12c-d). Similarly to Paper IV, reduced ectomycorrhizal biomass is often observed after N fertilization or in systems with elevated N deposition (Nilsson & Wallander 2003; Demoling et al. 2008; Kjøller et al. 2012; Bahr et al. 2013; Högberg et al. 2014a).

The diverging responses between regions support the idea that endogenous and anthropogenic N supply would affect ectomycorrhizal fungi differently (Figure 4b), and that anthropogenic N always has a negative effect. Just like in the study by Sterkenburg et al. (2015), ectomycorrhizal abundance remained stable across a natural fertility gradient, also at sites with high nutrient availability. This would imply that ectomycorrhizal fungi play an essential role in boreal forest fertility, analogous to how the fungal community can drive fertility in temperate forests (Mayer et al. 2021). In contrast, the decline in biomass in the nemoral region corroborates the idea that anthropogenic N plays an important regulatory role for ectomycorrhizal abundance (van der Linde et al. 2018). Increased N availability in the nemoral region is influenced by external inputs, which are likely to disrupt natural flows of C and N through the system. Trees may respond to increased N availability by decreasing C allocation to ectomycorrhizal roots (Mäkelä et al. 2008; Franklin et al. 2014; Baskaran et al. 2017), likely leading to decreased ectomycorrhizal fungal biomass. Further, N deposition with concomitant shifts in pH may cause additional stress on the fungal community.

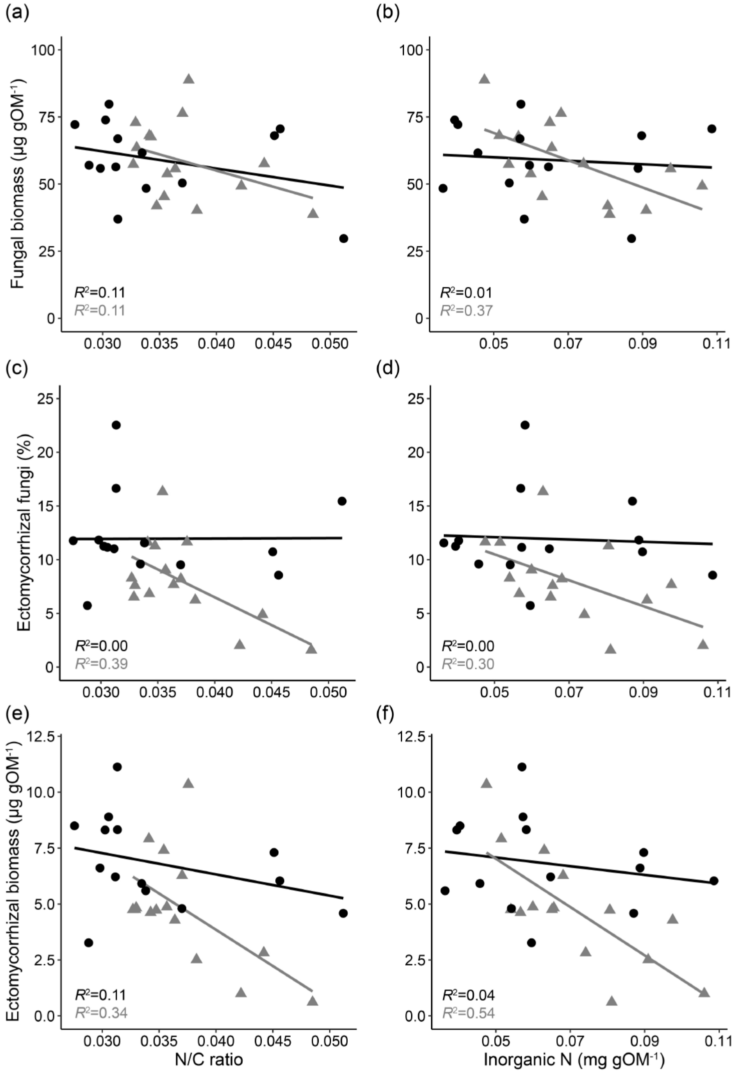

Figure 12. Concentrations of ergosterol (µg gOM-1) in the organic horizon and top 7 cm of mineral soil across gradients of (a) N/C ratio and (b) inorganic N (mg gOM-1), abundance of ectomycorrhizal fungi (% of sequence reads based on PacBio Sequel sequencing of ITS2 markers) across gradients of (c) N/C ratio and (d) inorganic N and ectomycorrhizal biomass (µg gOM^{-1}) across gradients of (e) N/C ratio and (f) inorganic N in the organic horizon and top 7 cm of mineral in boreal Picea abies forests in two regions in Sweden. Lines represent fitted linear regressions. Points and shapes denote sampled regions: Boreal (solid black circles) and nemoral (solid grey triangles).

Contrary to the results in Paper IV, increased fungal biomass after fertilization seemingly counteracted a decline in relative abundance of ectomycorrhizal fungi in Paper II (Figure 13d, Table 2), resulting in stable estimates of ectomycorrhizal biomass (data not shown). Increased total fungal biomass and sustained ectomycorrhizal biomass would support the hypothesis that increased N availability, regardless of source, is beneficial to ectomycorrhizal fungi up to a threshold where the C cost to maintain the symbiosis is likely too high for the tree host (Figure 4a) (Näsholm et al. 2013; Baskaran et al. 2017; Högberg et al. 2021). The low N/C ratio in the *Pinus sylvestris* forests could indicate strong N limitation of ectomycorrhizal funig (Högberg et al. 2021) and, like in the study by Clemmensen et al. (2006) in arctic tundra systems, alleviation of the limitation could stimulate biomass production.

Alternatively, the different effects of N fertilization and atmospheric N deposition on total fungal and ectomycorrhizal biomass in this thesis could relate to the input frequency or effects on other soil properties. While atmospheric N deposition is a continuous source of N to a system, often associated with soil acidification (Falkengren-Grerup 1987), the forest fertilization in Paper II occurred at longer intervals and did not decrease soil pH. Fertilization took place every 5-7 years, and it is possible that the relatively long time in between N pulses was enough for the ectomycorrhizal biomass to recover (Högberg et al. 2011, Högberg et al. 2014a). In contrast, continuous addition of N through atmospheric deposition may put constant stress on the ectomycorrhizal community by decreasing the soil pH and simultaneously allow trees to maintain high inorganic N uptake without investment in ectomycorrhizal fungi.

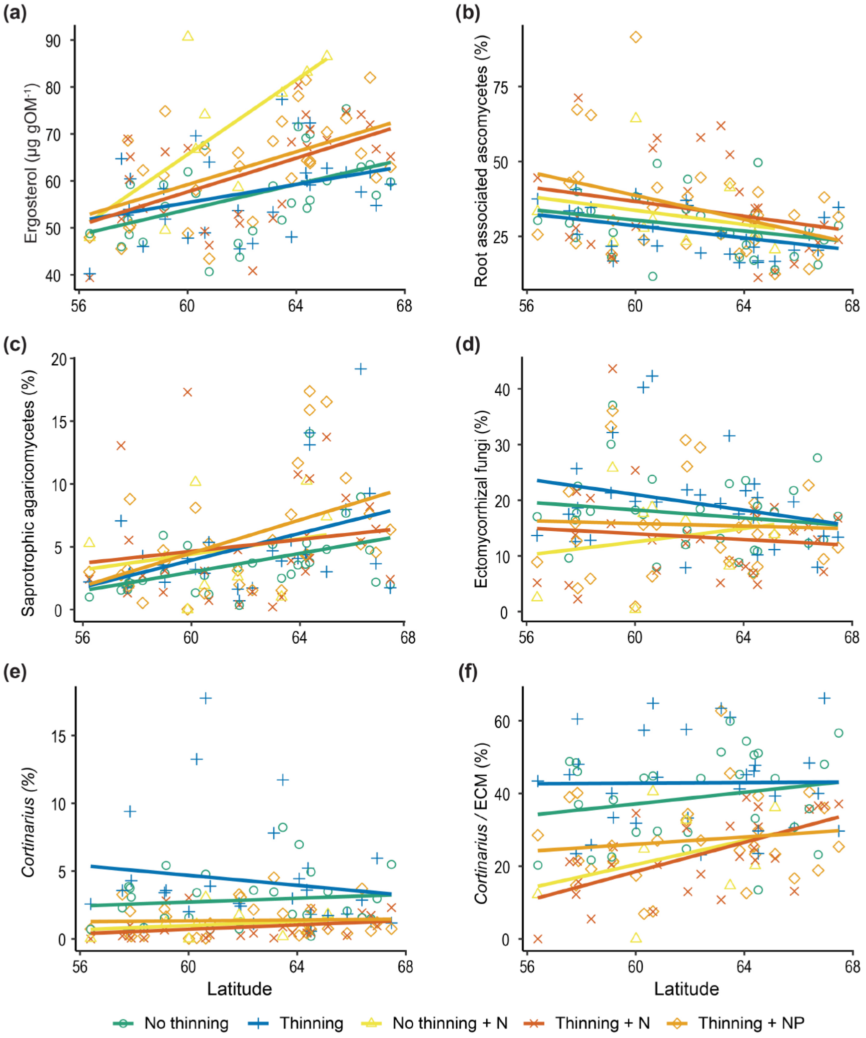

Figure 13. Ergosterol concentrations and relative abundances of fungal guilds in the organic topsoil of pine forest plots subjected to thinning and/or fertilization across a latitudinal transect in Sweden. (a) Ergosterol concentration (µg gOM^{-1}) estimated by measurements of ergosterol concentration in the organic topsoil, relative abundance (% of fungal sequences based on PacBio Sequel sequencing of ITS2) of (b) root-associated Ascomycetes, (c) saprotrophic Agaricomycetes, (d) ectomycorrhizal fungi and (e) *Cortinarius* species, and (f) the proportion of *Cortinarius* in the ectomycorrhizal (ECM) community. Lines are fitted regression lines from linear models.

Table 2. *Effects on ergosterol concentration (µg gOM^{-1}) and the square root-transformed relative abundance of fungal guilds (% of fungal sequences from PacBio Sequel sequencing of ITS2 amplicons) were analysed by linear mixed-effects models with N and P loads normalized by the average load. For latitude, the most southern site was used as a zero reference. Interaction effects of N fertilization and thinning, and N fertilization and latitude were included in the ergosterol model. Numbers in parentheses represent 95% CI. Numbers in bold denote significant effects ($P < 0.05$).*

	Ergosterol concentration (µg gOM^{-1})		Root associated ascomycetes (%)		Saprotrophic agaricomycetes (%)		Ectomycorrhiza (%)		*Cortinarius* (%)		*Cortinarius*/ECM	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	50.08 (44.41 – 55.76)	**<0.001**	0.57 (0.52 – 0.63)	**<0.001**	0.13 (0.08 – 0.17)	**<0.001**	0.44 (0.38 – 0.50)	**<0.001**	0.18 (0.14 – 0.21)	**<0.001**	0.38 (0.32 – 0.45)	**<0.001**
N fertilization	7.76 (1.24 – 14.28)	**0.020**	0.05 (0.01 – 0.08)	**0.020**	0.01 (-0.02 – 0.04)	0.523	-0.08 (-0.12 – -0.05)	**<0.001**	-0.09 (-0.12 – -0.07)	**<0.001**	-0.18 (-0.22 – -0.13)	**<0.001**
P fertilization	1.16 (-2.74 – 5.05)	0.560	0.01 (-0.03 – 0.06)	0.605	0.00 (-0.03 – 0.04)	0.807	0.02 (-0.02 – 0.06)	0.399	0.01 (-0.02 – 0.04)	0.440	0.03 (-0.02 – 0.08)	0.300
Thinning	0.14 (-3.75 – 4.03)	0.944	-0.01 (-0.05 – 0.03)	0.758	0.02 (-0.01 – 0.05)	0.198	0.03 (-0.01 – 0.06)	0.162	0.02 (-0.01 – 0.04)	0.166	0.02 (-0.03 – 0.07)	0.366
Latitude	1.20 (0.39 – 2.01)	**0.004**	-0.01 (-0.02 – -0.00)	**0.022**	0.01 (0.00 – 0.02)	**0.001**	-0.01 (-0.01– 0.00)	0.215	-0.00 (-0.01 – 0.00)	0.381	0.00 (-0.01 – 0.01)	0.628
N:Thinning	-8.51 (-14.92 – -2.10)	**0.009**										
N:Latitude	1.11 (0.23 – 1.98)	**0.013**										

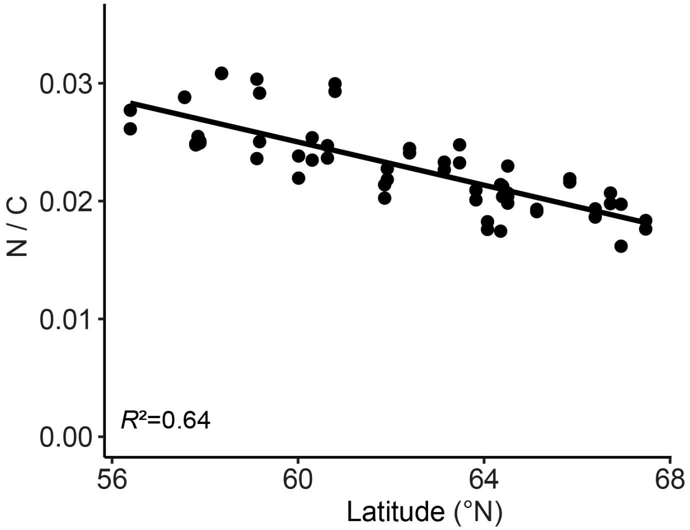

Figure 14. N/C ratio in the organic topsoil in all unfertilized plots across a latitudinal transect in Swedish *Pinus sylvestris* forests.

5.2.3 Ectomycorrhizal fungal community composition

Across gradients of N availability

Ectomycorrhizal fungi vary in their sensitivity to N (Lilleskov et al. 2011; van der Linde et al. 2018), and it is relevant to explore common traits of taxa that behave similarly. Based on the results of Paper III, I classified ectomycorrhizal genera as either “fast-growing” or “slow-growing” depending on their ability to colonize the ingrowth bags relative to their abundance on roots. Fast-growing genera (*Amanita*, *Amphinema*, *Tomentella*, *Tomentellopsis* and *Tylospora*) were characterized by high abundance in bags while slow-growing genera (*Cenococcum*, *Cortinarius*, *Hyaloscypha*, *Piloderma* and *Russula*), in contrast, were poor colonizers of bags. In Paper IV, the responses of these groups (as a proportion of the ectomycorrhizal community) were tested.

The relative abundance of fast-growing genera increased at high N/C, but only in the nemoral region (Figure 15a). Slow-growing genera decreased with increased N availability in both regions and were most abundant in the N-poor end of the nemoral gradient (Figure 12c-d).

Among the fast- and slow-growing genera, four (*Hyaloscypha*, *Piloderma*, *Tomentella* and *Tylospora*) displayed diverging responses to the N/C ratio between the two regions. The fast-growing *Tomentella* and *Tylospora* correlated positively with the N/C ratio in the nemoral region but were unresponsive in the boreal region (Figure 13c-d). The slow-growing *Piloderma* declined in abundance along the N/C gradient in both regions, but the negative response was stronger in the nemoral region (Figure 13b). These results, thus, concurred with the patterns observed for the consolidated groups of fast- or slow-growing ectomycorrhizal genera. In contrast, *Hyaloscypha* displayed diverging responses compared to other slow-growing genera, with relative abundance correlating negatively with the N/C ratio in the boreal region but strongly positively in the nemoral region (Figure 13a). *Hyaloscypha* is abundant in seedling nurseries (Menkis et al. 2005) and has also been found to be more abundant on rich sites than poor ones (Peltoniemi et al. 2021). Possibly, this implies that *Hyaloscypha* is adapted for environments with high inorganic N availability but are non-competitive in systems that rely on organic N cycling. Possibly this is a sign of a stress-tolerant or ruderal strategy.

There was relative increase of fast-growing genera (Figure 15a) despite decreased ectomycorrhizal biomass (Figure 12e-f) in response to increased N availability in the nemoral gradient, indicating that they are less sensitive to N than other ectomycorrhizal genera. While these genera supposedly have extensive mycelial production (Paper III), they may also have high CUE_A. Since trees are expected to allocate less C to their ectomycorrhizal partners as N availability increases (Mäkelä et al. 2008; Franklin et al. 2014; Baskaran et al. 2017), high CUE_A would be a beneficial trait for overcoming C limitation in N rich environments.

