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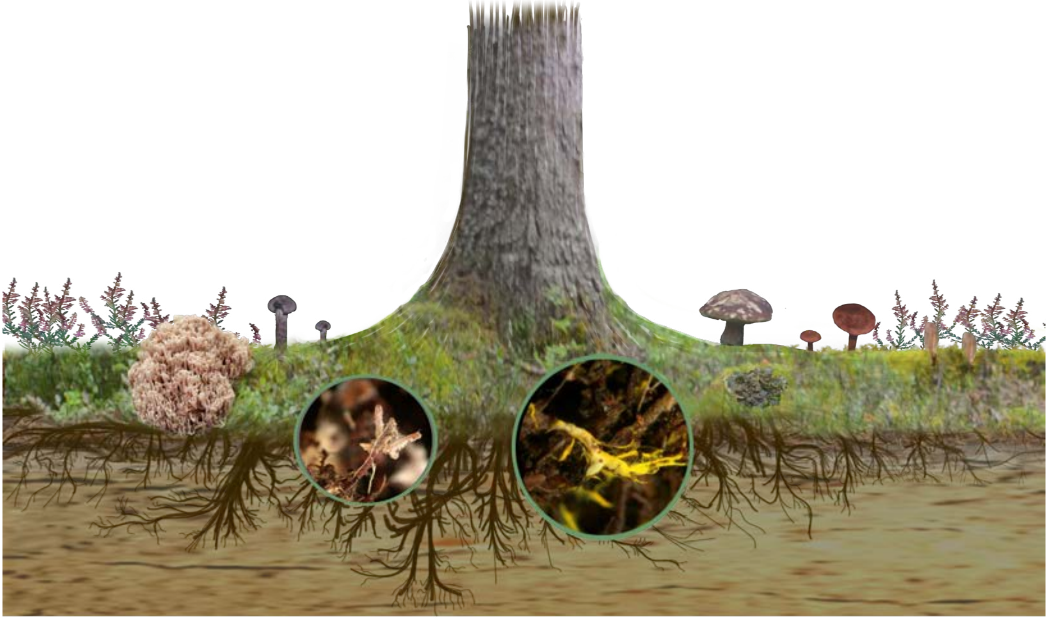

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Abstract

The immense diversity and biomass of ericoid-, ectomycorrhizal, and saprotrophic fungal guilds in boreal forest soils make them vital components of conservation and ecosystem processes, and in particular, many ectomycorrhizal fungi are considered species of conservation concern. However, amalgamated information on the functions and relationships of soil fungi to perceived forest conservation values, and how inter and intra-guild interactions affect the accretion and decomposition of soil organic matter is lacking. In a long-term factorial shrub removal and pine root exclusion experiment, I assessed guild contributions to soil respiration and decomposition of organic substrates guided by ecological theory. Then in the northern and southern boreal forest, I evaluated whether forest conservation values are aligned with the diversity of ectomycorrhizal fungi. Overall, the ericoid guild makes a significant contribution to total soil respiration (11 ± 9%), and ericoid activities appeared to be more sensitive to periods of drought compared to ectomycorrhizal (43 ± 1%) and saprotrophic (53 ± 5%) guilds. Saprotrophic-ectomycorrhizal interactions during decomposition led to a modest, yet inconsistent Gadgil effect (10%) for early-stage litter decomposition. Ericoid and ectomycorrhizal guilds interactions were determined to be more important for late-stage organic matter balance in boreal forest soils. Ectomycorrhizal species richness was significantly higher in the southern boreal forest compared to the north. Furthermore, forest conservation values across the boreal forest were not adequately related to ectomycorrhizal diversity through DNA-metabarcoding. Instead, soil fertility, corresponding to tree species basal area, was the clearest indicator of ectomycorrhizal diversity and composition in both regions. Mycorrhizal guilds may be underappreciated and understudied in terms of conservation, but their functional roles in the accumulation and decomposition of organic matter in long-term soil carbon pools emphasizes the importance of evaluating the many dimensions of fungal conservation in boreal forests.

Keywords: Mycorrhizal fungi, soil, boreal forest, functional guild, respiration, decomposition, conservation values, ITS, high throughput sequencing, biodiversity

Funktionella grupper och bevarandevärden hos mykorrhiza i boreala skogar

Abstract

Marksvampar spelar en central roll för uppbyggnad och nedbrytning av organiskt material i boreal skogsmark och innehåller både ett stort antal nedbrytarsvampar och symbiontiska mykorrhizasvampar. Eftersom skogsmark lagrar mer kol än de sammanlagda förråden i växterna och atmosfären kan en förändrad artsammansättning och funktion av marksvampsamhället få stora konsekvenser. Många mykorrhizasvampar är dessutom rödlistade och därmed skyddsvärda. Kunskapen om marksvamparnas funktioner och hur funktionerna är relaterade till skogens generella naturvärde är dock bristfällig. Därför undersökte jag hur olika funktionella grupper av svampar bidrar till markrespiration och nedbrytning av organiskt material. Jag använde mig av ett faktoriellt försök där ljungväxter togs bort och trädrötter exkluderades. Jag undersökte även om dagens metoder för bedömning av naturvärden kan användas för att identifiera skogar med hög mångfald av marksvampar. Jag jämförde ektomykorrhizasvampars artrikedom i de södra och norra boreala skogsregionerna genom markprovtagning och massekvensering. Mina resultat visar att interaktioner mellan nedbrytarsvampar och mykorrhizasvampar är av liten betydelse för de senare faserna av förnanedbrytning. Däremot är interaktioner mellan ljungassocierade och ekto(träd)mykorrhiza viktiga för senare stadier av förnanedbrytningen, vilket i sin tur påverkar mängden organiskt material (och kol) som blir kvar i marken. Gruppen ljungassocierad mykorrhiza bidrar till markrespirationen (11 ± 9 %) och kan även påverkas av klimatförändringar. Artrikedomen av ektomykorrhizasvampar var signifikant högre i skogar från den södra boreala regionen än i den norra regionen. Den fanns ingen eller mycket svag koppling mellan bedömda naturvärden och mångfald av ektomykorrhizasvampar vilket visar att dagens sätt att bedöma naturvärden inte kan användas för att bevara mångfald av mykorrhizasvampar. Även om kunskapen kring hur olika funktionella grupper av mykorrhizasvampar påverkar kolomsättningen i boreala skogar är bristfällig och att de i dagsläget inte inkluderas i skogliga naturvårdsstrategier, visar mina resultat att förändrad artsammansättning kan ha betydelse för boreala skogars kolinlagring. Detta understryker betydelsen av en bättre förståelse av marksvamparnas många olika bevarandebiologiska värden.