In intensely managed forests

Similarly to Paper IV, the relative abundance of ectomycorrhizal fungi decreased after external N input in Paper II (Figure 10d). A decline in ectomycorrhizal abundance can lead to increased proliferation of saprotrophic fungi due to decreased spatial competition – a "Gadgil effect" (Gadgil & Gadgil 1971, 1975). However, the saprotrophic Agaricomycetes did not respond to the fertilization treatment, although they increased in abundance towards the northern end of the latitudinal transect (Figure 13c). The lack of response to N fertilization stands in contrast to results from temperate ecosystems where saprotrophic Agaricomycetes have been shown to be sensitive to N additions (Entwistle et al. 2017).

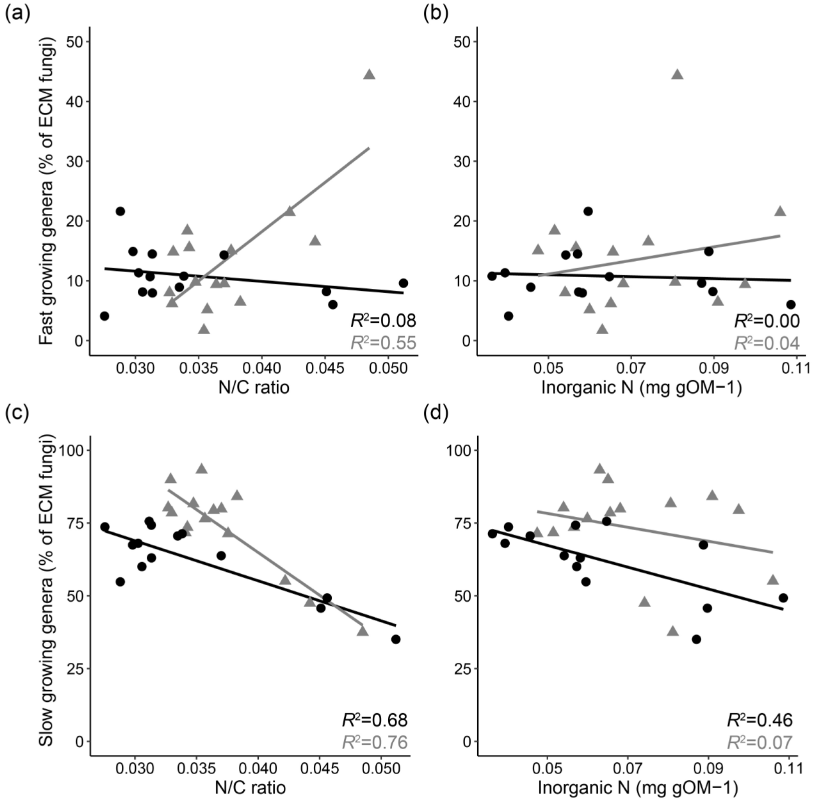

Figure 15. Relative abundance of (a-b) fast and (c-d) slow-growing ectomycorrhizal genera (% of ectomycorrhizal sequence reads of PacBio Sequel sequencing of ITS2 markers) in the organic horizon and top 7 cm of mineral soil across gradients of N/C ratio and inorganic N (mg gOM^{-1}) in boreal *Picea abies* forests in two regions in Sweden. Growth speed was determined based on results from the Cafeteria experiment (Paper III). Lines represent fitted linear regressions. Points and shapes denote sampled regions: boreal (solid black circles) and nemoral (solid grey triangles).

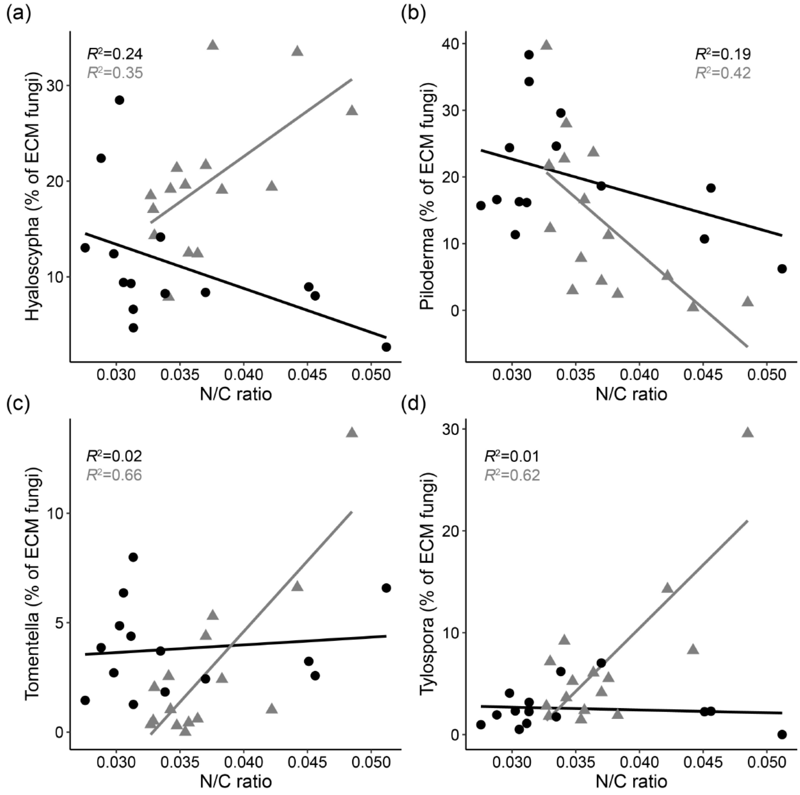

Figure 16. Relative abundances (% of ectomycorrhizal sequence reads of PacBio Sequel sequencing of ITS2 markers) of genera that responded differently to N availability (as determined by the N/C ratio) in the boreal and nemoral regions. Lines represent fitted linear regressions. Points and shapes denote sampled regions: boreal (solid black circles) and nemoral (solid grey triangles).

Nonetheless, the result corroborates previous studies in similar systems and questions the universal existence of a Gadgil effect in boreal forests (Sterkenburg et al. 2018).

While ectomycorrhizal fungi declined in relative abundance in response to fertilization, root-associated Ascomycetes (excluding ectomycorrhizal Ascomycetes) increased (Figure 13b), and the increase in fungal biomass (Figure 13a) could potentially be attributed to them. Particularly, two species within genus *Archaeorhizomyces* (Rosling et al. 2011) increased in the N fertilized plots (Figure 17).

Two ericoid mycorrhizal species responded negatively to fertilization, and one of them, *Hyaloscypha hepaticicola*, was positively affected by thinning (Figure 17). These responses may have been conditional on management effects on their plant host as both fertilization and thinning affect the abundance of *Vaccinium myrtillus* and *Vaccinium vitis-idaea* (Granath & Strengbom 2017). Among ectomycorrhizal species *Cortinarius acutus*, *Russula decolorans* and *Suillus variegatus* decreased in fertilized plots (Figure 17). This result is in line with the general perception of *Cortinarius* and *Suillus* as sensitive to increased N availability. However, species within genus *Russula* display a more mixed response with different species responding either negatively or positively (Lilleskov et al. 2011).

Although only one among the 40 most frequent species in Paper II, an unidentified Leotiomycete (sp.5, Figure 17), responded (negatively) to the supplementation of P together with N when evaluated with linear mixed effect models, CCA analysis of the whole fungal community showed that P addition affected community composition. Nitrogen fertilization alone caused a shift in fungal community composition, but additions of P together with N affected community composition further and seemingly made them more similar across the gradient (Figure 18). Since N is often the main limiting nutrient in the boreal forest, the relationship between soil fungi and P is not well studied. However, more boreal forests may start to balance between N and P limitation if N availability increases through fertilization or N deposition. In N enriched forests in southern Sweden (northern part of the nemoral region) P deficiency seemingly affects fungal community composition and functioning (Almeida et al. 2019). The functional implications of these shifts are not well understood and will likely be subject to more research in the future if N deposition increases as projected.

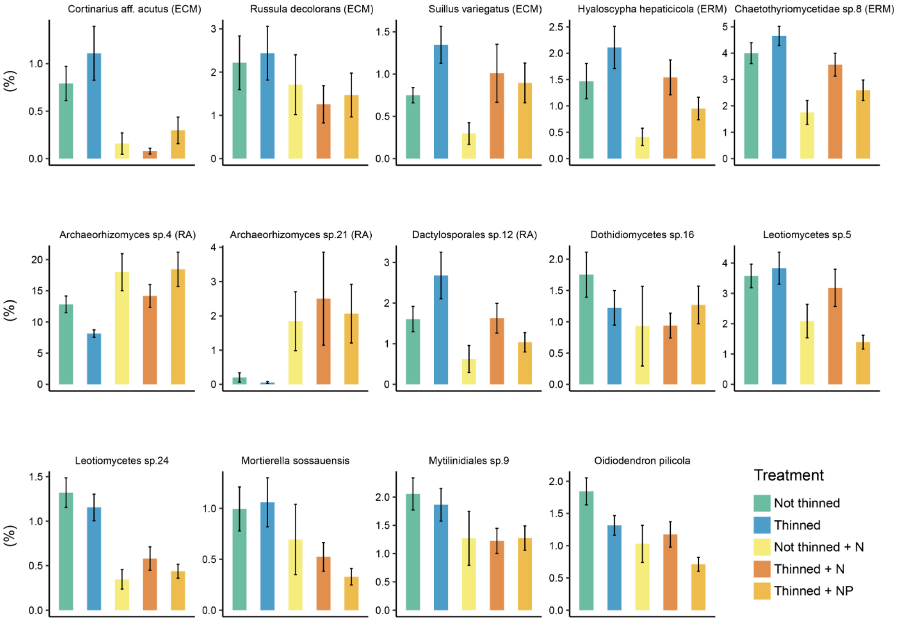

Figure 17. Relative abundance of species in the core fungal community of organic horizon of pine plots subjected to different combinations of thinning and fertilization. Relative abundance (% of fungal reads from PacBio Sequel sequencing of ITS2 amplicons) of species that had a statistically significant response (P ≤0.05 in the linear mixed-effects models) to any of the treatments. Abbreviations in parentheses denote functional guild: ECM = ectomycorrhiza, ERM = ericoid mycorrhiza, RA = root-associated ascomycete, no letters = unknown guild. Bars are mean abundance, and error bars denote SE.

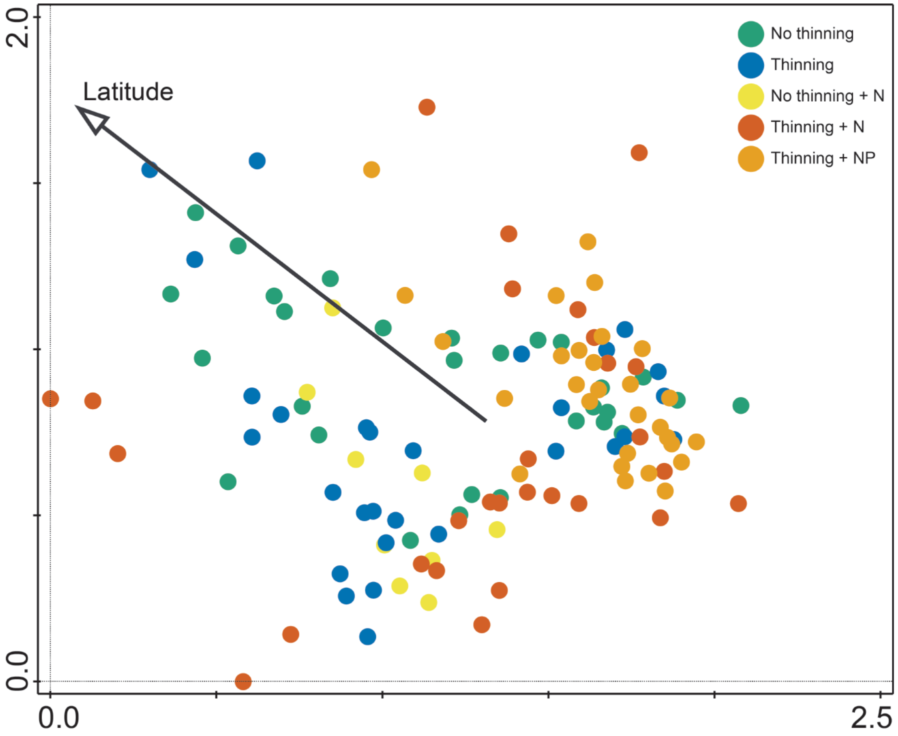

Figure 18. Sample plot of a detrended correspondence analysis (DCA) based on the composition of the fungal community in the organic topsoil of pine forests plots subjected to thinning and/or fertilization across a latitudinal transect in Sweden. The analysis is based on PacBio Sequel sequencing of ITS2 amplicons and encompasses 209 species that accounted for ≥1% of fungal sequences in any sample. Latitude was included as a supplementary variable in the analysis. DCA axis 1 explains 12.1% and axis 2 5.2% of the total variation

5.3 Effects of nitrogen on extracellular enzyme activities

Across gradients of N availability

In Paper IV, activities of manganese peroxidases were negatively correlated with N/C ratio and inorganic N, and were generally higher in the nemoral than in the boreal region (Figure 19a). The decline in activity along the N/C gradient in the boreal region stands in contrast with the study by Kyaschenko et al. (2017b), which found manganese peroxidase activities to correlate positively with ecosystem fertility. Activities of oxidative enzymes generally correlate positively with soil pH (Sinsabaugh 2010) which, in turn, is associated with ecosystem fertility (Sterkenburg et al. 2015). In a temperate forest, oxidative decomposition with phenol oxidases correlated positively with fertility (Mayer et al. 2021). Thus, oxidative decomposition seems to be a key contributor to ecosystem fertility. One major difference between Paper IV and the study by Kyaschenko et al. (2017b) is that their fertility gradient extended into poorer, *Pinus sylvestris* dominated sites while the gradient in Paper IV only consisted of *Picea abies* forests. Potentially, the positive correlation between fertility and manganese peroxidase activity is only detectable across much wider fertility gradients than the one used in Paper IV. Moreover, the most fertile sites in Paper IV may have located further towards the rich end compared to the study by Kyaschenko et al. (2017b). Decreased manganese peroxidase activity at high N availability in the *Picea abies* gradients in Paper IV (Figure 19a) is in line with the declined activities that are often observed after N amendments (Bödeker et al. 2014; Entwistle et al. 2018). Possibly, increased N availability, regardless of source, may supress oxidative decomposition at a certain point.

Activities of β-1,4-*N*-acetylglucosaminidase and acid phosphatase were generally higher in the boreal than in the nemoral region (Figure 19c-f). Acid phosphatase activities were consistent across the N/C gradient, but there was a marginally significant (P = 0.061) negative correlation between N-acetylglucosaminidase and N/C. While high ecosystem fertility is often associated with increased decomposition rates, it is still generally poorly correlated with hydrolytic enzymatic activity (Kyaschenko et al. 2017b; Mayer et al. 2021). However, the relative activity of different hydrolytic enzymes could potentially hint at the needs of the decomposing community. At high N availability, mining for organically bound N may be downregulated in relation to P acquiring enzymatic activity since organisms would have to maintain N:P stoichiometry. The ratios between acid phosphatase and β-1,4-*N*-acetylglucosaminidase were higher in the nemoral than in the boreal region (Figure 19g-h), with a marginally significant positive interaction (P=0.058) between N/C ratio and region. This could indicate an increased need for organically bound P relative to N in the nemoral region and could be related to the higher input of inorganic N (Heuck et al. 2018).

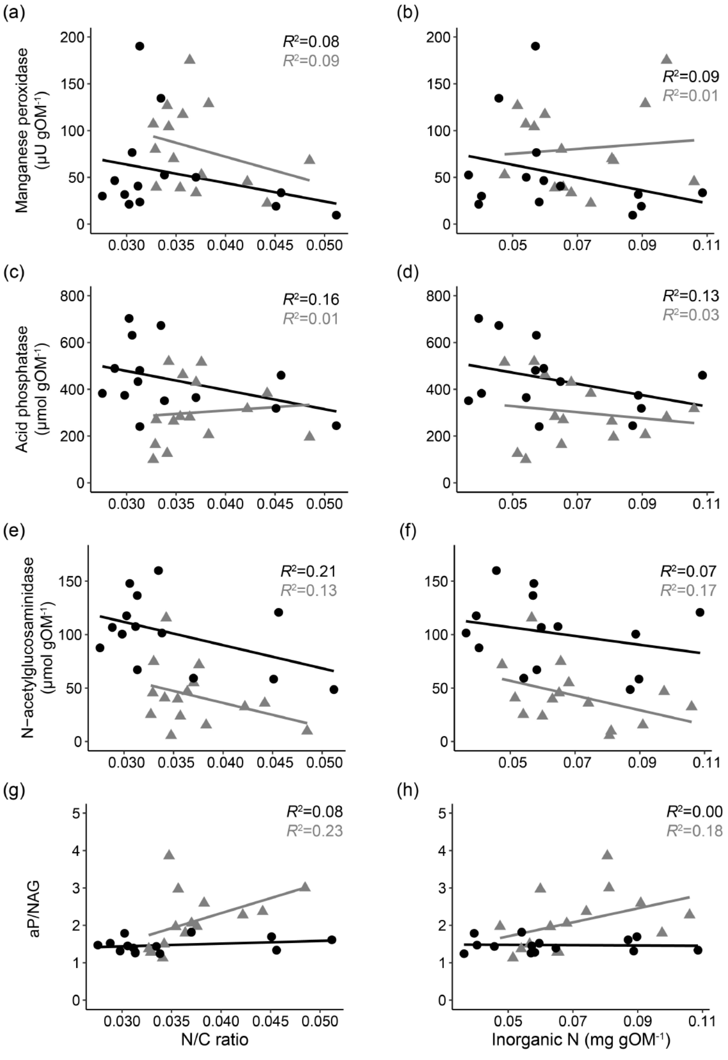

Figure 19. Activities of extracellular enzymes estimated from the organic horizon and top 7 cm of mineral soil of boreal *Picea abies* forests in two regions in Sweden. (a-b) Manganese peroxidase (μU gOM^{-1}) (c-d) acid phosphatase (μmol gOM^{-1}) and (e-f) β-1.4-*N*-acetylglucosaminidase (μmol gOM^{-1}) and (g-h) the acid phosphatase/ β-1,4-*N*-acetylglucosaminidase ratio plotted across gradients of N/C ratio and inorganic N (mg gOM^{-1}). Lines represent fitted linear regressions. Points and shapes denote sampled regions: boreal (solid black circles) and nemoral (solid grey triangles).

In intensely managed forests

Activities of β-glucosidase declined towards the north along the latitudinal transect in the intensely managed *Pinus sylvestris* forests. In the models used to evaluate the data, N addition did not have an effect on its own. Instead, I found an interaction between N fertilization and latitude that counteracted the latitude-effect and resulted in similar activities across the whole transect in fertilized plots (Figure 20b). This could be interpreted as a sign of a general N constraint on cellulose decomposition in the boreal forest that was alleviated by increased access to N.

Activities of β-1,4-*N*-acetylglucosaminidase were not affected by fertilization in Paper II, consistent with the relatively weak response to increased N availability in Paper IV. Activities were, however, stimulated by thinning (Figure 20c).

One tentative conclusion from Paper II was that N fertilization seemingly induced a greater need for organic P. Activities of acid phosphatase increased in plots where N fertilizer had been applied but were comparable to non-fertilized levels when P was added together with N (Figure 20d). This result is in line with previous studies from both boreal and temperate forests showing that N additions can stimulate the activity of phosphatases (Saiya-Cork et al. 2002; Heuck et al. 2018). However, it remains to be elucidated whether the increased acid phosphatase activities actually denote P limitation or represented a shift from inorganic to organic P acquisition (Widdig et al. 2019).

In Paper II, manganese peroxidase activities were highly variable across treatments, and no effects were detected across the latitudinal transect (Figure 20a). However, when comparing the relative change in activity between treatments, there was a tendency towards declined activities in the combined N and P fertilization treatment relative to the other treatments ($P = 0.072$) (Figure 21). Often, N amendments would supress manganese peroxidase activity (Bödeker et al. 2014; Entwistle et al. 2018). Potentially, sustained activity in the N fertilized plots could have been due to an increased demand for organically bound P. That would imply that oxidative decomposition of low-quality organic matter could also be used to access P. However, this tentative mechanism is largely unexplored and requires further research.

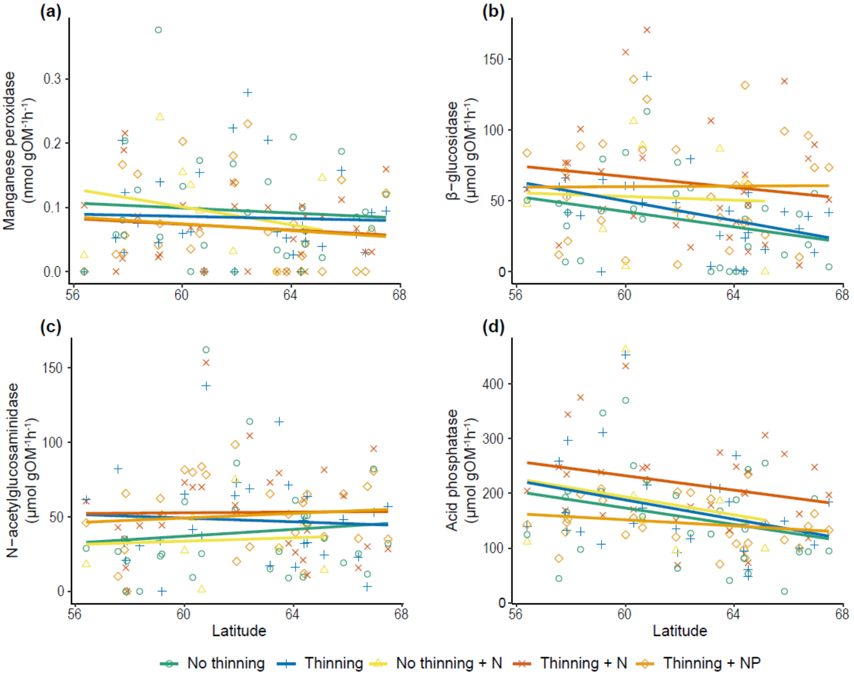

Figure 20. Enzyme activities in the organic topsoil of pine forest plots subjected to different combinations of thinning and fertilization along a latitudinal transect in Sweden. Enzyme activities of (a) manganese peroxidase, (b) β-glucosidase, (c) β-1,4-*N*-acetylglucosaminidase and (d) acid phosphatase. Lines are fitted regression lines from linear models.

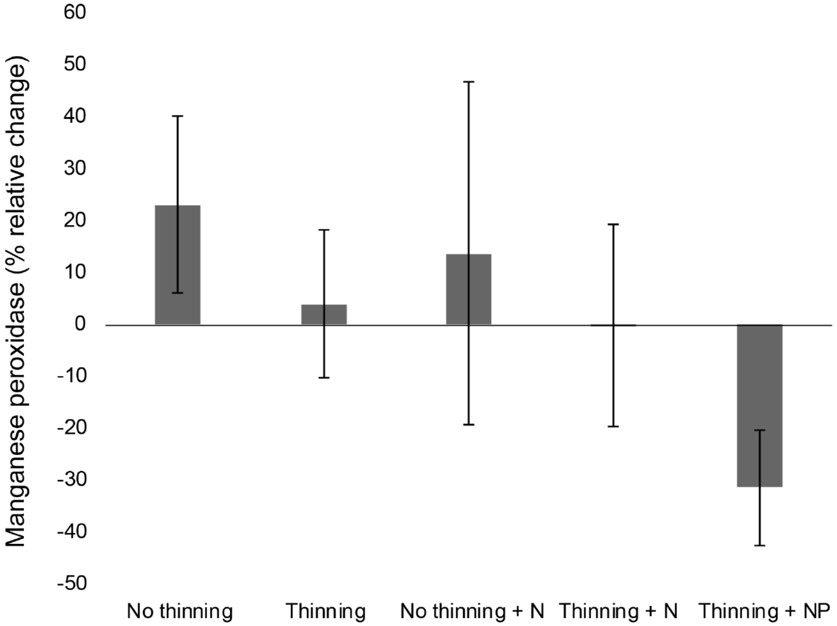

Figure 21. Activities of manganese peroxidases in boreal *Pinus sylvestris* forests across a 1300 km latitudinal transect in Sweden subjected to different forest management practices. The bars represent the change in activity in the different treatments relative to the site average. Bars denote standard errors.

5.4 Ecosystem C storage

5.4.1 Effects of intense forest management on ecosystem carbon stocks

In Paper I, the effects of different management scenarios on C storage in *Pinus sylvestris* forests were quantified and compared with the standard silvicultural practice of thinning. Abstention from thinning increased the ecosystem C stock in the stands by 50%. Nitrogen fertilization also had a stimulating effect on C sequestration and increased C stocks both in trees and in the organic topsoil. The combination of fertilization and abstention from thinning resulted in a positive interaction effect and generated the largest ecosystem C gain (Figure 22a). The higher C sequestration in non-thinned, fertilized stands was possibly due to a higher N uptake in stands with higher

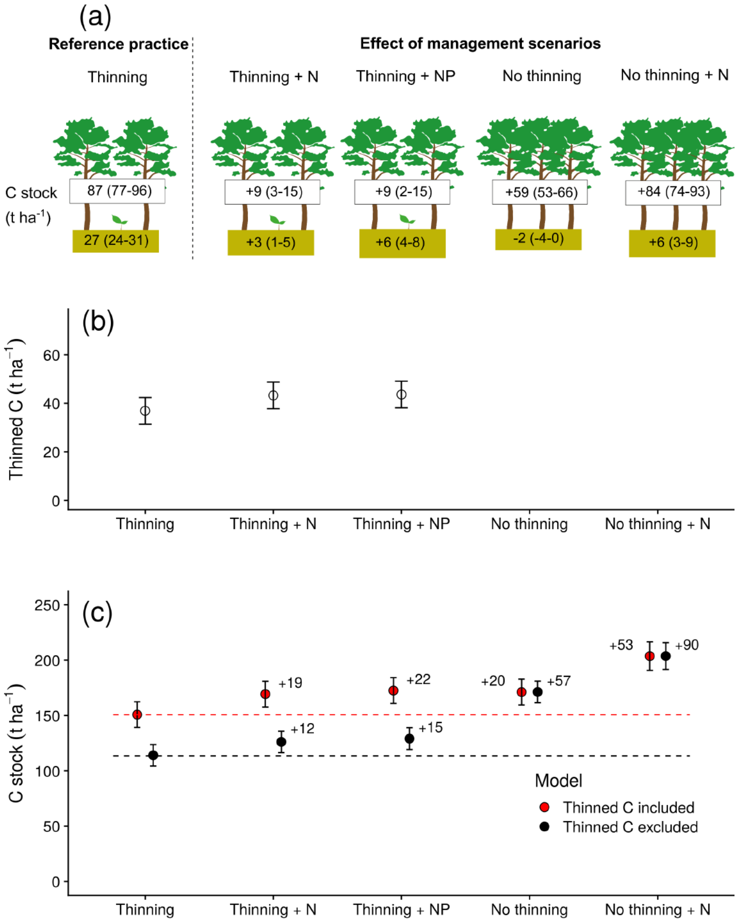

Figure 22. Effects of different management scenarios on C stocks (trees, organic horizon), C removed by thinning, and total ecosystem C gain. (a) Tree and organic horizon C stocks in stands subjected to the standard silvicultural practice of thinning and the effect of different alternative management scenarios, applied over a period of 40 years. (b) C removed during thinning operations. (c) Ecosystem C stocks under different management scenarios and associated effects on net C sequestration (numbers beside the points). Red symbols represent total C stocks that includes removed thinned wood, and black symbols represent standing C stocks without removed wood. Dashed lines show the standard silvicultural practice of thinning without fertilization. Points represent adjusted means and numbers in parentheses (a) and error bars (b,c) represent 95% confidence intervals. The average N application corresponds to 881 kg ha^{-1} applied over circa 40 years.

needle biomass, and thus, higher photosynthetic capacity. Combined fertilization with both N and P increased C sequestration in the organic topsoil twofold compared to N fertilization alone but did not affect tree productivity.

The observation of boosted tree productivity after N fertilization was not surprising given the strong N limitation on productivity and a vast body of previous studies reporting similar responses (Tamm 1991; Hyvönen et al. 2008; From et al. 2015; Högberg et al. 2017). Nonetheless, the interactive effect of thinning and fertilization highlights the importance to understand belowground processes, possibly mediated by tree roots, since the organic topsoil C stocks were also affected by the interaction.

In Paper I, we used an alternative approach in the estimates of stored C stocks and included the biomass that had been removed in thinning operations (i.e. assuming that all removed C remains stored in harvested wood products). Then, differences between thinned and non-thinned treatments were smaller but stimulated growth owing to decreased competition for resources for remaining trees was not enough to compensate for the outtake of biomass (Figure 22c). These results highlight a trade-off between maximal C uptake and quality of the timber in managed forests.

5.4.2 What regulates C storage below ground?

Similarly as for the tree C stocks, increased belowground C sequestration is often observed in N amended boreal forests (Magnani et al. 2007; Hyvönen et al. 2008; de Vries et al. 2014; Maaroufi et al. 2015; Tipping et al. 2017). However, the mechanisms behind stimulated C accumulation are not fully understood. Therefore, Paper II aimed at evaluating potential fungal mechanisms that may contribute to the results observed in Paper I.

In temperate forest ecosystems, increases in belowground C stocks after N amendment have been connected to decreased abundance of fungi with ligninolytic capacity (Entwistle et al. 2017) and decreased expression of peroxidase encoding genes (Entwistle et al. 2018; Zak et al. 2019). In Paper II, the relative abundance of peroxidase producing *Cortinarius* species (Bödeker et al. 2009, 2014) decreased after fertilization (Figure 13e). The decline was particularly pronounced for *Cortinarius acutus* (Figure 17), which has been highlighted as a key regulator and decomposer of C stocks in Swedish boreal forests (Lindahl et al. 2021). However, there was no support for declined ligninolytic decomposition (estimated by the activity of manganese peroxidase) (Figure 20a) in spite of decreased abundance of *Cortinarius*.

Despite the lack of a mechanistic explanation that entails the manganese peroxidase activity, there was a negative correlation between C stock and the relative abundance of *Cortinarius* (Figure 23f), but the different forest management treatments were a strong confounding factor. A growing body of research highlights *Cortinarius* as pivotal for organic matter turnover in boreal soils, and despite the lack of a direct connection in Paper II, I would still argue that the results obtained in this thesis further underscore their role in boreal forest functioning.

I think that the lack of a direct connection between C stock, *Cortinarius* and oxidative enzyme activity may be due to the different scales of the experiment in Papers I and II. Carbon stocks were quantified after around 40 years of management, while analyses of fungal community composition and enzyme activities provided a snapshot of species composition and activities at the sampling time. The build-up of C stocks on a site scale likely depends on the outcome of long-term dynamics and may not necessarily be reflected in current enzymatic activity. Further, the longevity and size of mycorrhizal mycelia may lead to spatial heterogeneity of enzymatic activities since the fungi may be more actively exuding extracellular enzymes at hotspots of mycelial activity than in other parts.

In addition to decreased oxidative decomposition, increased organic matter input may also contribute to higher C stocks after fertilization. In Paper II, standing tree biomass did not correlate with the organic topsoil C stocks. This suggests that aboveground litter input has minor influence on long-term C storage, in line with previous research (Janssens et al. 2010; Wardle et al. 2012). Instead, increased fungal biomass (Figure 23b), which was partly attributed to an increase in the relative abundance of root-associated ascomycetes (Figure 13b), may have contributed to the stimulated C stock. Root-associated ascomycetes, among them many ericoid mycorrhizal species, often form melanised mycelia that are difficult to decompose and thereby add to a stable C sink. There may also have been additional input of increased *Pinus sylvestris* fine root biomass into the soil through, albeit only in the combined NP treatment (Figure 24).

Soil respiration rates declined in fertilized plots in Paper I, consistent with previous research (Janssens et al. 2010; Maaroufi et al. 2015; Forsmark et al. 2021). Decreased soil CO_2 efflux rates despite observed increases in biomass in the soil would suggest increased CUE of roots and fungi. Forsmark et al.

(2021) observed increased fine root production of *Pinus sylvestris*, coupled with decreased autotrophic respiration rates after N fertilization.