Keywords: mykorrhizasvampar, naturvårdsbedömning, ljungväxter, kolbindning, sandtallskog, tajga, skyddsvärd skog, biologisk mångfald, skogsbiologernas

Dedication

To my grandmother – for your wonderful spirit and support.

> "*Biological relations attending the formation of mycorrhiza are so complicated that there are almost as many interpretations of the nature of mycorrhiza as there are scientists working on this*"
>
> -Erik Björkman (1970)

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. Mielke, L. A., Ekblad, A., Finlay, R. D., Fransson, P., Lindahl, B. D., & Clemmensen, K. E. (2022). Ericaceous dwarf shrubs contribute a significant but drought-sensitive fraction of soil respiration in a boreal pine forest. Journal of Ecology, 00, 1–14.

II. Mielke, L. A., Lindahl, B., Klein, J., Finlay, R., Ekblad, A., Clemmensen, K. Mycorrhizal guild interactions, rather than the Gadgil effect, slow decomposition of mor-layer humus. (manuscript)

III. Mielke, L. A. Hekkala, A.-M., Jönsson, M., Sjögren, J., Kärvemo, S., Strengbom, J., Clemmensen, K. Conservation value assessments do not reflect ectomycorrhizal fungal diversity in the boreal forest. (manuscript)

The contribution of Louis A. Mielke to the papers included in this thesis was as follows:

I. Maintained the long-term experiment, performed field and laboratory work, and did all the data analysis. Interpreted results with support from co-authors. Wrote the manuscript and made revisions with support from co-authors. Responsible for journal correspondence.

II. Maintained the long-term experiment, designed and performed field and laboratory work, and did all the data analysis. Interpreted results with support from co-authors. Wrote the manuscript and made revisions with support from co-authors.

III. Contributed to the design of the study. Designed, organized and conducted all soil sampling field work, performed all laboratory work and data analysis. Interpreted results with support from co-authors. Wrote the manuscript and made revisions with support from co-authors.

Abbreviations

BA	Basal area
C	Carbon
CCA	Constrained correspondence analysis
CO_2	Carbon dioxide
CUE	Carbon use efficiency
DCA	Detrended correspondence analysis
DD	Data Deficient
ITS	Internal transcribed spacer
LC	Least Concern
MnP	Manganese peroxidase
N	Nitrogen
NE	Not Examined
NT	Near Threatened
PCR	Polymerase chain reaction
VU	Vulnerable
$\delta^{13}C$	Isotopic signature of Carbon
$\delta^{15}N$	Isotopic signature of Nitrogen

1. Peeling up the mycelial mat

An introduction

An ecosystem consists of the interactions and processes of all the living and non-living components, and everything in-between (Tansley 1935). Our observations of natural phenomena are essential to understanding why nature is the way it is, what patterns emerge across different scales, and ultimately, how dynamics on Earth might alter our ecology. What people learn from ecology – or the study of eco, meaning our "home," depends on the questions we ask, the theory that has developed, and the assumptions we make. I urge any reader to peel up a mat of moss and humus covering the bedrock of pine or spruce forest to look for the hyphal threads of fungal mycelium (*Figure 1*). There is a lot going on beneath our feet, and people have been postulating the significance of how these hidden organisms shape our soils for well over a century.

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Figure 1. Mycelial mat of a *Piloderma sp.* on the underside of a lifted moss and litter layer in Norbotten, Sweden. The hymenia (i.e. spore-bearing surface) of the fungus is facing down in the soil, and it may facilitate spore dispersal when the wind moves the trees, and consequently the roots, so that spores get pushed out from the air in the soil into the atmosphere around. Photo by Karina Clemmensen.

The soil is both a home – and a grave – to millions of species and multitudes of individual organisms. In its totality, soil is one of the most important resources for society whether we realize it or not (Bardgett & van der Putten 2014). Land use change from expanding food production, increasing bioenergy consumption, warming temperatures, eutrophication, and increasing frequency and/or severity of droughts, are some of the important factors that will continue to effect soil dwelling organisms. Essentially, soil organisms, and their response to environmental change, are critical for the balance of soil organic matter formation and decomposition, and in effect, the stability of atmospheric concentrations of CO_2 (Averill et al. 2022). In the midst of Anthropocene, nature-based solutions are considered an integrated approach to mitigate climate change and biodiversity loss; however, both synergies and trade-offs between biodiversity and carbon storage need to be considered because they may not be mutually inclusive (Seddon et al. 2021).

The organic matter retained in topsoil, while providing important habitat for many species, holds more carbon globally than plants and the atmosphere combined (Jackson et al. 2017). In the boreal forest, this pattern is amplified: for example, the amount of soil organic matter is up to 5 times larger in the soil compared to aboveground biomass (Pan et al. 2011). At the same time, there is a much higher fungi:plant ratio in boreal forests than other parts of the world (Tedersoo et al. 2014; Wardle & Lindahl 2014). Underneath the boreal canopy and understory, soil carbon is held mainly in the form of particulate organic matter, making it an important resource especially vulnerable to disturbances compared to mineral-associated organic matter in other soils on the European continent (Lugato et al. 2021).

The accretion and decomposition of soil organic matter is not a passive process, but vitally dependent on the traits of different organisms, climate, soil type, and complexity of the organic matter itself (Chapin et al. 2011; Lehmann et al. 2020). Organisms do not just adapt to their environment, but rather they actively shape their surroundings through time (*Figure 2*). From this perspective, organismal-environmental interactions are dialogical and a co-evolutionary process.

What are fungi, and what is a guild?

In this thesis, I focus on fungi. A common misconception, due to historical groupings of organisms published and popularized by Carl von Linné, is that fungi are more closely to plants than animals. However, phylogenetic approaches comparing ribosomal small subunit RNA demonstrated more genetic similarity of fungi to animals (Wainright et al. 1993). Like animals, fungi are an extremely diverse group of heterotrophic organisms that rely on other organisms and substrates for energy. Fungi acquire energy by extracellularly breaking down and/or incorporating simple sugars from dead or living tissues from a wide range of organisms into cells in dissolved forms. The spore is a basic unit of reproduction, relying on passive forms of dispersal by wind, water or other creatures to move around. Unlike plants, fungi do not photosynthesize, and their cell walls are composed of chitin. Some fungi are unicellular, single-celled organisms, such as yeasts. Filamentous fungi contain hyphae, which are micro-meter thin linear structures of one or more cells that form multicellular conglomerates called mycelium, and in many cases forming some of the largest and oldest terrestrial organisms on the planet (Smith et al. 1992). The highest estimated amounts of fungal biomass on the planet are found within boreal forests and tundra ecosystems (He et al. 2020). Ecologists and biologists tend to categorize fungi into functional groups or ‘guilds’ based on what they do (Nguyen et al. 2016), and into phylogenies based on how closely related they are. The guild is a well-established concept that groups species by particular functions or lifestyles that are not necessarily related phylogenetically (Schimper & Fisher 1902). In this thesis, I will focus on three fungal guilds: saprotrophs, ectomycorrhizas and ericoid mycorrhizas, because of their diversity and importance in nutrient cycling in boreal forest soils (Read 1991; Wardle & Lindahl 2014).

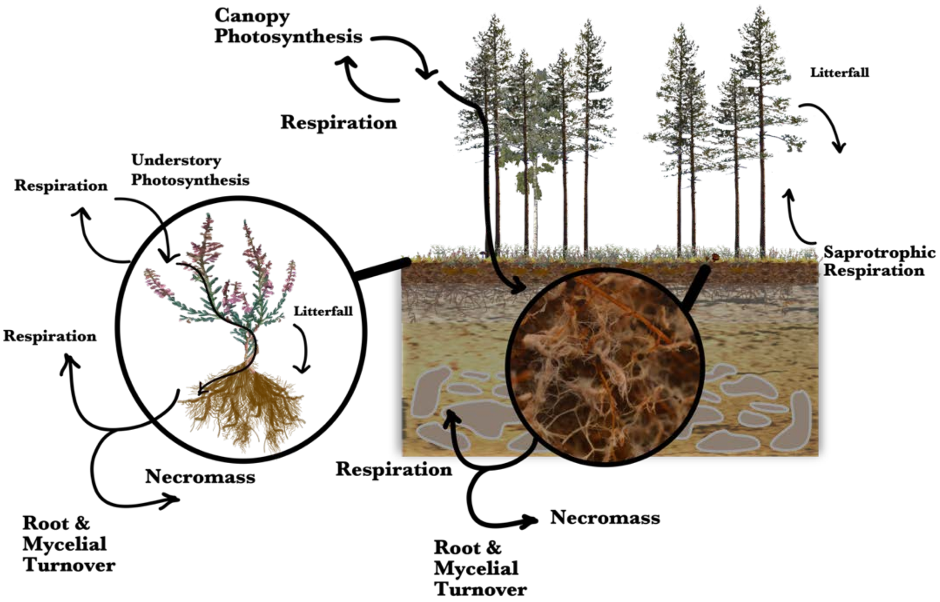

Figure 2. Saprotrophic, ericoid and ectomycorrhizal functional guild biomass, necromass and activities (turnover and respiration) contribute to carbon cycling in a boreal forest.

1.1 The ecology of saprotrophic soil fungi

Saprotrophic fungi specifically live by breaking down dead organic matter. The word saprotroph is derived from Greek ‘saprós’ meaning rotten and ‘trophē’ meaning nourishment. Some saprotrophic fungi have a filamentous form such as the bread mould *Neurospora crassa* (Shear & Dodge) or the common button mushroom *Agaricus bisporus* (J.E. Lange) Imbach. Some have a unicellular yeast form, such as species in the genus *Exophiala* (Carmich) that can live in extremely arid environments in melanized forms (Rosa & Péter 2006; Warren et al. 2019). Within the large diversity of fungi, most are known as saprotrophs (Põlme et al. 2021), and they are the most well-studied group.