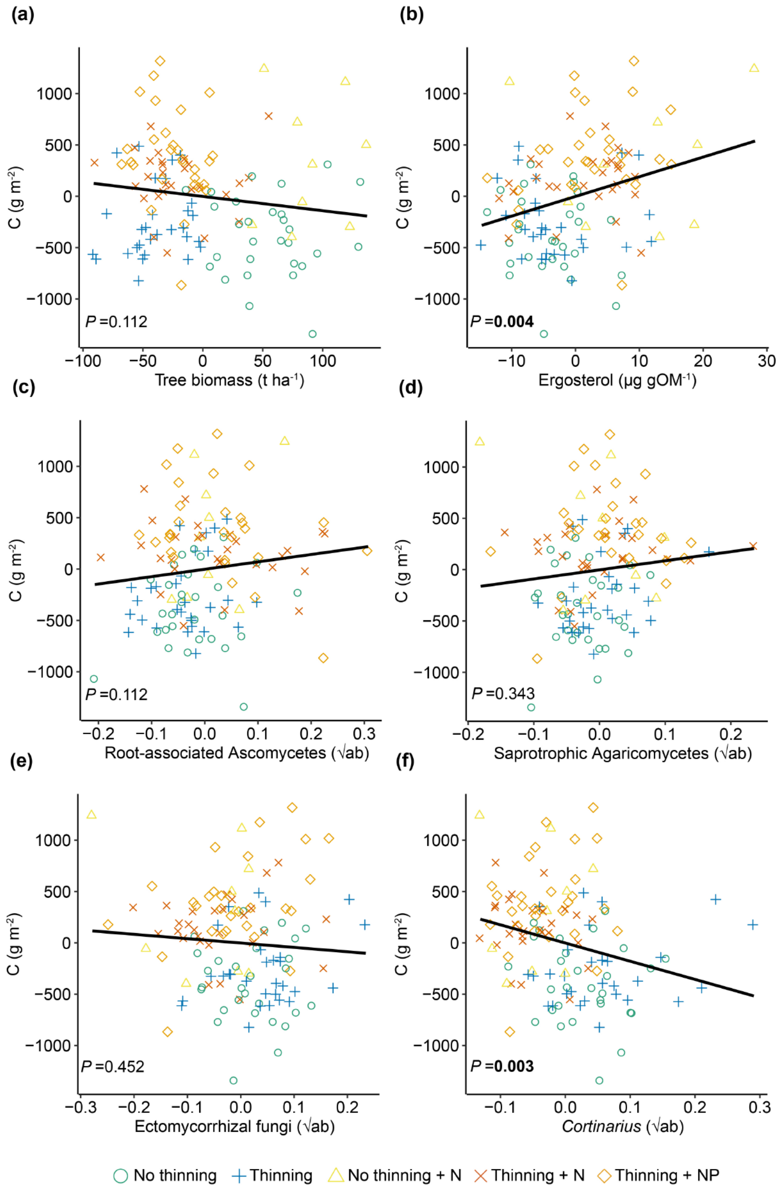

Figure 23. Correlations between organic topsoil C stocks and potential regulatory factors across pine forests plots subjected to different combinations of thinning and

fertilization along a latitudinal transect in Sweden. Partial residuals, after accounting for site-specific random effects, of C in the organic topsoil (g m^{-2}) plotted against (a) standing biomass of trees (tonnes), (b) ergosterol (µg gOM^{-1}), or square root transformed relative abundances (√ab) of: (c) root-associated ascomycetes, (d) saprotrophic agaricomycetes, (e) ectomycorrhizal fungi and (f) Cortinarius species. Significant effects ($P \leq 0.05$), evaluated by linear models, are highlighted in bold text in the figure. N=124.

Based on this, the authors concluded that the N amendments increased the efficiency of fine root production – possibly the same shift occurred in the forests in Paper I and lead to an increased CUE_A of roots and associated fungi.

To conclude, the results of Papers I and II corroborate the idea that boreal forest soil C stocks are mainly regulated by belowground processes (Janssens et al. 2010; Clemmensen et al. 2013) rather than aboveground plant litter input (proxied by standing tree biomass). In non-fertilized plots, output rates (i.e. decomposition) seemingly played an important role in the regulation of C stocks. In fertilized plots, the combination of decreased C output and increased input of root derived C may have contributed to increased C accumulation. Thus, the results suggest that multiple mechanisms interplay to regulate C storage in the organic topsoil, particularly in N amended systems.

The fertilization-induced shifts in fungal community composition further highlight that there are trade-offs between different values of boreal forest, since gains in biomass and C storage lead to decreased abundance of ectomycorrhizal fungi and losses presumably pivotal taxa for natural nutrient turnover, such as *Cortinarius*.

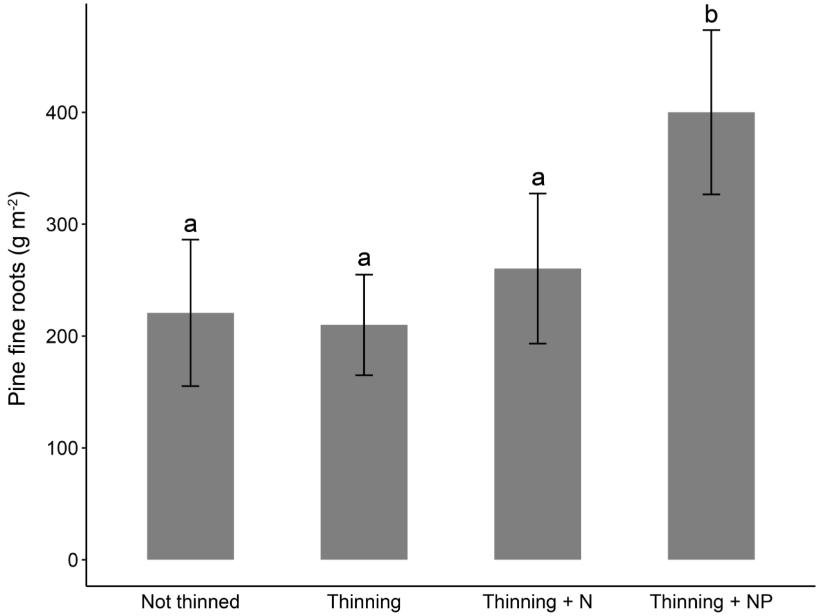

Figure 24. Pine fine roots (g m^{-2}) in the organic horizon of a subset of 13 sites (total n=52) in the long-term fertilization study. Bars indicate mean root concentration and bars show standard error. Letter above bars denote statistically significant effects ($P < 0.05$) between treatments according to post-hoc Tukey HSD test.

5.5 Ecological tipping points and stabilizing mechanisms

In ecosystems subjected to environmental changes, such as external N additions, a general concern is that they may reach a tipping point, at which ecosystem function rapidly shifts into a possibly irreversible alternative state (Dakos et al. 2019). Recently, the concept of ecological tipping points was applied to ectomycorrhizal fungi (Suz et al. 2021), and the authors describe a positive feedback that may lead up to a point where ectomycorrhizal function drastically changes in N amended systems. In short, increased N availability would lead to a declined abundance of nitrophobic species with high biomass and an ability to mine nutrients from organic matter, while lower biomass, nitrophilic species would be favored due to their capacity to take up and tolerate higher levels of mineralized N the soil.

Some of the results presented in this thesis follow the projected shifts that may eventually lead to ectomycorrhizal tipping points described by Suz et al. (2021). The relative abundance of *Hyaloscypha, Tomentella* and *Tylospora*, genera that can be classified as being nitrophilic (Lilleskov et al. 2011, 2019; van der Linde et al. 2018), increased towards the rich end of the gradient in the region subjected to elevated rates of atmospheric N deposition (Paper IV; Figure 16a,c-d). However, according to Paper III, *Tomentella* and *Tylospora* seem to produce extensive mycelia and may therefore take part in a negative feedback in which N is immobilized in fungal biomass. This would be a mechanism to maintain N limitation and, thus, counteract the positive feedback leading to the tipping point.

Meanwhile, the relative abundance of slow-growing fungi (Figure 15c-d) and particularly the nitrophobic *Piloderma* (Figure 16b), decreased at higher N availability in both the boreal and the nemoral region. Assuming that the slow-growing genera have low $\mathrm{CUE}_{\mathrm{A}}$, this response would be aligned with the idea that increased N availability leads to reduced C investment from trees in expensive ectomycorrhizal partners (Näsholm et al. 2013; Baskaran et al. 2017).

I find it plausible that forests in the nemoral zone are more prone reaching an ectomycorrhizal tipping point than the forests in the boreal region due to the historically higher N deposition rates in the nemoral than the boreal region (Figure 2). However, in Paper IV, similar inorganic N levels across the fertility gradients in both regions disputes that the N amended forests in the nemoral region would have reached N saturation. Instead, N uptake seems to have been sustained, despite a general decline of ectomycorrhizal biomass. While increased N losses cannot be ruled out, sustained inorganic N levels despite higher input of atmospheric N deposition in the nemoral gradient were possibly also sustained by a switch towards more direct uptake of inorganic N from trees and a shift towards ectomycorrhizal species with high $\mathrm{CUE}_{\mathrm{A}}$ and high N retention capacity.

The tipping point framework assumes a positive feedback that leads to a rapid shift in ecosystem functioning. In Paper I and II, however, the dramatic loss of *Cortinarius* after fertilization could, in contrast, induce a negative feedback on N availability in which more N is locked up in organic matter. Because of their pivotal role in turnover of low-quality organic matter in the deeper layers of organic topsoils (Bödeker et al. 2014; Lindahl et al. 2021), loss of the ligninolytic function could make nutrients unavailable to organisms that only possess hydrolytic enzymatic capacity.

Similar shifts, with lowered rates of organic matter turnover and subsequent build-up of organic stocks – ecosystem retrogression – can be observed in ecosystems that have not been subjected to any major disturbances for a very long time (Wardle et al. 2003, 2012; Peltzer et al. 2010). One major difference between naturally retrogressing systems and the fertilized *Pinus sylvestris* forests in Paper I and II is that plant productivity was sustained and even stimulated by N fertilization. Likely, tree uptake of N shifted towards inorganic fertilizer N, rather than uptake through ectomycorrhiza, and subsequently, trees could reallocate more C towards growth than to ectomycorrhizal N miners (Bödeker et al. 2014). In a retrogressing ecosystem, maintenance of tree productivity would have been impossible since high fertility and plant productivity is associated with fast turnover of organic matter (Kyaschenko et al. 2019; Mayer et al. 2021). In contrast, plant productivity in the *Pinus sylvestris* forests was maintained through anthropogenic N input that likely compensated for the "retrogression-like" process that went on below ground.

While the comparisons between ecosystem retrogression – a decadal to century-spanning process – and relatively rapid shifts induced by anthropogenic activities, such as in Paper I and II, may be problematic due to differences in temporal scale, both examples highlight the dual function of boreal forest soils as both sinks for organic matter and sources of nutrients. These two functions are tightly linked, and this becomes evident in boreal forests where roots and associated fungi to such a large degree contribute to both decomposition and build-up/stabilization of organic matter (Clemmensen et al. 2013; Adamczyk et al. 2019; Kyaschenko et al. 2019).

6. Conclusions and a new postulation

In this thesis, the effects of endogenous and anthropogenic N on the composition and functioning of the ectomycorrhizal fungal community were compared. In addition, I investigated whether morphological traits of ectomycorrhizal extraradical mycelia corresponded to patterns of soil colonization, and whether these patterns varied depending on N availability.

In Paper III, I found that ectomycorrhizal exploration types were poorly correlated with soil colonization patterns. An alternative theory, supported by Paper III, is that biomass production and colonization of new substrates may be related to how “expensive”, in terms of host C, ectomycorrhizal fungi are relative to their biomass.

My impression is that external N disrupts natural boreal ecosystem functioning. However, the implications of the disruption likely vary depending on the ecological context. In Paper I and II, N fertilization decreased the abundance of ectomycorrhizal fungi and altered the composition of the fungal community. Further, increased N availability in the nemoral region (Paper IV), where atmospheric N deposition rates are elevated, caused rapid declines in ectomycorrhizal abundance while simultaneously shifting the fungal community. In contrast, the relative abundance of ectomycorrhizal fungi remained stable across a wide fertility gradient in the boreal *Picea abies* forests. Increased endogenous N availability seemingly had a less pronounced effect on ectomycorrhizal fungal community composition than in the N amended system. I, therefore, find it reasonable to conclude that the effects of endogenous and anthropogenic N supply have different effects on ectomycorrhizal fungi and that the impact of external N is large compared to natural supply.

One of the overarching hypotheses (a) in this thesis (presented in section 1.9) was that all N, regardless of source, has the same effect on ectomycorrhizal fungi. Based on the results presented in Paper IV, hypothesis (a) can be refuted.

The alternative hypothesis (b) assumed that endogenous and external N supply have fundamentally different effects and that natural fertility would be positively correlated with ectomycorrhizal performance while external supply would have a negative effect. This hypothesis is largely supported by the results from Paper IV. However, contrary to the hypothesis, ectomycorrhizal abundance and biomass did not increase in the boreal gradient despite increased N supply, instead it remained stable. Moreover, declined abundance of slow-growing genera and enzymes used for organic N mobilization (manganese peroxidase and β-1,4-*N*-acetylglucosaminidase) seems to be a general feature of N-rich systems regardless of region and source of N.

Across fertility gradients in *Picea abies* forests, differences between the regions were more pronounced at high N availability. In addition, ectomycorrhizal biomass in fertilized *Pinus sylvestris* forests was stable despite a rather high N load. This suggests that N dose and/or initial nutrient availability at the site may determine the direction of the impact of additional N. Further, I observed a relative increase of fast-growing genera with assumed high CUE_A in the N amended *Picea abies* forests in the nemoral region (Paper IV). In Paper II, *Cortinarius* decreased in abundance after fertilization. Both these shifts imply a potential negative feedback on N availability wherein more N is locked up in the organic topsoil and in fungal mycelia.

Based on these observations, a postulation about the relationship between ectomycorrhizal performance and N availability can be made (Figure 25). In low fertile systems, N limitation would be strong and increased N supply, regardless of source, would have a positive effect on ectomycorrhizal performance. At intermediate levels of N availability, responses to endogenous and anthropogenic N would start to diverge. At this stage though, ectomycorrhizal performance could be maintained by a negative feedback on inorganic N availability, through the proliferation of ectomycorrhizal fungal genera with high mycelial production relative to host-derived C (high CUE_A). However, as anthropogenic N availability continues to increase, a tipping point may be reached and a, possibly irreversible, shift in ecosystem functioning could occur.

The decline in ectomycorrhizal fungal abundance and biomass in the nemoral gradient in Paper IV indicates that a tipping point may have been reached at the rich end of the gradient. Yet, the proposed negative feedback on inorganic N availability may have mitigated the effects of anthropogenic N additions, and consequently likely decreased the risk of e.g. N leaching while simultaneously causing a build-up of soil C. However, I did not observe any signs that suggested that a tipping point related to N availability had been reached in the boreal gradient, despite N/C ratios and inorganic N levels that were comparable to the nemoral gradient.

The results also underscore that anthropogenic N, both in the form of atmospheric N deposition and fertilization, largely influences ectomycorrhizal community composition and processes in boreal forest soils. Particularly the loss of ectomycorrhizal genera with oxidative enzymatic capacity, such as *Cortinarius*, could largely disrupt organic matter turnover, and potentially lead to decreased tree productivity if input of external N stops and *Cortinarius* does not recover or is replaced by taxa with a similar function.

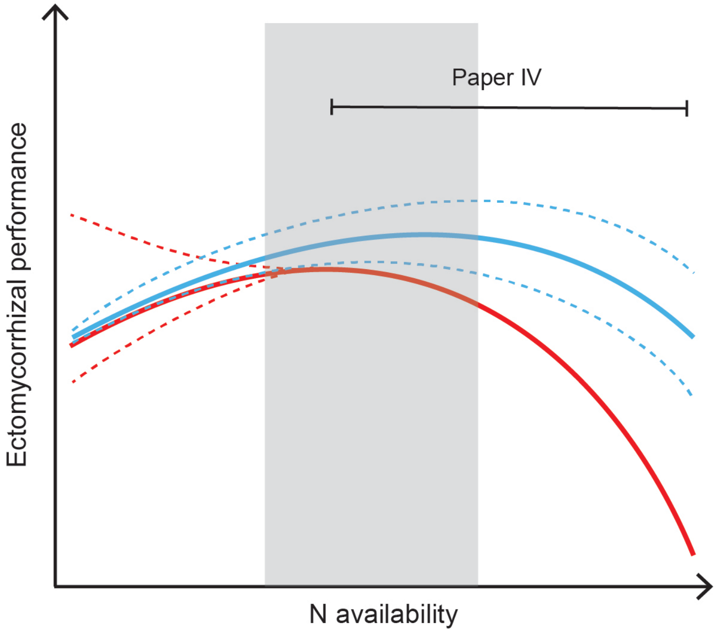

Figure 25. Postulated responses of ectomycorrhizal performance to increased N availability. The blue line represents response to endogenous N supply while the

red line represents response to anthropogenic N supply (fertilization, N deposition). At low N availability the effects of increased endogenous and anthropogenic N supply would be the same, but they start to diverge as N availability increases. The grey area represents a state wherein ecosystem N limitation (despite anthropogenic additions) is maintained through a negative feedback where N is immobilized in mycelial biomass.

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Popular science summary

The boreal forests, dominated by coniferous trees that cover large parts of the northern hemisphere are characterized by very strong nutrient limitation. The influx of nutrients from outside the system (for instance through nitrogen fixation) is very low. Therefore, nutrients must be recycled from organic matter in the soil. Fungi play an important role in this recycling since they can decompose organic matter and thereby make nutrients available. Ectomycorrhizal, fungi that live in symbiotic relationships with tree roots, central for nutrient turnover in boreal forests since they deliver nutrients – mainly nitrogen – to their host trees in exchange for carbon. In addition, ectomycorrhizal fungi and other fungi that associate with plant roots contribute to a large degree to the long-term storage of carbon in forest soils.