In the boreal forest, free-living saprotrophic fungi reside mainly in the surface litter layer where they exploit recently shed plant litter for high-quality organic molecules (Lindahl et al. 2007). Accordingly, saprotrophic fungal biomass regulates leaf litter decomposition across spatial scales (Bradford et al. 2017). Although some species may dominate, decomposition does not typically happen in isolation. There may be several saprotrophic fungi that persist in dead moss or on a pine needle. Species of saprotrophic fungi may also be specialized to certain substrates depending on their niche.

Saprotrophic functional diversity

The diversity of decomposing strategies among saprotrophic fungal species is important in regulating decomposition (van der Wal et al. 2013; Baskaran et al. 2019). Opportunistic saprotrophic fungi, such as those in the orders *Mortierellales* and *Eurotiales*, are fast-growing moulds in the phyla *Mucoromycota* and *Ascomycota*, respectively, and persist on simple sugars. Many of these fungi are involved in the decomposition of fungal necromass (Kennedy & Maillard 2022). Saprotrophic fungi are also the main decomposers of coniferous wood and litter (Boddy 1999), and are therefore important in nutrient cycling in the boreal forest. Initially, fungi in the phylum *Ascomycota* dominate pine litter, such as the anamorphic taxa *Chalara longipes* (Preuss) Cooke. These fungi typically have hydrolytic enzymes that are involved in the breakdown of cellulose (Boberg et al. 2011). As litter or wood becomes depleted in hydrolysable carbon by primary decomposers, co-metabolic oxidation by secondary basidiomycetous decomposers is required to break-down recalcitrant complexes, advancing

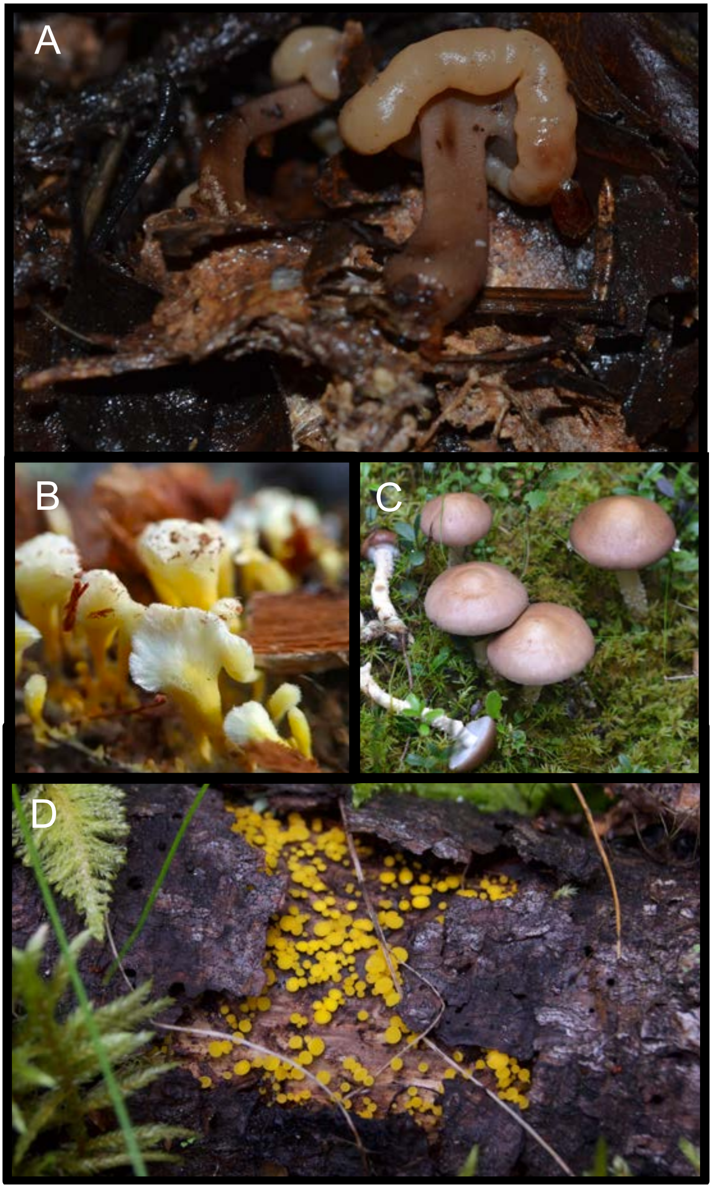

Figure 3. Saprotrophic fungi including *Cudonia* Fr*.*, the litter ascomycete (**A**), the threatened, putative saprotroph (VU) *Stereopsis vitellina* under decaying pine deadwood (B), the soil dwelling basidiomycete *Stropharia hornemanii* (**C**) and the commonly found *Bisporella citrina* on decayed wood (**D**).

decomposition (Baldrian & Valaskova 2008; Boberg et al. 2011; Lindahl & Tunlid 2015). Many saprotrophic fungi, especially those in the phylum *Basidiomycota*, such as species in the genus *Mycena* (Pers.) Roussel, are able to grow extensively across substrates by forming mycelial chords, or rhizomorphs, which are a tube-like aggregation of hyphae that can connect distinct nutrient patches (Boddy 1999). *Mycena* are a part of a larger class of fungi, known as the Agaricomycetes, where lignin-degrading peroxidases had evolutionarily emerged during the Carboniferous period (Floudas et al. 2012). White-rot fungi (*e.g., Phanerochaete* and *Mycena spp.*) excrete extracellular enzymes such as Manganese (Mn) peroxidases, which reduce H_2O_2 while oxidizing Mn^{2+} to Mn^{3+} and can degrade all components of plant cell walls, including lignin (Eriksson et al. 1990). Brown rot fungi, (*e.g.* such as several independent lineages in the order *Boletales*), alter the composition of organic substrates by non-enzymatic oxidation, which selectively removes structural carbohydrates from lignin (Presley & Schilling 2017). This mechanism does not lead to mass loss *per se*, but is important for the progression of lignin rich substrates without the support of white rot mechanisms (Lindahl & Tunlid 2015). Ultimately, there are many different features of saprotrophic fungi, and the diversity in decomposing strategies of particular species have been widely studied, but the interactions between other functional guilds and even between multiple organisms is lacking.

1.2 The ecology of mycorrhizal fungi

Current research and concepts about mycorrhizas and their functions are based on a long heritage of observations, theory, and experimentation. Before international publishing standards in the 1980s, most studies concerning local plant and fungal communities were written up in national or regional journals, making biological literature extremely polyglot (Summerbell 2005). Still there is a lot of evidence from early literature building a solid foundation of mycorrhizal ecology.

Fungal cells on roots were first microscopically observed on non-chlorophyllus orchid roots like *Neottia nidus avis* (L.) Rich. and other plants from early in the 19th century (Rayner 1926), but the term 'mycorrhiza' was originally used by Alfred Herbert Frank in a seminal paper in 1885. The species he had been studying were a group of hypogeous-gasteroid false truffles, *Elaphomyces* (T. Nees), which were thought to be parasites of Scots pine *Pinus sylvestris* (L.) (Frank 1885; Rayner 1926; Frank & Trappe 2005). Through a pot experiment, Frank found evidence for what was believed to be a benefit for both the plant and fungus, and therefore instigated the view that mycorrhizas represent a mutualistic symbiosis where both the fungus and plant host rely nutritionally on one another (Frank 1885; Frank & Trappe 2005). Mycorrhiza is derived from Greek where 'mykes' means fungus and 'rhiza' means root (Frank 1885; Read et al. 2000). The newly formed concept of symbiosis at that time from de Bary encompassed both mutualist and antagonistic interactions (de Bary 1879; Oulhen et al. 2016), but fungi on roots had been previously been considered parasites or agents of disease and decay. Some researchers had modified their view of the relationship between plants and fungi from parasitism to a mutualism in the mid 1800s before Frank (Tulasne & Tulasne 1862); however, it was Franks terminology and output that eventually popularized the concept. The concept of beneficial fungal-root mutualisms festered a few decades before it was broadly accepted (Rayner 1926) possibly due to scientific white-outs during periods of war (Summerbell 2005). de Bary had described that there was every possible gradation between parasitic and supportive organisms at the time of defining symbiosis, and as time went on, the term was used more and more to describe a mutualistic beneficial relationship between two very different organisms (Harley & Smith 1983). In the same manner, the perception of parasites has diverged to be synonymous with that of pathogens (Harley & Smith 1983; Kaishian et al. 2022).

Over time, the fundamental physiological differences between free-living saprotrophic fungi and mycorrhizal fungi have been distinguished and blurred. In some instances, mycorrhizal fungi are grouped with autotrophs because they provide an extension of the root system that can access nutrients in microscale spaces smaller than the diameter of fine roots in exchange for carbon from their photosynthetic hosts (Melin 1925; Finlay & Read 1986), while in other cases they are thought of more as heterotrophic because they decompose and depend on exogenic carbon (Lindahl & Tunlid 2015). It has been argued that instead of a categorical assessment, fungal species should be placed along a biotrophy-saprotrophy continuum (Koide et al. 2008). Similarly, fungal decomposition across the biotrophy-saprotrophy continuum has also been difficult to assess. A fungus could be very dependent on recent, photosynthetically-derived host carbon and efficient at decomposing. The opposite could be true; a fungus could be less dependent on host carbon and inefficient at decomposing organic substrates. In this regard, fungi could also fit into a spectrum of varying decomposition capacity, which may or may not be aligned with saprotrophic habits. In a way, there are multiple different classifications of mycorrhizal types that have evolved independently throughout evolutionary history (Harley & Smith 1983).

1.2.1 The ectomycorrhizal guild

The ectomycorrhizal or ‘ectotrophic’ symbiosis is formed between a wide phylogenetic range of soil fungi and the fine roots of plants that are typically woody perennials (Harley & Smith 1983), although there are some interesting exceptions in graminoids (Michelsen et al. 1998). Ectomycorrhizal fungi have independently evolved an estimated 80 times from saprotrophic ancestors, including at least 36 independent evolutions in the mushroom forming class of Agaricomycetes (Sanchez-Garcia et al. 2020). Once forming a ectomycorrhizal habit, it seems unlikely, but not impossible, to reverse to saprotrophism or parasitism, although the fungal fossil record and functional annotations of many species are still lacking (Ryberg et al. 2022).

Within the root, the fungus grows between the root epidermal and cortical cells forming a Hartig net, enlarging the surface area of contact between host and fungus to allow efficient transfer of metabolites; on the outside of the root, the mantle or sheath develops with contiguous hyphae, emanating into surrounding substrates (Agerer 2001; Read & Smith 2008). The web-like structure on roots was originally described as purely antagonistic by the forest pathologist Theodor Hartig decades before the theory of the beneficial mycorrhizal symbiosis was developed (Frank 1885; Rayner 1926). Later on, Melin's laboratory experiments demonstrated that photosynthetically derived sugars were allocated to mycorrhizal fungi, which were essential for their development (Melin 1925). In parallel studies, they were found to be important conduits for nitrogen from organic soils to plant hosts (Melin 1925; Lindeberg 1989). Accumulating evidence suggested that the carbon supply of the fungus is derived from the host tree (Björkman 1970). During those times, there was more focus on what constituted a beneficial symbiosis, rather than the repercussions of mycorrhizal fungal involvement in decomposition, although this was still emphasized as a future research direction of interest (Melin 1925; Lindeberg 1989).