The degree of nutrient limitation in boreal forests vary and greatly affects the species composition and growth of plants. A boreal forest can, thus, be either a low productive, nitrogen poor pine forest or a highly productive, nitrogen rich spruce forest with some contribution of broad-leaf trees.

Species composition of ectomycorrhizal fungi also vary depending on the nitrogen availability since they are adapted for different environments. Their adaptation is likely dependent on how “expensive” they are for trees to associate with. Form the tree’s perspective, carbon is the currency they use to trade for nitrogen from the ectomycorrhizal fungi. Depending on the traits of the fungus, the price for nitrogen may differ and the inclination of trees to invest in ectomycorrhiza may also differ depending on the nitrogen availability in the soil – it usually decreases if nitrogen becomes more available.

Nutrient turnover in boreal forests is usually a closed process which is largely governed by relationships between plants and decomposers. However, during the past century we humans have intensified our use of natural resources – something that has not only had detrimental effects on the climate but also increased the amount of reactive nitrogen. Reactive nitrogen is easily assimilated in biological processes and, thus, has a large potential to affect ecosystems. Although this may not necessarily be negative. The use of mineral fertilizer is a prerequisite for food production and fertilization is, albeit to a small degree, used in forestry to boost tree production. There are, however, undesired side effects of globally increased levels of nitrogen. Atmospheric nitrogen deposition contributes to eutrophication of oceans, but also to terrestrial systems – among them the boreal forest, in which additions of external nitrogen may disrupt natural processes.

In this thesis, I have investigated how ectomycorrhizal fungi are affected by variation in nitrogen availability. My overarching research question was if ectomycorrhizal fungi are affected in the same way by increased nitrogen availability in naturally fertile systems and in forests subjected to anthropogenic nitrogen additions. I have also examined how fungi are affected by forest fertilization and investigated whether changed carbon storage after fertilization was connected to shifts in the composition and functioning of the fungal community.

To answer my questions, I compared how ectomycorrhizal biomass and community composition changed along gradients of nitrogen availability in spruce forests in two regions, one in central and one in central Sweden. A key difference between the regions is that nitrogen deposition rates are much higher in the south than in the central part of Sweden. I also compared fungal communities in fertilized and non-fertilized pine forests.

The amount of ectomycorrhizal fungi decreased with increased nitrogen availability in the region with nitrogen deposition, but did not change along the natural fertility gradient. Different ectomycorrhizal fungi were affected in different ways by increased nitrogen availability. The abundance of species that could be considered expensive (from the trees point of view) along both gradients while another group, that are supposedly cheaper to associate with, seemingly benefitted from nitrogen deposition.

Forest fertilization increased carbon storage in both trees and soil. In addition, it supressed the abundance of ectomycorrhizal fungi, and one genus – *Cortinarius* – were particularly sensitive. Species within this genus have the ability to decompose low quality organic matter and the loss of these species after fertilization likely contributed to the increased soil C stock. Increased C storage in soils could be desirable as a means to mitigate climate change, but in the case of forest fertilization it would also entail negative effects on biological and functional diversity in boreal forests.

To conclude, my results suggest that nitrogen has different effects on ectomycorrhizal fungi depending on if the source is turnover of organic matter och if it has been supplied through anthropogenic activities. Ectomycorrhizal fungi seem to be positively affected by increased soil fertility, but negatively affected by anthropogenic nitrogen.

Populärvetenskaplig sammanfattning

De barrträdsdominerade boreala skogarna som täcker stora delar av norra halvklotet kännetecknas av mycket stark näringsbegränsning. Tillförseln av näringsämnen utifrån systemet (till exempel genom kvävefixering) är normalt väldigt liten och istället måste näringsämnen återvinnas från organiskt material i marken. Här spelar svampar en viktig roll eftersom de har förmågan att bryta ned organiskt material och därmed göra näringsämnen tillgängliga. Ektomykorrhizasvampar, som lever i symbios med trädrötter, är särskilt centrala i skogens näringsomsättning eftersom de transporterar näringsämnen, framför allt kväve, direkt till sina värdväxter i utbyte mot kol. De och andra svampar som lever i anslutning till växtrötter bidrar också i stor utsträckning till att kol långsiktigt lagras in i marken.

Graden av näringsbegränsning i boreala skogar varierar och har en stor påverkan på både artsammansättningen och tillväxten hos vegetationen. Boreala skogar kan därför vara allt från mycket kvävefattiga, lågproduktiva system som ofta är dominerade av tall, till kväverikare, högproduktiva grandominerade system med inslag av lövträd.

Artsammansättningen av ektomykorrhizasvampar varierar också beroende på kvävetillgängligheten. Detta beror på att olika ektomykorrhizasvampar är anpassade till att leva i olika typer av miljöer och dessa anpassningar har troligtvis att göra med hur ”dyra” de är för träd att leva i symbios med. Från ett träds perspektiv utgör kolet de investerar i ektomykorrhizasvampar en betalning för det kväve de får tillbaka från svampen. Beroende på olika egenskaper hos svamparna kan kolkostnaden variera och utöver det kan trädens benägenhet att investera i ektomykorrhizasvampar skifta beroende på kvävetillgängligheten i marken – den brukar minska om mängden tillgängligt kväve ökar.

Näringsomsättning i boreala skogar är en väldigt sluten process och regleras i stor utsträckning av olika samband mellan växter och nedbrytare av organiskt material. Dock har vi människor under det senaste århundradet intensifierat användandet av naturresurser vilket inte bara fått enorma konsekvenser för klimatet utan också bidragit till att mer reaktivt kväve tillgängliggjorts. Reaktivt kväve går snabbt att inkorporera i biologiska processer och har därför stor potential att påverka ekosystem. Detta är inte nödvändigtvis negativt – till exempel är användning av mineralgödselmedel en förutsättning för livsmedelsproduktion. Mineralgödsel används också, om än i relativt liten utsträckning, i skogsbruk för att stimulera trädtillväxt. Det finns dock tydliga negativa sidoeffekter av den ökade globala kvävetillgängligheten. Kvävedeposition bidrar till övergödning av hav, men också till att terrestra ekosystem, ibland dem boreala skogar får tillskott av kväve som riskerar att rubba de naturliga processerna i ekosystemet.

I den här avhandlingen har jag undersökt hur ektomykorrhizasvampar påverkas av förändrad kvävetillgänglighet. Min övergripande frågeställning har varit om ektomykorrhizasvampar i boreala skogar påverkas på samma eller olika sätt av ökad kvävetillgänglighet beroende på om ökningen beror på naturliga eller antropogena orsaker. Jag har även tittat på svampar påverkas av skogsgödsling samt undersökt om förändrad kollagring efter gödsling var kopplat till skiften i hur svampsamhället var uppbyggt och fungerade.

För att få svar på mina frågor undersökte jag hur biomassan och artsammansättningen av ektomykorrhizasvampar ändrades längs kvävetillgänglighetsgradienter i granskogar i två regioner, en i mellan- och en i södra Sverige. En avgörande skillnad mellan dessa regioner är att kvävedepositionen i södra Sverige är mycket högre än i Mellansverige. Jag jämförde även svampsamhällen i gödslade och ogödslade tallskogar.

Mängden ektomykorrhizasvampar minskade när kvävetillgängligheten ökade på grund av kvävedeposition men inte längs gradienten i naturlig bördighet. Olika sorters ektomykorrhizasvampar påverkade på olika sätt av ökad kvävetillgänglighet. Till exempel skedde en minskning av arter som kan betraktas som dyra i drift (från trädens perspektiv) längs båda gradienterna, medan det fanns en grupp, förmodat billigare svampar, som tycktes gynnas av kvävedeposition.

Skogsgödsling gjorde förutom att öka kollagringen i både träd och mark också att andelen ektomykorrhizasvampar minskade och ett släkte – *Cortinarius* (spindelskivlingar) – var särskilt känsliga. Arter inom dessa släkte har förmågan att bryta ned extra svårtillgängligt organiskt material och förlusten av dessa svampar i gödslade skogar bidrog sannolikt till att kollagringen i marken ökade. Ökad kollagring i marken skulle kunna vara eftersträvansvärt som ett sätt att försöka motverka klimatförändringarna men i fallet med skogsgödsling innebär det också negativa effekter på skogens biologiska och funktionella mångfald.

Sammanfattningsvis tyder resultaten i den här avhandlingen på att kväve påverkar ektomykorrhizasvampar på olika sätt beroende på om kvävet härstammar från omsättning av organiskt material eller om det introducerats till skogen på grund av mänsklig aktivitet. Ökad markbördighet verkar i allmänhet gynna ektomykorrhizasvampar medan antropogent kväve har en negativ effekt.

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Appendix

Table 3. *Area (1000 ha) fertilized productive forest in Sweden 2009-2019. Statistics for 2018 are missing since no data collection was made that year. Source: Swedish forest agency (Internal reference code JO1650).*

	2009	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**
Total	55.5	80.4	52.9	45.6	23.9	22.5	33.2	29.3	25.1	..	35.9
North Norrland	17.7	23.7	19.1	21.0	3.6	2.7	6.0	5.3	9.9	..	4.4
South Norrland	26.7	38.0	23.1	12.9	13.3	11.8	15.8	13.1	6.3	..	21.1
Svealand	10.7	15.3	10.7	11.6	6.8	7.6	11.1	9.9	8.2	..	10.0
Götaland	0.4	3.4	0.0	0.1	0.2	0.4	0.3	1.0	0.8	..	0.3

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REGULAR ARTICLE

Forest management to increase carbon sequestration in boreal *Pinus sylvestris* forests

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Abstract

Background and aims Forest management towards increased carbon (C) sequestration has repeatedly been suggested as a "natural climate solution". We evaluated the potential of altered management to increase C sequestration in boreal *Pinus sylvestris* forest plantations.

Methods At 29 forest sites, distributed along a 1300 km latitudinal gradient in Sweden, we studied interactive effects of fertilization and thinning on accumulation of C in standing biomass and the organic horizon over a 40 year period.

Results Abstention from thinning increased the total C stock by 50% on average. The increase was significant (14% on average) even when C in the removed timber was included in the total ecosystem C pool. Fertilization of thinned stands increased stocks similarly regardless of including (11%) or excluding (12%) removed biomass, and fertilization combined with abstention from thinning had a synergistic effect on C stocks that generated an increase of 79% (35% when removed timber was included in the C stock). A positive effect of fertilization on C stocks was observed along the entire gradient but was greater in relative terms at high latitudes. Fertilization also reduced soil respiration rates.

Conclusion Taken together, our results suggest that changed forest management practices have major potential to increase the C sink of boreal forests. Although promising, these benefits should be evaluated against the undesired effects that such management can have on economic revenue, timber quality, biodiversity and delivery of other ecosystem services.

Keywords Forestry · Fertilization · Thinning · Climate mitigation · Latitudinal gradient · Nitrogen

Introduction

The goal of net zero emissions of carbon dioxide (CO_2) by 2055 calls for drastically decreased emissions of greenhouse gasses, but also measures that increase removal of carbon (C) from the atmosphere (IPCC 2018). Forests are important global C sinks that annually sequester an estimated 2.4 Gt of C from the atmosphere (Pan et al. 2011), and thereby pivotal

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components of "natural climate solutions" (Canadell and Raupach 2008; Griscom et al. 2017). Recently, global afforestation to counter historical deforestation has been promoted as a possible means to mitigate climate change (Bastin et al. 2019), but mitigation can also be achieved by changed silvicultural practices (Canadell and Raupach 2008; Griscom et al. 2017). Since the capacity of forests to bind C is linked to stand dynamics and soil fertility, which can both be manipulated by directed management (Magnani et al. 2007; Tamm 1991), optimization of forest management towards increased C sequestration might be an important measure to reach net zero emissions within the next couple of decades.

The boreal forest is one of the largest terrestrial biomes, and about two thirds of the area is managed in some way (Gauthier et al. 2015). Young, actively growing trees in plantations have a major potential to store C in newly formed wood (Bastin et al. 2019). In addition, a combination of low temperature, recalcitrant litter and competitive inhibition of decomposers leads to accumulation of a purely organic O-horizon clearly separated from, and overlying, the mineral soil (hereafter: organic horizon) (Berg and McClaugherty 2014; Steidinger et al. 2019; Tamm 1991).The organic horizon, which is characteristic of boreal forests and sometimes referred to as the "mor layer", consist of both litter- and root-derived C (Clemmensen et al. 2013) and constitutes a significant store of C (Pan et al. 2011; DeLuca and Boisvenue 2012). The C stock of the organic horizon is relatively dynamic and possible to manipulate into increased C sequestration within decades (Moldan et al. 2006), whereas the around two thirds of the total soil organic C (SOC) that is stored in the mineral soil (Nilsson et al. 2017) are relatively stable (Rumpel et al. 2002; Schulze et al. 2009).

Most forests in Fennoscandia are intensely managed for biomass production, and thinning is a commonly used practice to improve timber quality and increase economical revenue during a forestry rotation period (Royal Swedish Academy of Agriculture and Forestry 2015). In essence, thinning shifts stand structure from many trees with low individual biomass towards fewer but larger trees by reducing competition and should not, at least theoretically, have a major effect on long-term C accumulation. This process occurs also without active management, as outcompeted trees die (i.e. self-thinning), but to a lesser extent than during active thinning operations (Westoby 1984). Nevertheless, thinning always has momentary negative effects on the standing biomass, and commonly results in lower standing biomass compared to when forests are left for self-thinning (Bergh et al. 2014). Despite that thinning reduces tree biomass, and presumably also C input into the soil, thinning effects on soil C stocks have appeared to be minor (Mayer et al. 2020). However, when evaluating over-all effects of thinning on long-term C accumulation, both standing biomass and soil, as well as C potentially stored in removed timber have to be included.

Although not as common as thinning, nitrogen (N) fertilization is a management alternative that can promote net C uptake in trees due to strong N limitation in most forests (Högberg et al. 2017; Hyvönen et al. 2008; Tamm 1991; Vitousek and Howarth 1991). Increased N input, through atmospheric deposition or fertilization, can also promote C sequestration in the organic horizon (Magnani et al. 2007; Hyvönen et al. 2008; de Vries et al. 2009; Maaroufi et al. 2015; Tipping et al. 2017). Fertilization can promote belowground C sequestration by stimulating inputs, but also by reducing decomposition (Janssens et al. 2010). This can be achieved either via changes in composition and activity of decomposer communities (Entwistle et al. 2017), or by interference with mycorrhizal symbiosis (Baskaran et al. 2017). However, as extensive thinning may decrease photosynthesis and C fluxes to biomass and soils, and thereby reduce the potential of fertilization to stimulate C sequestration, interactive effects between thinning practices and fertilization are likely and potentially important when guiding management towards increased C sequestration.

Increased N input can shift nutrient stoichiometry towards higher N:P ratios, which may lead to phosphorus (P) limitation and impaired growth, at least in more nutrient rich, temperate systems (Braun et al. 2010). Twenty years of N additions increased the N:P ratio in *Picea abies* needles, but not to such extent that growth was reduced, with no indication of P limitation (Palmqvist et al. 2020). On the other hand, Hyvönen et al. (2008) observed lower N use efficiency on tree growth in forests fertilized with N only compared to combined N, P and potassium (K) fertilization.

Although there is a prospect to manage boreal forests towards improved CO_2 withdrawal through thinning and fertilization, C sequestration is not the only ecosystem service that boreal forests provide. For example, production of high quality saw-timber may not always go hand in hand with maximized biomass production and C sequestration, and forest management often interacts and/or interferes with a number of other ecosystem properties with potentially conflicting goals (Strengbom et al. 2018). Leaching of nutrients, both during and after the forest rotation period, is of particular concern. Although boreal forests are strongly N limited (Högberg et al. 2017), additions of N may render them N saturated and cause leaching and eutrophication of water (Aber et al. 1989). There is also a major risk that forest thinning and fertilization operations lead to losses of biodiversity (Strengbom and Nordin 2008).

Here, we used a 40-year field trial that studied interactive effects of fertilization (N and P) and thinning across a boreal latitudinal gradient in Sweden to estimate the potential of managing boreal Scots pine (*Pinus sylvestris*) dominated forests towards increased C sequestration. We aimed to assess effects on C accumulation in standing tree biomass and the organic horizon, as well as in timber removed during thinning operations. To explore the mechanisms behind changes in belowground C stocks, we investigated the balance between C losses in the form of soil respiration and C inputs based on tree productivity. We also measured levels of inorganic N (nitrate and ammonium) in the soil.