Ectomycorrhizal associations are the most common in boreal and temperature forest ecosystems, which constitute around 11% of the terrestrial biosphere (Read 1991). Many ectomycorrhizal sporocarps (*i.e.* sexual spore-bearing structures), are well recognized as important food sources that are difficult to culture, such as chantarelles and porcini mushrooms (Arora 2000). Observations of ectomycorrhizal root tips in the boreal forest have estimated that several hundred thousand to a few million mycorrhizal root tips occupy a square meter of the forest floor (Dahlberg et al. 1997; Taylor & Alexander 2005). The amount of extramatrical ectomycorrhizal mycelial dry biomass in a hectare of boreal forest may weigh as much as a moose (Finlay & Clemmensen 2016; Ekblad et al 2013). In temperate forests, it appears that the production of extra-radical mycelium may be more important for the overall standing biomass (Cheeke et al. 2021), while rates of turnover may be more essential to biomass maintenance in boreal forests (Hagenbo et al. 2017). Because of their extensive mycelial network, and influence on other soil microorganisms, all mycorrhizal fungi essentially make their own 'mycorrhizosphere' (Foster & Marks 1967).

Figure 4. Ectomycorrhizal fungi in the class of *Agaricomycetes*, including the threatened (VU) *Ramaria boreimaxima* Kytöv. & M. Toivonen in Jädraås, Sweden (**A**), *Cortinarius sanguineus* (Wulfen) Gray (**B**), *Hygrophorus camarophyllus* (Alb. & Schwein.) Dumée, Grandjean & Maire (**C**) and mat-forming signal species (S) *Hydnellum ferrigineum* (Fr.) P. Karst. (**D**).

Ectomycorrhizal functional diversity

The decomposition capacity of ectomycorrhizal fungi is not a straightforward concept, and depends on what sort of comparisons have been made given global, regional or smaller scale contexts. It could be very well assumed that ectomycorrhizal fungi are directly involved in decomposition, as they initially were considered parasites or pathogens essentially causing necrosis on plant roots, and even Frank developed the 'mycorrhizal decomposition theory' in the mid-1880s (Lindahl & Tunlid 2015). Romell (1938) concluded that mycorrhizal fungi were practically unable to break down organic matter without the support of trees *in situ*, but still their importance in decomposition was apparent. Results of microcosms experiments revealed that ectomycorrhizal fungi are able to utilize major components of plants and thus can contribute to litter decomposition in the forest floor, but it has been concluded that ectomycorrhizal fungi generally have a low ability to perform decomposition compared to saprotrophic fungi grown under similar conditions (Trojanowski et al. 1984; Read et al. 2004).

Saprotrophic ancestors generally lost their ability to produce both hydrolytic and oxidative enzymes involved in the degradation of plant cell walls, and this is thought of as a partial explanation as to how they transitioned into ectomycorrhizal symbiosis (Kohler et al. 2015). Saprotrophic fungi have been regarded to have higher decomposition capacity compared to ectomycorrhizal fungi, however, there are some early exceptions with the capacity for the ectomycorrhizal fungus *Tylospora fibrillosa* (Burt) Donk to express Mn II peroxidase (Chambers et al. 1999). Furthermore, it has been demonstrated that the ectomycorrhizal habit has independently retained oxidative capacities in several lineages (Bödeker et al. 2009; Kohler et al. 2015). Growing evidence for ectomycorrhizal involvement in directly affecting decomposition has reinvigorated efforts to think of ectomycorrhizal fungi as mutualists, yet also heterotrophic organisms that decompose (Bödeker et al. 2014; Lindahl & Tunlid 2015; Zak et al. 2019). Boreal forest soils are characterized by low pH with limited available nitrogen (Tamm 1991), and energetically expensive oxidative decomposition may have been retained precisely in these types of forests by ectomycorrhizal fungi to access the masses of complexed soil organic nitrogen with the support of simple sugars from plant hosts (Lindahl & Tunlid 2015).

A few investigated ectomycorrhizal species in the genus *Cortinarius* (L.) possess a genetic potential for oxidative decomposition similar to white-rot wood decomposers, using class II manganese peroxidases (MnP). It has been demonstrated that the transcription of *Cortinarius* (L.) MnP genes, such as by *Cortinarius glaucopus* (Schaeff.) Gray, is correlated with high peroxidase activity in soils supporting the hypothesis that *Cortinarius* species can decompose complex humic-rich substrates in the mor layer (Bödeker et al. 2014). There are several hundred species in the family Cortinariaceae known to reside in Fennoscandia and over 2,000 thousand species worldwide (Liimatainen et al. 2022). In a pine chronosequence, where a space for time substitution allows researchers to track successional stages over time, the relative abundance of Cortinaraceae is nearly zero in young stands (<34 years) and they become more dominant forests with longer tree continuity, at least up to 158 years (Kyaschenko et al. 2017a). The ectomycorrhizal genera *Lactarius* and *Russula* Pers. are also 'late stage' fungi found in the same order as the partially necrotrophic genus *Heterobasidion* Bref. with well-known lignin degrading abilities. *Lactarius* have been found to produce polyphenol oxidases (Giltrap 1982) and in a few cases both *Russula* and *Lactarius* have retained copies of MnPs (Looney et al. 2022). Some species of *Russula* are affected by nitrogen deposition, but the intolerance varies across the genus (Suz et al., 2015).

Piloderma spp. are resupinate, chord and mat forming ectomycorrhizal fungi in the order *Atheliales*. They dominate the ectomycorrhizal community in young pine forests before canopy closure until forests reach harvest age (Kyaschenko et al. 2017a), they are 'organophilic' *i.e.* have an affinity to incubated organic substrates, and are 'nitrophobic' *i.e.* are negatively affected by high nitrogen concentrations (Lilleskov et al. 2011). Therefore, they prefer substrates such as humus with low inorganic nitrogen availability (Jörgensen 2021). The sequenced genome of *Piloderma croceum* J. Erikss. & Hjortstamhas has no known encoding enzyme for lignin degradation (Miyauchi et al. 2020). *Piloderma olivaceum* (Parmasto) Hjortstam, has a similar encoding enzyme to MnP, but has mutated in parts coding for the active site of the enzyme, which is therefore not functional as a MnP (Björn Lindahl, pers. communication). Therefore, it does not have a white rot decomposition capacity. *Tomentella* species are resupinate species like *Piloderma*, and probably have little decomposition capacity, generally being nitrophilic. They are estimated to be the most species rich genus on the planet (Tedersoo et al. 2022).

Cenococcum geophilum coll. (Fr.) is an extremely common, culturable, and globally distributed ectomycorrhizal fungus without any known sexual reproductive stage. The fungus is characterized by melanized cell walls that are difficult to decompose (Fernandez & Koide 2014), and may contribute to long-term organic pools because they likely cannot decompose their own melanized necromass (Kohler et al 2015). In one study, the age of granules of *Cenococcum* sclerotia were assessed with C^{14} dating, and found them to possibly be over 1,000 years old (Watanabe et al. 2007). *Paxillus involutus* (Batsch) Fr. is another very common, mushroom-forming species that has broad host associations (*i.e.* a generalist), and is related to decay fungi the Boletales (Kohler et al. 2015). *Paxillus involutus* is easily culturable, which is unusual for ectomycorrhizal fungi. Multiple lab studies have indicated that it has retained a similar set of enzymes involved in brown rot, but it lacks plant cell wall-degrading enzymes such as glycoside hydrolases (Eastwood et al. 2011; Rineau et al. 2012); Therefore, it has been demonstrated that it participates in non-enzymatic oxidation of lignin.

Ectomycorrhizal fungi display a diverse array of functions, even though they are classified in the same guild. It may be appropriate to group species together into guilds to understand ecosystem processes, but to fix general patterns to assign species functions and vice-versa may be an ecological fallacy.

1.2.2 The ericoid mycorrhizal guild

Some of the primary systematic studies of ericoid mycorrhizal fungi examined the mycelial state from root-cultured isolates in *Calluna vulgaris* (L.) in the early 1900s (Rayner 1915; Harley & Smith 1983). The first demonstration of Koch’s postulate for this group (*i.e.* the isolation of the mycobiont and resynthesis of ericoid mycorrhizas from the same fungal strain) was in the mid 20th century (Read & Pearson 1973). Ericoid mycorrhizal fungi form hyphal coils inside ericaceous shrub root cells of genera such as *Vaccinium* (L.) (*e.g.* lingonberry, bilberry). Generally, the ericoid guild dominates in acidic soils with high organic matter content, and the hosts have a range of forms as epiphytes in tropical forests, or as trees in temperate forests (Read 1991).

Ericoid mycorrhizal fungi are a far less studied group compared to the ectomycorrhizal guild, as there is an entire order of magnitude difference in the number of publications between ericoid and ectomycorrhizal studies on the effects of carbon and nitrogen cycling in soils (Ward et al. 2022). Plants in the Ericaceae have been recently delimited with the advent of DNA sequencing (Kron & Lutyen 2005), but share morphological traits like specialized distal roots. These roots are situated away from the point of attachment or 'branch' where the epidermal cells of fine, hair-like roots, are colonized by ericoid mycorrhizal fungi (Read & Smith 2008). Ericoid associations are less well studied, but they still cover extensive regions of the planet (Lahti & Väisänen 1987; Kron & Lutyen 2005) with estimated cover reaching 96% in the boreal forest (Ward et al. 2022). In boreal forest podzols, the symbiosis occurs in the shrub root zone and spanning from just below the intact litter layer into the deepest humus layers of the mor horizon in sandy soil (Lindahl et al. 2007).

Ericoid diversity and functions

Ericoid mycorrhizal fungi can degrade organic matter with laccases and cellulolytic enzymes, and they assimilate and pass organic nitrogen to the host plant (Read et al. 2004). For this reason, it has been concluded that ericoid mycorrhizal fungi are more potent decomposers than ectomycorrhizal fungi (Read et al. 2004). However, ericoid mycorrhizal fungi lack the capacity to produce the most potent oxidative enzymes found in some ectomycorrhizal Agaricomycetes, such as *Cortinarius spp.* (Bödeker et al. 2009; Martino et al. 2018). Thus, although ericoid mycorrhizal fungi have saprotrophic capacities and access organic nitrogen using hydrolytic decomposition, they also appear to build up particulate organic matter from their recalcitrant necromass (Fernandez & Koide 2014; Clemmensen et al. 2015; Adamczyk et al. 2016). Recent studies *in situ* have found that soil organic matter stocks increase along gradients from ectomycorrhizal to ericoid mycorrhizal dominance in multiple local and regional gradients in subarctic (Hartley et al. 2012; Clemmensen et al. 2021), boreal (Clemmensen et al. 2015) and temperate ecosystems (Ward et al. 2021), indicating that the ericoid mycorrhizal guild has specific traits that support soil organic matter accumulation (Clemmensen et al. 2015; Ward et al. 2022).