Material and methods

Experimental design and tree monitoring

We used 29 Scots pine (*Pinus sylvestris* L.) dominated forest stands (percent pine of total stem volume: min 84%, median 100%) from a long-term thinning and fertilization experiment established along a 1300 km latitudinal gradient (56–67°N) in Sweden. The experiment was initiated between 1969 and 1982, when the sites were at canopy closure, i.e. at the time when operational thinning would be performed according to standard silvicultural practice. Depending on latitude, this occurred when the stands were between 32 and 54 years old. At the time of our field campaign in 2016, the stands were between 65–99 years old. The experimental plot net size was typically 25 × 40 m (0.1 ha) with a surrounding buffer zone of 10 m, and we sampled four treatments at each of the 29 sites: "no thinning", "thinning", "thinning + N" and "thinning + N + P". At nine sites distributed along the gradient, an additional treatment of "no thinning + N" was also sampled, making the total number of plots 125. Thinning operations occurred one to four times during the experimental period (Supplementary table 1), with the first thinning conducted when the trees were between 12 and 16 m high. Due to variation in initial basal area, the intensity of the initial thinning varied among sites, but corresponded on average to a 20–25% reduction in basal area. Subsequent thinning aimed to keep the basal area constant over time at around 18 m^2 ha^{-1} (Nilsson et al. 2010). Since the aim of the thinning was to achieve an even spatial distribution of healthy residual stems, trees with low vitality and severe damage were removed at the time of thinning as well as other species than pine. Only stems were removed, and tops and branches (including needles) were left in the plots. Nitrogen was added as ammonium nitrate (NH_4NO_3) at a rate of 100–150 kg N ha^{-1} every 5th year for the first 25 years, and thereafter every 7th year. Phosphorus was added as superphosphate ($CaSO4 + Ca(H_2PO_4)_3$) corresponding to 100 kg P ha^{-1} at the initiation of the experiment, and thereafter the same dose every 20–21 years. The experimental set-up is described in more detail in Nilsson et al. (2010) and Bergh et al. (2014), as well as in Supplementary table 1.

All trees within the plots were individually marked, and measured (height and diameter at breast height) at the initiation of the experiment as well as at the end of the study period. The diameter of trees removed during thinning, or by accidental windfalls, were measured at five or six occasions over the experimental period.

Sampling and chemical analyses of the organic horizon

All stands in the study had podzol soils with an organic horizon thickness ranging 2.5 to 11.7 cm (average: 5.9 cm), which had a C:N ratio of 25.2–62.8 (average: 40.6), and a pH of 3.3–4.6 (average: 3.7). In July 2016, we sampled the organic horizon (O-horizon) at 26 of the sites by pooling 25 soil cores (3 cm in diameter) collected in a grid pattern across each 0.1 ha plot. Due to logistic problems, 3 sites were sampled during the same period the following year. Living plants and litter (equivalent to the Oi horizon, commonly accounting for less than 5% of the C in the organic horizon (Sterkenburg et al. 2018)) were removed from the soil surface before sampling. The underlying mineral soil layer was removed together with larger roots (> 2 mm), and the organic horizon (Oe and Oa) of the 25 cores was pooled into a single composite sample per plot. The transition from organic horizon to mineral soil was generally sharp, but in cases where the transition was more gradual we chose to include the uppermost part of the mineral soil together with the organic horizon rather than discarding parts of the organic horizon. The soil samples were put in a cooler and frozen (-20 ℃) within 48 h.

Organic matter concentration (OM), mineral N availability, and C and N concentration in the organic horizon were determined from subsamples after homogenization of the frozen composite sample. OM content was determined by accounting for water content (drying at 105 ° for 24 h). A subsample was freeze-dried and finely ground in a ball mill before determination of C and N concentration in 0.4 g of soil in a combustion elemental analyzer (TruMac CN, LECO, Saint Joseph, MI, USA). Ammonium (NH_4^+-N) and nitrate (NO_3^--N) concentrations were analyzed from extracts with a 1:2.5 mass ratio of freshly frozen soil and 2 M KCl in an Autoanalyzer (BRAN-LUEBBE XY-2 Sampler, SEAL Analytical Inc., Australia) and scaled to mg N m^{-2} (sampled soil dry weight × inorganic-N concentration, divided by the area of the 25 sampling cores).

Carbon stocks

The organic C stock of the organic horizon was calculated as soil dry weight (DW) × C concentration, and scaled up to tonnes (t) ha^{-1} based on the added area of the 25 sampling cores. Biomass of trees was estimated using allometric functions based on plot average tree stem diameter and height (Marklund 1988) (Supplementary Material and Method) for Scots pine, Norway spruce and birch (as a proxy for all deciduous trees) multiplied by the number of trees. For biomass of trees removed during thinning, the height of the trees was estimated (Laasasenaho 1982) before using the allometric functions. For belowground biomass of the trees, we used the allometric functions developed by Petersson and Ståhl (2006). Our allometric functions provided biomass estimates of wood, branches and needles, stump and coarse roots (> 2 mm). The fine root biomass (< 2 mm) was included as part of the organic horizon pool. The above allometric functions have been shown to be very robust along latitudinal and fertility (i.e., stand index) gradients in Sweden. Marklund (1988) reported only marginally improved predictability when adding one of these predictors. For example, the R^2 value of the pine stump model increased from 0.972 to 0.978 when including latitude as a predictor. Hence, our biomass estimates should not be biased across the latitudinal gradient or treatments, with the cautionary note that the allometric functions have not been tested for highly fertilized trees.

We estimated C stocks in standing and removed biomass by assuming a 50% C-content (Neumann et al. 2016). In the ecosystem calculations, we included C stock in trees (including coarse roots) and in the organic horizon but assumed no changes in C stocks in the mineral soil and shrub layer. Changes in C stocks in response to management were calculated in two different ways. In the first approach, we only considered C that was stored at site, i.e. in standing biomass, roots and organic horizon. In an alternative approach, we also included C in wood removed during thinnings, which potentially may be stored outside the forest, in timber or forestry products. In reality, the degree and durability of C storage in removed wood depend on its subsequent fate. A proper life cycle analysis (LCA) was outside the scope of this study, and our two approaches can be interpreted as boundary estimates. However, assuming a 30–46-24% distribution of harvested biomass between wood products, paper/pulp and fuel for energy production, respectively (Lundmark et al. 2014), and an average half time of 30 years for C in wood products and 2 years for C in paper/pulp or fuelwood (IPCC 2006), the expected half-time of C stored in forest products can be estimated to around 10 years.

Soil respiration

Soil CO_2 flux (R_s) measurements were recorded in 2017 on rain-free days between June 29th and July 29th in all treatments except "thinning + NP", summing up to 96 measured plots. We measured soil R_s from south to north to track phenological advancement of the vegetation, so that all sites were measured at a similar phenological stage. In each plot, we measured R_s at ten evenly distributed positions, distanced at least 0.5 m from the plot edge and from the nearest tree. Based on a pilot power analysis on R_s measurements from 20 locations within a 0.1 ha plot, similar to the experimental sites, we concluded that ten samples would be sufficient to detect a 15% change in R_s from 100 mg C m^{-2} h^{-1}.

R_s was measured in a closed chamber constructed by a PVC collar (diameter = 30 cm, height = 14.5 cm, internal volume = 10.2 dm^3). To minimize the influence of aboveground plant respiration, we removed all living ground vegetation before gently pushing the chamber 0–1 cm into the soil. R_s was estimated by use of a portable soil respiration chamber and a portable infrared CO_2 gas analyzer (Vaisala GMP343). CO_2 concentration was recorded at 15-s intervals during 3 min. To calculate CO_2 exchange rate, we fitted a quadratic relationship between CO_2 and time, where the linear term is the estimated exchange rate. A quadratic model gives a more accurate exchange rate (Kutzbach et al. 2007), and during field measurements, we noticed that the increase in CO_2 concentration levelled off with time. R_s was calculated on an area basis (mg C m^{-2} h^{-1}) accounting for chamber temperature and volume according to standard equations (Kutzbach et al. 2007).

After each R_s measurement, we recorded soil water content (WC) and soil temperature within the collar. We measured WC with a soil moisture sensor (Meter GS3 probe with a Pro-check reader) and used the mean value of four recordings. To measure soil temperature, a digital lab thermometer (TFA LT-101) was inserted into the organic soil layer and left for one minute before recording the temperature.

Statistical analysis

To test the effect of treatments and environmental variables on response variables, we used linear mixed models (LMM) in the lme4 package (Bates et al. 2015) of R 3.5 (R Core Team 2018). Thinning was included as a fixed factor, while N application was included as a continuous variable corresponding to the accumulated N load at the time of the field sampling, to account for variation in N load between sites. To make interpretation easier, N application was standardized by the mean N application. Thus, one unit change in N application (i.e. the effect size in our models) corresponded to the mean N application (881 kg ha^{-1}) and the standardized range (0.6 – 1.7) corresponded to an actual range of (500 – 1200 kg ha^{-1}). We included P addition as a factor (not for the R_s model), but as this treatment always was applied in combination with the N and thinning treatments, its additive effect was modelled in combination with these treatments. To assess if the effect of the management regimes changed across the latitudinal gradient, we ran models where we included the interactive effect of treatment and latitude. Site was included as a random factor, and for the R_s model, treatment plot was added as an additional random factor nested under site to account for multiple samples within a plot. R_s is modified by soil temperature and moisture (Davidson et al. 1998), and we therefore included these variables as covariates to control for local effects. As expected, these variables had an influence on R_s, but due to a technical failure, soil water content was not recorded at two sites. To be able to include these sites in the full model, we employed an imputation method implemented in the R package amelia (Honaker et al. 2011), which creates many data sets with imputations, run individual models, and finally average these models to account for extra variation due to imputation. Given the high replication, we calculated effect-size uncertainties (95% confidence interval) and P-values based on Wald statistic. Residuals were visually checked for homogeneity and normality. The residual variance of the R_s model increased with R_s, wherefore R_s was log-transformed. We also plotted the soil temperature at the time of R_s measurement against latitude to evaluate if there were any systematic differences in temperature across the latitudinal transect (Supplementary Fig. 1). Tables were produced using the sjPlot package (ver 2.6.2) (Lüdecke 2018). Treatment effects are evaluated relative to thinning without fertilization, which is the standard silvicultural practice in Swedish forestry.

Results

First we only considered C stored at the site, as a boundary estimate that assumes that all C in removed biomass is rapidly returned to the atmosphere. Fertilization combined with abstention from thinning increased the C stock by 90 t C ha^{-1} (CI: 80–100) (+79%) over a 40-year period, compared to the standard practice of thinning without fertilization (Fig. 1a, Supplementary table 2). A major fraction of the increased sequestration (84 t C ha^{-1}, CI: 74–93) occurred through increased production and retention of standing biomass, while increased C storage in the organic horizon accounted for a smaller proportion (6 t C ha^{-1}, CI: 3–9). Abstaining from thinning alone increased the total C stock by 57 t C ha^{-1} (CI: 50–64) (+50%) but had no significant effect on soil C. Both biomass production and organic horizon C storage were stimulated by N additions. The increase was most pronounced when fertilization and abstention from thinning were combined, as indicated by a significant interaction effect. Repeated N fertilization, on average 881 (500 – 1200) kg N ha^{-1} over the 40 year period, increased the ecosystem C stock by 12 t C ha^{-1} (CI: 5–19) (+11%) in thinned stands, and by 33 t C ha^{-1} (CI: 23–42) (+19%) without thinning (Fig. 1c). Complementation of the N fertilization with P had no effect on tree growth, but increased the organic horizon C stock in thinned stands by 3 t C ha^{-1} (CI: 1–5) relative to N fertilization only (Fig. 1a, Supplementary table 2).

In a second scenario, we included the C that had been removed from the stands during thinning in the total C stocks, as a boundary case where all removed C was assumed to remain sequestered in stored wood and forest products (Fig. 1c, Supplementary table 2). Fertilization combined with abstention from thinning then increased the C stock by 53 t ha^{-1} (CI: 45–61) (+35%) compared to the standard practice of thinning without fertilization. Inclusion of the C storage in thinned timber did not eliminate the positive effect of abstention from thinning, indicating that total production was higher in stands left without thinning. Furthermore, the positive effect of fertilization on C sequestration was again higher without thinning compared to thinned stands.

When we modelled the potential of changed management regimes across latitudes, we found that the positive effect of N addition on above- and below-ground C sequestration was highest in the low-productive ecosystems in the northern parts of the boreal zone (Fig. 2, Supplementary table 3). The interaction between latitude and thinning was associated with a large uncertainty (Organic horizon: $P=0.69$, Net tree production: $P=0.13$). For simplicity and robustness of the model, we therefore removed this interaction term from the model.

The combination of fertilization and abstention from thinning decreased soil respiration by 19% (CI: 5–31) compared to the unfertilized, thinned stands (Fig. 3, Supplementary table 4). The other treatments had no significant effect. Within treatments, soil C stocks were not positively linked to tree growth; in fact, there was a marginally significant negative correlation (Fig. 4, Supplementary table 5). Latitude had no influence on soil respiration ($P=0.85$) and was excluded from the model.

Mineral N levels were slightly elevated, although not statistically significant, after N fertilization. This increase was entirely ascribed to ammonium, whereas the contribution of nitrate was low and generally close to the detection limit (Fig. 5, Supplementary table 6).

Discussion

Compared to the standard silvicultural practice of thinning, both abstention from thinning and fertilization had a major influence on C accumulation in Scots pine forests. While the main purpose of thinning in forestry is to increase timber quality and provide early economic revenue during a forest rotation cycle, thinning may also reduce competition for nutrients and light, which in turn is expected to increase growth of the remaining trees (Royal Swedish Academy of Agriculture and Forestry 2015). Here, thinning reduced over-all C accumulation in stands irrespective of whether C in harvested timber was included or not. This result is in accordance with other thinning trials in boreal Fennoscandian (Mäkinen and Isomäki 2004), Mediterranean (Bravo-Oviedo et al. 2015), Central European (Seidl et al. 2007), and temperate regions of central North America (Powers et al. 2011). Without fertilization, we found no effect of thinning on the C stock in the organic horizon. Similarly, a meta-analysis concluded that, although thinning commonly reduces litter input in coniferous

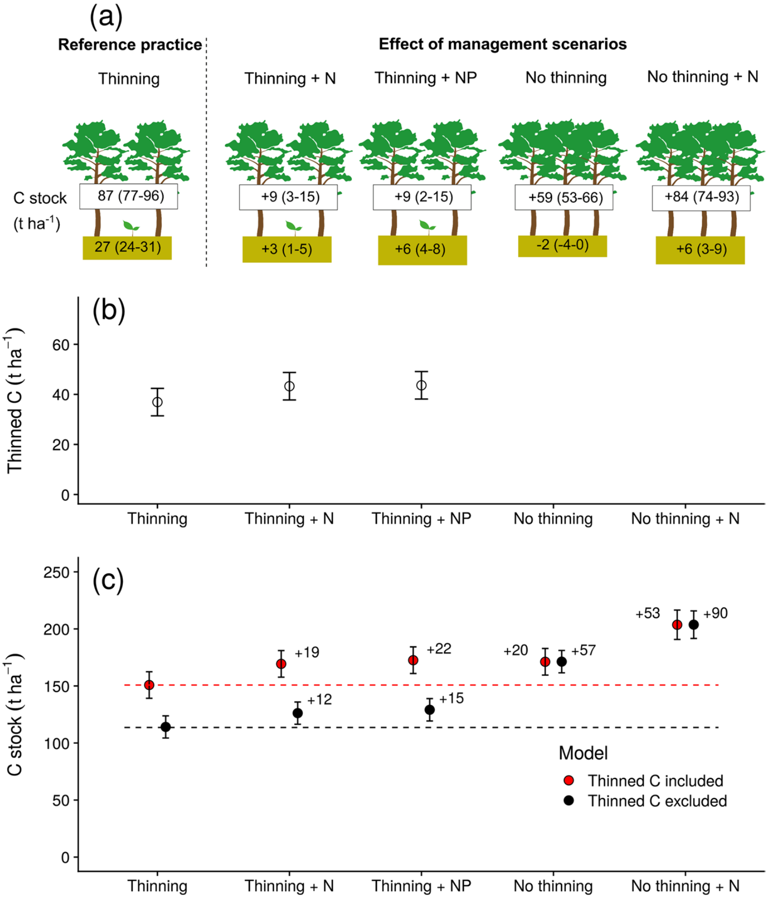


Fig. 1 Effects of different management scenarios on C stocks (trees, organic horizon), C removed by thinning, and total ecosystem C gain. (**a**) Tree and organic horizon C stocks in stands subjected to the standard silvicultural practice of thinning and the effect of different alternative management scenarios, applied over a period of 40 years. (**b**) C removed during thinning operations. (**c**) Ecosystem C stocks under different management scenarios and associated effects on net C sequestration (numbers beside the points). Red symbols represent total C stocks that includes removed thinned wood, and black symbols represent standing C stocks without removed wood. Dashed lines show the standard silvicultural practice of thinning without fertilization. Points represent adjusted means and numbers in parentheses (**a**) and error bars (**b**, **c**) represent 95% confidence intervals. The average N application corresponds to 881 kg ha^{-1} applied over circa 40 years. See table S2 for statistics

forests, the effects on the soil C stock are often small (Zhang et al. 2018).

The strong positive effect of fertilization on tree growth confirms earlier findings from boreal forests (Bergh et al. 2014; Högberg et al. 2017; Hyvönen et al. 2008; Tamm 1991; Valinger et al. 2000). The stimulation of C sequestration in trees was, however, not large enough to compensate for C losses during thinning. Similarly, Valinger et al. (2000) found that C stocks in thinned stands matched those in self-thinned stands only under moderate thinning intensities, even when productivity was boosted by fertilization. The C sink in tree biomass was also more responsive to fertilization without thinning, presumably as the larger standing biomass and larger leaf area could exploit more of the additional N to increase productivity.

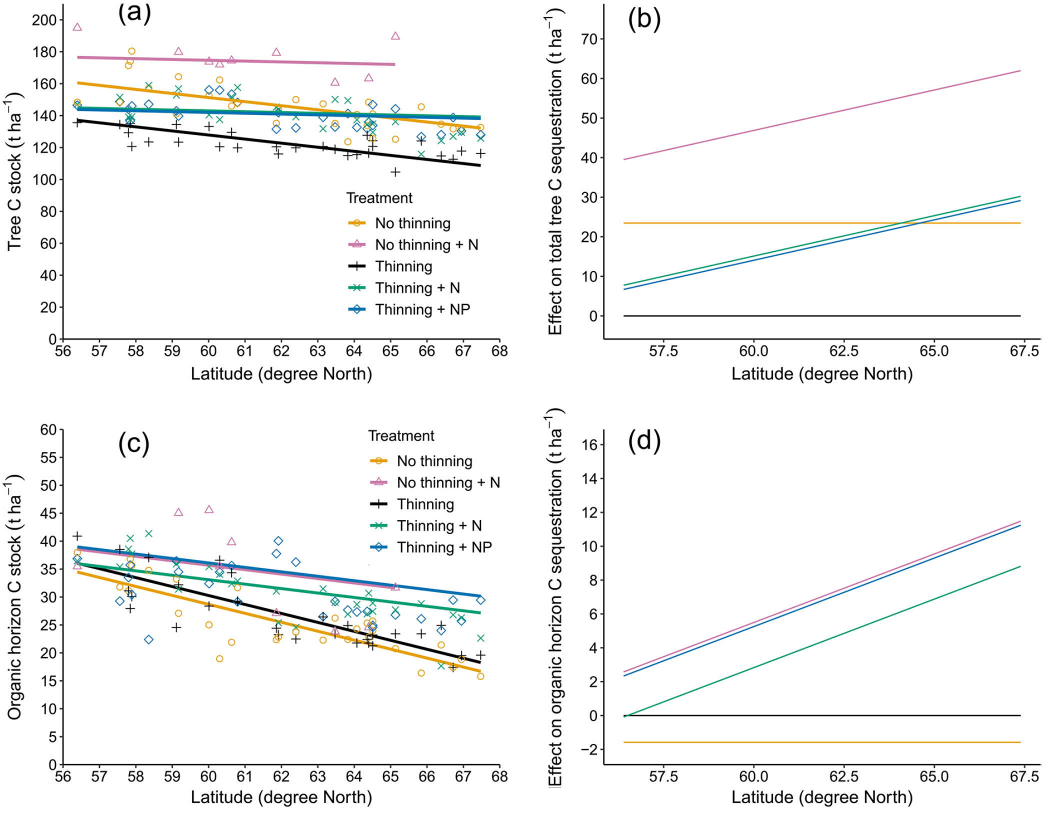


Fig. 2 C stocks in (**a**) trees (including C removed by thinning) and (**c**) organic horizon, and model estimates of treatment effects on (**b**) tree net C uptake, and (**d**) organic horizon C accumulation. Colors represent different management scenarios and their effects compared to the standard silvicultural practice of commercial thinning without fertilization, indicated in black. See Supplementary table S3 for statistics

The observed increases in organic horizon C stocks after nutrient addition are in line with previous studies (Hyvönen et al. 2008; Janssens et al. 2010; Maaroufi et al. 2015). Although the belowground C sink was much smaller than sequestration in tree biomass, changes may still be more important from a climate mitigation perspective, as they can be assumed to represent a more stable and long-lasting form of C sequestration (Kyaschenko et al. 2019; Rumpel et al. 2002; Schulze et al. 2009). Nevertheless, the long-term stability of the soil C sink induced by fertilization remains an open question.

The observed decrease in respiration following fertilization (Fig. 3) and the increase in the organic horizon C stock (Fig. 1a) indicate decreased rates of decomposition, corroborating previous studies (Janssens et al. 2010; Maaroufi et al. 2015; Zak et al. 2008). If anything, soil C stocks correlated negatively with tree productivity within treatments (Fig. 4), supporting the idea that variation in C input has marginal influence on belowground C accumulation (Lajtha et al. 2018; Terrer et al. 2021). Rather, variation in decomposition rates (Janssens et al. 2010; Kyaschenko et al. 2017b; Stendahl et al. 2017), in particular of root derived C (Clemmensen et al. 2013; Kyaschenko et al. 2019) seems to play a dominant role in regulating the below-ground C pool. Increased N availability after fertilization may decrease the need for microbial exploitation of organic stocks for nutrients, leading to decreased

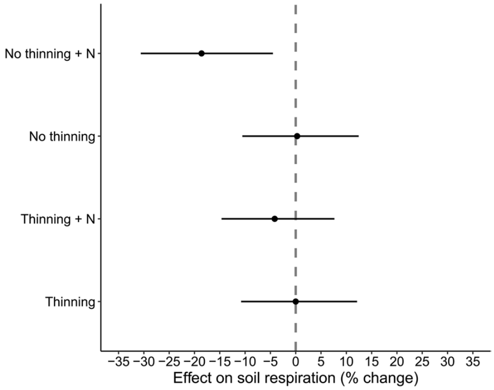
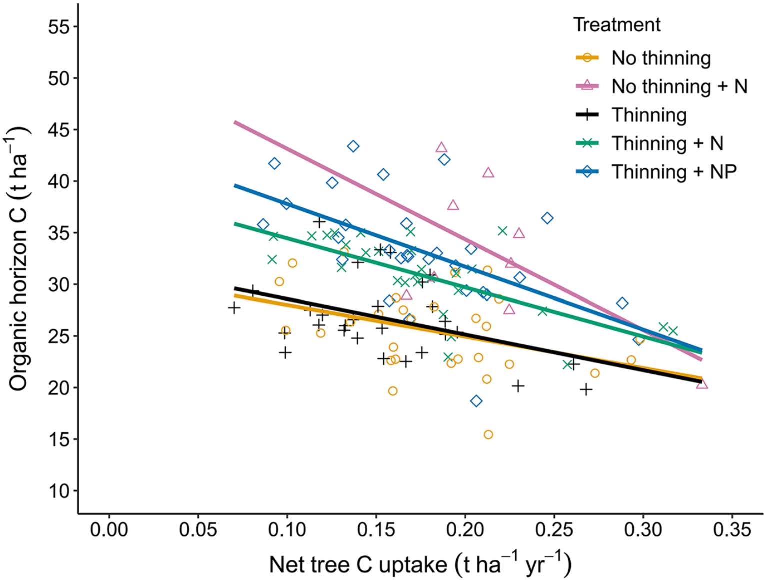


Fig. 3 Model estimates of treatment effects on soil respiration, calculated on a per area (R_s) basis, are adjusted for variation in soil temperature and water content. Effects are related to the standard silvicultural practice of thinning without fertilization. Bars indicate 95% confidence intervals. See Supplementary table S4 for statistics and Supplementary file 3 for estimates

decomposition (Entwistle et al. 2018; Terrer et al. 2021). In boreal forests, ectomycorrhizal fungi have been proposed to regulate belowground C accumulation through their mining (i.e. decomposition) of organic matter for N (Clemmensen et al. 2013; Lindahl and Tunlid 2015; Sterkenburg et al. 2018). Negative effects of fertilization on mycorrhizal fungi specialized in exploitation of organic nutrients (Baskaran et al. 2017) may shift both functioning and composition of fungal communities (Högberg et al. 2010; Lilleskov et al. 2002; Maaroufi et al. 2019). However, we observed no decrease in respiration after fertilization of thinned stands, which is in agreement with the lower C accumulation observed in these stands. Potentially, thinning increases availability of high C:N substrates in the form of dead roots as well as branches and needles left in the plot, which may maintain C supply and N limitation of the decomposer community. Thus, similarly to aboveground responses, the positive effect of fertilization on below-ground C sequestration was highest in the absence of thinning.

Surprisingly, supplementation of N fertilization with additional P increased organic horizon C sequestration even further (Fig. 1a). We do not have respiration data for the plots supplemented with P, but we speculate that the higher C stock is a result of further reduced decomposition. When N limitation is alleviated by fertilization, P may become a limiting factor (Aber et al. 1989; Braun et al. 2010) that could stimulate microbial decomposers to mine P from organic matter (Forstner et al. 2019; Widdig

Fig. 4 Relationship between soil C stocks and tree productivity (t yr^{-1} ha^{-1}) within different treatments. See Supplementary table S5 for statistics



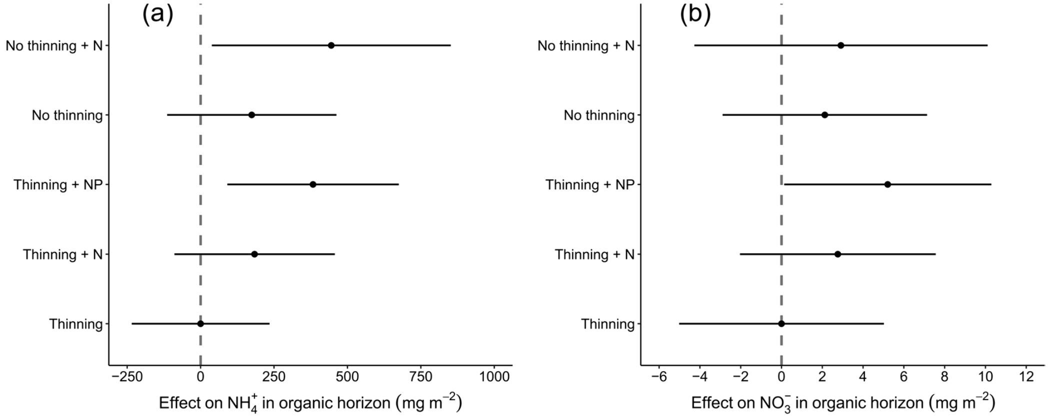


Fig. 5 Treatment effects on extractable **a**) ammonium and **b**) nitrate in the organic horizon. Bars indicate 95% confidence intervals. See Supplementary table S6 for statistics

et al. 2019). However, (Forsmark et al. 2020) did not find any evidence for that higher microbial P mobilization in response to N enrichment would affect soil C storage.

A potential problem with adaptation of forest management to increase soil C stocks is the possibility that a substantial part of the accumulated soil C stock may be lost after clear-felling (James and Harrison 2016; Nave et al. 2010). Soil temperature and moisture are key factors controlling decomposition rates (Swift et al. 1979), and clear-felling influence both (Jansson 1987), with a risk of destabilization of soil C. Below-ground C stocks also depend on plant-soil feedbacks and are expected to be sensitive to the removal of mycorrhizal trees at harvest (Kyaschenko et al. 2017a). However, whether loss of mycorrhizal activity stabilize or destabilize the organic matter, remains unresolved (Frey 2019; Sterkenburg et al. 2018).

N fertilization affected C sequestration at all latitudes, but the marginal effect of additional N supply was larger at higher latitudes (Fig. 2). Nutritional constraints on C cycling may be stronger at higher latitudes, with nutrients locked up in recalcitrant organic pools and mycorrhizal interactions playing a central role in organic matter dynamics (Clemmensen et al. 2013; Lindahl and Tunlid 2015). Fertilization may reduce the impetus for mycorrhiza-driven exploitation of organic nitrogen and associated organic matter oxidation (Bödeker et al. 2014). In addition, anthropogenic N-deposition at the southern sites has altered soil C:N ratios, which may lower effects of additional nutrient enrichment at the southern sites in our experiment (Van Sundert et al. 2018).

Taken together, our results support previous findings and demonstrate that changed management can promote C sequestration, achieving significant gains within decades and, potentially, contribute towards the zero net-emission goal. We identified abstention from commercial thinning combined with fertilization as a particularly efficient way to increase C sequestration. Such management seems to have great potential as a “natural solution” to counter climate change. The effect of management on C stocks differed substantially depending on whether the C in harvested timber was included or not (Fig. 1). This highlights the importance of the downstream use of extracted biomass (Baul et al. 2017), *i.e.* if the removed wood is used primarily in products with a long residence time, or to produce short-lived products, such as paper. A rough calculation estimates that C in harvested biomass returns to the atmosphere after, on average, 10 years, but substitution of fossil fuels may add further to climate change mitigation. A proper LCA is needed to fully assess the climate change mitigation potential of forestry, a task that, however, goes beyond the scope of this paper. In this study, we have only examined the effects on C stocks in the system, without considering the C-cost of implementing the different management options. However, LCAs on the production of NH_4NO_3 as a fertilizer in agriculture estimated the C footprint to circa 1.7 kg C kg N^{-1} (6.2 CO_2 eq.), which would amount to circa 1.5 t C ha^{-1} in our study (Skowrońska and Filipek 2014), thus having a rather small impact on the net C accumulation.

Due to their strong N limitation, boreal forests have high capacity to retain external N input (Binkley and Högberg 2016; Kjønaas and Wright 2007; Näsholm et al. 2013). However, at some point of N input the N retention capacity will be saturated, resulting in increased nitrification and the risks of gaseous or leaching losses of N, with subsequent increased risk of eutrophication of neighboring ecosystems (Aber et al. 1989). The high mobility of nitrate in soils makes nitrification especially problematic, but the high N retention of boreal forests likely makes them resistant to high rates of N input (Ring et al. 2011). Although we observed somewhat elevated ammonium concentrations in the fertilized plots, nitrate levels were generally below the detection limit (Fig. 5). Thus, we suspect that most of the fertilizer applied during the 40-year experimental period (500 – 1200 kg N ha^{-1}) was retained in soil and biomass, and that the experimental sites remain below N saturation.

Although promising, management alterations to increase C sequestration should be evaluated against trade-offs in the delivery of other ecosystem services provided by boreal forest. For example, there is a risk that forestry directed towards increased C sequestration, via fertilization and abstention from commercial thinning, leads to biodiversity loss (Allison et al. 2007; Strengbom et al. 2011), as well as reduced delivery of other ecosystem services, such as production of wild berries and reindeer fodder (Strengbom et al. 2018). However, the net loss of such services can probably be reduced if C-oriented management is constrained to secondary forests, exposed to clear-cut forestry, which presumably have already lost large parts of their original values.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-021-05038-0.

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Authors' contributions JS, GG and BL conceived the study. GG, JS and KJ collected the data and GG and KJ performed the analyses. KJ wrote the first draft and all authors contributed to interpretation and writing.

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Data availability Data supporting this article are available through Zenodo: https://doi.org/10.5281/zenodo.4971647

Code availability Code to reproduce the analyses is avaibable through Zenodo: https://doi.org/10.5281/zenodo.4971647

Declarations

Competing interests The authors declare no competing interests.

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Supplementary information

Supplementary table 1. *Site information.*

Site ID	Latitude (°N)	Birth of stand	Experiment established (1st thinning)	N load (kg) (dose in parenthesis)	Last N fertilization	Total P load (kg)	Last P fertilization	Last Revision	Thinnings	No thinning + N treatment
787	60,8	1941	1973	1050 (150)	2011	200	1997	2015	1981, 1988, 1997, 2015	
895	60,3	1936	1970	1050 (150)	2015	200	2001	2011	1977,1987, 1999, 2011	x
900	58,4	1931	1966	1200 (150)	2011	300	2011	2007	1974, 1987, 1999, 2007	
902	60,0	1933	1969	1200 (150)	2014	300	2014	2007	1978, 1989	x
912	57,8	1930	1969	1200 (150)	2014	300	2014	2016	1981, 1995, 2006	
918	60,6	1923	1970	1200 (150)	2015	300	2015	2007	1984, 2000	x
922	57,9	1922	1967	1200 (150)	2012	300	2012	2007	1975, 1991, 2007	
923	57,8	1933	1973	1050 (150)	2011	200	1997	2007	1985, 1997, 2007	
926	64,4	1921	1969	800 (100)	2014	300	2014	2015	1982, 1996	x
927	65,1	1925	1969	800 (100)	2014	300	2014	2015	1982, 1993	x
929	59,2	1926	1970	1200 (150)	2015	300	2015	2016	1982, 1998, 2006	x
931	57,6	1936	1973	1050 (150)	2011	300	1997	2006	1982, 1995	
933	59,1	1920	1973	1050 (150)	2011	200	1997	2015	1990, 2003	
935	61,9	1935	1972	700 (100)	2016	200	2002	2007	1978, 1992, 2007	
936	61,9	1939	1974	700 (100)	2012	200	1998	2007	1985, 1998	x
938	64,1	1917	1974	700 (100)	2012	200	1998	2016	1991, 2005	
940	56,4	1922	1969	1200 (150)	2014	300	2014	2013	1982, 1996, 2007	x
945	63,5	1952	1973	600 (100)	2016	200	2009	2015	1985, 1993, 2001	x
946	64,5	1943	1976	700 (100)	2014	200	2000	2015	1985, 1993	
947	64,5	1943	1978	700 (100)	2016	200	2002	2015	1987, 1996	
951	66,9	1927	1975	700 (100)	2013	200	1999	2015	1988, 2005	
952	66,7	1922	1975	700 (100)	2013	200	1999	2015	1992, 2007	
991	64,4	1937	1976	700 (100)	2014	200	2000	2015	1985, 1996,	
994	67,5	1928	1978	700 (100)	2016	200	2002	2015	1994	
996	62,4	1946	1973	600 (100)	2012	200	2005	2016	1981, 1988, 1997	
999	63,1	1942	1976	700 (100)	2014	200	2000	2015	1985, 1994	
1000	65,8	1946	1981	600 (100)	2012	200	2005	2016	1986, 1992, 2000	
1005	63,8	1944	1980	600 (100)	2011	200	2004	2016	1987, 1996	
1006	66,4	1950	1978	500 (100)	2010	200	2010	2015	1986, 1996, 2005	

Supplementary table 2. *Treatment effects on C stocks (t ha^{-1}) of results presented in Fig. 1a. Numbers in parentheses indicate 95% CI intervals. Significant p-values in bold.*

	Standing tree C		**Soil C (t ha-1)**		**Removed tree C**		**Total ecosystem C gain - excluding removed C**		**Total ecosystem C gain – including removed C**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
Intercept (Thinned)	86.91 (77.39 – 96.43)	**<0.001**	27.19 (23.87 – 30.51)	**<0.001**	36.91 (31.44 – 42.39)	**<0.001**	114.08 (104.37 – 123.79)	**<0.001**	150.82 (139.19 – 162.45)	**<0.001**
Nitrogen	8.89 (2.70 – 15.08)	**0.005**	3.17 (1.01 – 5.34)	**0.004**	6.34 (3.48 – 9.21)	**<0.001**	12.10 (5.38 – 18.82)	**<0.001**	18.52 (13.36 – 23.69)	**<0.001**
No thinning	59.13 (52.71 – 65.55)	**<0.001**	-1.93 (-4.18 – 0.31)	0.092			57.22 (50.24 – 64.20)	**<0.001**	20.37 (15.01 – 25.72)	**<0.001**
P added (Thinned, Nitrogen)	0.05 (-6.41 – 6.52)	0.987	2.90 (0.64 – 5.16)	**0.012**	0.37 (-2.57 – 3.30)	0.806	2.93 (-4.09 – 9.95)	0.414	3.23 (-2.16 – 8.61)	0.240
Nitrogen x No thinning	15.78 (5.22 – 26.35)	**0.003**	4.55 (0.85 – 8.25)	**0.016**			20.31 (8.83 – 31.78)	**0.001**	13.93 (5.11 – 22.74)	**0.002**
Observations	125		125		87		125		125	

Supplementary table 3**.** *Latitudinal effects on C stocks of results presented in Fig. 2. Latitude is centralised at the middle of the gradient. Numbers in parentheses indicate 95% CI intervals. Significant p-values in bold.*

	Soil C (t ha^{-1})		Total tree net C	
Predictors	*Estimates*	*p*	*Estimates*	*p*
Intercept (Thinned)	26.90 (23.84 – 29.95)	**<0.001**	122.48 (111.11 – 133.85)	**<0.001**
Nitrogen	4.53 (2.26 – 6.80)	**<0.001**	19.42 (14.57 – 24.27)	**<0.001**
No thinning	-1.58 (-3.73 – 0.57)	0.150	23.46 (18.87 – 28.05)	**<0.001**
P added (Thinned,Nitrogen)	2.50 (0.33 – 4.67)	**0.024**	-0.99 (-5.61 – 3.64)	0.676
Latitude	-1.60 (-2.50 – -0.71)	**<0.001**	-2.55 (-6.03 – 0.92)	0.150
Nitrogen x No thinning	4.17 (0.65 – 7.69)	**0.020**	8.22 (0.70 – 15.74)	**0.032**
Nitrogen x Latitude	0.81 (0.32 – 1.30)	**0.001**	2.04 (1.00 – 3.09)	**<0.001**
Random Effects				
σ^2	18.02		82.04	
τ_{00}	52.92 $_{\text{site}}$		895.44 $_{\text{site}}$	
N	29 $_{\text{site}}$		29 $_{\text{site}}$	
Observations	125		125	

Supplementary table 4**.** *Effects on soil respiration of results presented in Fig. 3. Numbers in parentheses indicate 95% CI intervals. Soil respiration data was log-transformed prior to statistical analysis. Significant p-values in bold.*

	Soil respiration	
Predictors	*Estimates*	*p*
Intercept (Unthinned)	5.85 (5.74 – 5.96)	**<0.001**
Nitrogen	-0.04 (-0.15 – 0.07)	0.441
Thinned	0.003 (-0.11 – 0.11)	0.959
Nitrogen x Thinned	- 0.17 (-0.35 – 0.02)	0.075
Soil water content	-1.33 (-1.82 – -0.83)	**<0.001**
Soil temp (deg C)	-0.04 (-0.09 – -0.01)	**0.013**
Random Effects		
σ^2 - within_plot	0.207	
γ – within_site	0.024	
τ_{00} site	0.054	
Observations	931	

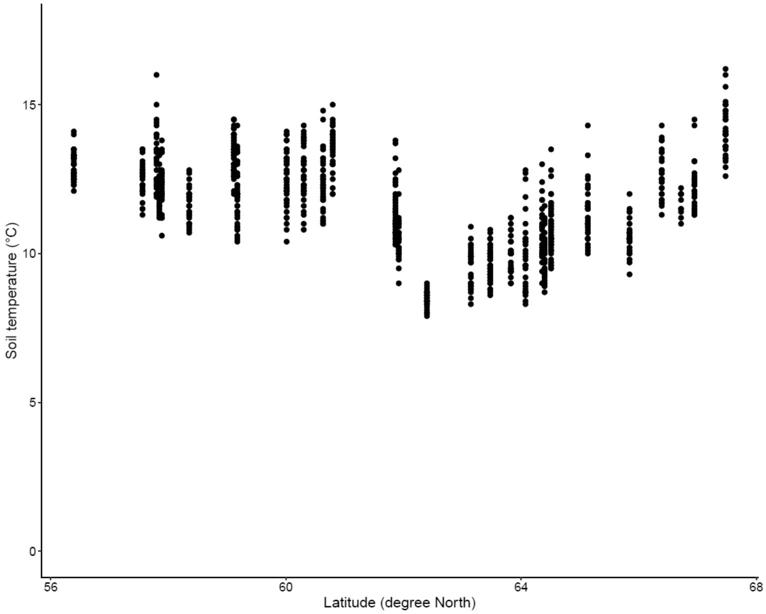
Supplementary table 5. *Relationship between O-horizon C and biomass C of results presented in Fig. 4. Numbers in parentheses indicate 95% CI intervals. Significant p-values in bold.*

	Soil organic carbon (t ha^{-1})	
Predictors	*Estimates*	*p*
Intercept (No thinning)	31.05 (20.23 – 41.87)	**<0.001**
Nitrogen	20.86 (4.33 – 37.40)	**0.013**
Thinned	0.97 (-7.32 – 9.26)	0.819
Nitrogen x Thinned	8.16 (-0.13 – 16.45)	0.054
P (Thinning, Nitrogen)	12.81 (4.35 – 21.28)	**0.003**
Net tree C uptake	-30.55 (-86.74 – 25.64)	0.287
Nitrogen x Net tree C uptake	-57.28 (-133.22 – 18.66)	0.139
Thinned x Net tree C uptake	-3.89 (-51.61 – 43.82)	0.873
Nitrogen x Thinned x Net tree C uptake	-17.01 (-61.15 – 27.12)	0.450
P added (Thinned, Nitrogen) x Net tree C uptake	-30.30 (-75.85 – 15.26)	0.192
Random Effects		
σ^2	19.83	
$\tau_{00\ \mathrm{site}}$	65.89	
N $_{\mathrm{site}}$	29	
Observations	125	

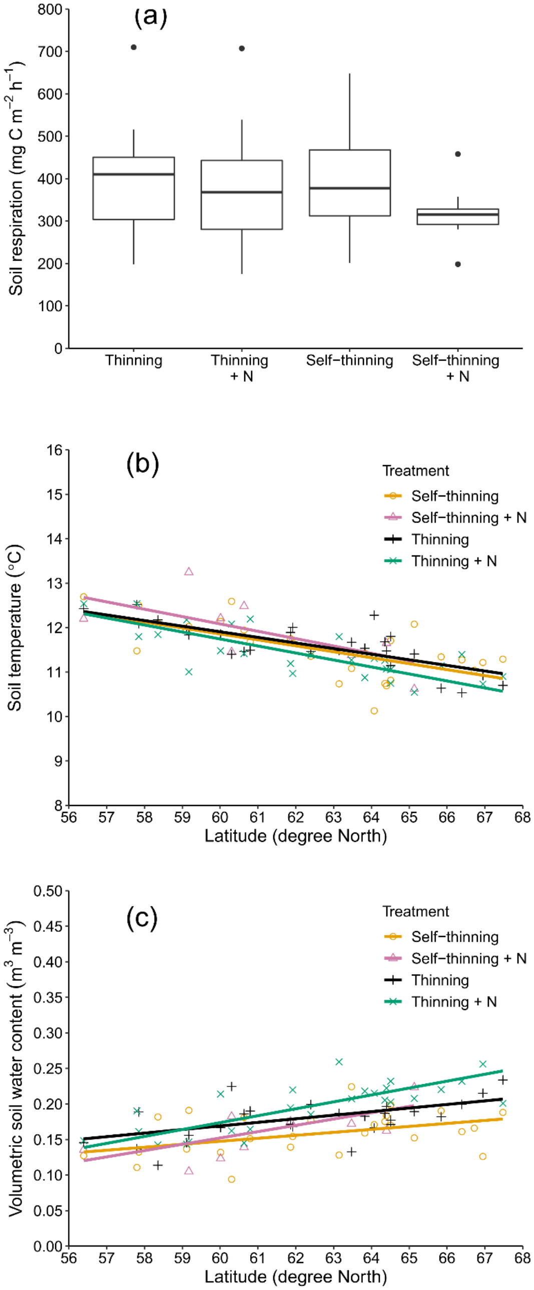
Supplementary table 6. *Effects on mineral N availability of results presented in Fig. 5. Numbers in parentheses indicate 95% CI intervals. Significant p-values in bold.*

	NH_4^+-N (mg m^{-2})		NO_3^--N (mg m^{-2})	
Predictors	*Estimates*	*p*	*Estimates*	*p*
Intercept (thinned)	278.7523 (44.4237 – 513.0809)	**0.020**	1.2876 (-3.7287 – 6.3038)	0.615
Nitrogen	183.7991 (-89.0324 – 456.6306)	0.187	2.7605 (-2.0360 – 7.5569)	0.259
No thinning	174.1885 (-113.7707 – 462.1477)	0.236	2.1235 (-2.8852 – 7.1322)	0.406
P added (Thinning, Nitrogen)	199.0190 (-90.5577 – 488.5958)	0.178	2.4477 (-2.5914 – 7.4867)	0.341
Nitrogen x No thinning	86.9168 (-382.6980 – 556.5316)	0.717	-1.9710 (-10.1850 – 6.2430)	0.638
Random Effects				
σ^2	331341.08		99.77	
τ_{00}	112830.40 $_{site}$		98.03 $_{site}$	
N	29 $_{site}$		29 $_{site}$	
Observations	125		125	

Supplementary figure 1. Organic horizon temperature at the time of R_s measurement plotted against latitude.



Supplementary figure 2. Measured R_s data



Supplementary Material and Methods

Additional M&M regarding estimation of standing biomass based on allometric functions:

Standing biomass was estimated based on the functions listed below. All functions estimate the biomass for an individual tree, and to get biomass per hectare, we multiplied the tree values with the stem density (per hectare) for each plot. Aboveground biomass is the sum of stem, living- and dead branches, and stump and we used equations from Marklund (1988). For belowground biomass, coarse roots (>2mm) were estimated applying equations from Petersson and Ståhl (2006) and fine roots (<2mm), were included in the soil cores sampled for estimates of soil C storage). Standing biomass of deciduous trees were estimated based on functions for birch. Deciduous trees were uncommon, and on average, their aboveground biomass never exceeded 3% of the aboveground biomass of Scots pine.

Standing biomass for Scots pine

$$\text{Stem (including bark): Biomass (kg)} = e^{7.5939\frac{d}{d+13}+0.0151h+0.8799\log_e h-2.6768}$$

$$\text{Living branches (incl. needles): Biomass (kg)} = e^{13.3955\frac{d}{d+10}-1.1955\log_e h-2.5413}$$

$$\text{Dead branches: Biomass (kg)} = e^{7.127\frac{d}{d+10}-0.0465h+1.106\log_e h-5.8926}$$

$$\underset{\text{(from ground to 1\% of tree height)}}{\text{Stump}}\text{: Biomass (kg)} = e^{11.06537\frac{d}{d+15}-3.9657}$$

$$\text{Coarse roots (>2 mm): Biomass (kg)} = \frac{e^{11.06537\frac{10d}{10d+113}+3.44275}}{1000}$$

Standing biomass for Norway spruce

$$\text{Stem (including bark): Biomass (kg)} = e^{7.4690\frac{d}{d+14}+0.0289h+0.6828\log_e h-2.1702}$$

$$\text{Living branches (incl. needles): Biomass (kg)} = e^{10.9708\frac{d}{d+13}-0.0124h-0.4923\log_e h-1.2063}$$

$$\text{Dead branches: Biomass (kg)} = e^{3.6518\frac{d}{d+18}-0.0493h+1.0129\log_e h-4.6351}$$

$$\underset{\text{(from ground to 1\% of tree height)}}{\text{Stump}}\text{: Biomass (kg)} = e^{10.6686\frac{d}{d+17}-3.3645}$$

$$\text{Coarse roots (>2 mm): Biomass (kg)} = \frac{e^{10.44035\frac{10d}{10d+138}+4.58761}}{1000}$$

Standing biomass for birch

$$\text{Stem (including bark): Biomass (kg)} = e^{8.2827\frac{d}{d+7}+0.0393h+0.5772\log_e h-2.67689}$$

$$\text{Living branches (incl. needles): Biomass (kg)} = e^{10.2872\frac{d}{d+10}-3.3633}$$

$$\text{Dead branches: Biomass (kg)} = e^{11.2872\frac{d}{d+30}-0.3081h+2.6821\log_e h-6.6237}$$

$$\underset{\text{(from ground to 1\% of tree height)}}{\text{Stump}}\text{: Biomass (kg)} = e^{11.0481\frac{d}{d+5}-3.9657}$$

$$\text{Coarse roots (>2 mm): Biomass (kg)} = \frac{e^{10.01111\frac{10d}{10d+225}+6.17080}}{1000}$$

d= diameter at breast height (1.3m) in centimetres, h= tree height in meter.

ACTA UNIVERSITATIS AGRICULTURAE SUECIAE

DOCTORAL THESIS NO. 2021:69

Ectomycorrhizal fungi are pivotal for nutrient cycling in boreal forests, and variation in endogenous and anthropogenic nitrogen supply influences their community composition and functioning. Ectomycorrhizal communities and their ecological roles were found to be responsive to shifts in nitrogen availability based on DNA-based characterization and estimates of biomass, enzymatic activities and ecosystem carbon stocks. The main conclusion is that anthropogenic nitrogen supply has a major and different impact on ectomycorrhizal fungi compared to variation in natural soil fertility.

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