Studies of the mycelial lifestyle in absence of sporocarps have made the taxonomic classification of ericoid fungi difficult, and there has been frequent renaming. Multiple species can even occupy the same fine root (Sietio et al. 2018). Most of these ericoid mycorrhizal fungi have been described as helotialean ascomycetes or comprising fungi in the ascomycetous order Chaetythriales (Allen et al. 2003), which is heavily based on cultured isolates (Read & Smith 2008). More recently, ericoid mycorrhizal fungi in the Basidiomycota have been described in a clade of the Sebacinales (Selosse et al. 2007; Weiss et al. 2016), and potentially there may be other ericoid mycorrhizal Agaricomycetes in the Trechisporales (Vohnik et al. 2012). In the helotialean clade, the previously known genera *Meliniomyces* and *Rhizoscyphus*, are a part of a well-studied aggregate group historically known as the *Pezeloma* or *Rhizoscyphus ericae* aggregate (Read & Smith 2008), and have been recently described as congeneric to *Hyaloscypha* (Fehrer et al. 2019). These names had never been formally associated because the sexual and asexual morphotypes had been studied in isolation by different research communities in mycology and ecology. Within the broadened genus *Hyaloscypha*, the functional lifestyles vary dramatically from plant pathogens, to soil and litter saprotrophs, ericoid mycorrhizal fungi, and ectomycorrhizal fungi (Fehrer et al. 2019). Also, certain species have dual-associations with trees and ericaceous shrubs, such as *Hyaloscypha variabilis* (Read & Smith 2008). A large proportion of the fungal community is made of helotialean fungi in the boreal forest, although their taxonomy and evolutionary history are still unresolved (Johnston et al. 2019). The widespread abundance and diversity of ericoid- fungi and these putative root-associated ascomycetes in the boreal forest makes them an important component of soil nutrient cycling and carbon sequestration (Wardle & Lindahl 2014), and more research into their diversity and functions is needed (Kohout 2018).

1.3 Functional guild effects on ecosystem processes

The ecosystem concept was not formally developed until the mid-1930s, (Tansley 1935; Willis 1997). Around that time, a trenching experiment was established in north central Sweden to see whether a release of available nitrogen from cutting roots would affect plant-soil processes (Romell 1938). Metal barriers were put in place to cut-off the tree roots from an isolated patch of soil, and in effect, plants that remained within the trenches had turned a darker shade of green. This 'green manuring' of the forest was one of the early observations of the release from nitrogen limitation. Additionally, the experiment also demonstrated that the production of *Lactarius glyciosmus* (Fr.) Fr. sporocarps was dependent on living roots, since the sporocarps were found just outside the metal barriers where roots and mycelium remained intact. No sporocarps were found inside the barriers, and perhaps this was one of the early experimental validations of the symbiosis *in situ* (Romell 1938) after Melin had found support for mycorrhization of *Lactarius* (Pers.) *in vitro* (Melin 1925).

1.3.1 The Gadgil effect

High-latitude forest ecosystems with colder climates, more recalcitrant plant litter, and acidic soils are dominated by ectomycorrhizal symbioses. For these reasons, they have been described to accumulate more soil organic matter than forests dominated by arbuscular mycorrhizal symbioses (Read 1991). One proposed process partially responsible for the large accumulation of soil organic matter, under nitrogen limited conditions, is that ectomycorrhizal fungi slow decomposition in litter by competing with free living saprotrophs for nitrogen (Gadgil & Gadgil 1971; Averill et al. 2014; Steidinger et al. 2019). This phenomenon – known as the '**Gadgil effect**' – emerged at a time where the prevailing view was that aboveground litter sources were the main inputs to soil organic matter, and that saprotrophs had a higher decomposition capacity than ectomycorrhizal fungi.

The first experiment explicitly examining the 'Gadgil effect' was in an introduced, 22 year-old *Pinus radiata* plantation in temperate New Zealand. After 12 months, more decomposition of pine needle litter was found when carbon allocation to ectomycorrhizal fungi and pine root were terminated by trenching (Gadgil & Gadgil 1971, 1975). Similar studies were developed thereafter in different environmental contexts. In temperate or hemi-boreal southern Sweden, leaf litter of *Fagus sylvatica* L., otherwise known as

European beech, was incubated in 6 mm mesh over two consecutive seasons for one to two years in 90-110 year old beech stands with varying 'mor' (more organic associated organic matter) and 'mull' (more mineral associated organic matter) soil types (Staaf 1988). There was no difference found in decomposition between trenched and control plots, and soil type did not have a strong effect, indicating that the Gadgil effect was not universal. However, it is quite possible that roots were able to enter the bags, since European beech roots can be smaller than 6 mm in diameter. Enchytrid worms have been stimulated by trenching and may enter into mesh sizes larger than 1mm in diameter (Babel 1977). In a young, regenerated forest of *Pinus rigida* (Miller) 'pitch pine' by seed trees in north eastern United States, the decomposition of litter in 1 mm mesh bags was higher in plots without trenching over one and two years of incubation (Zhu & Ehrenfeld 1996). A majority of these pitch pine plots contained 4 year-old pine saplings, so the stand age, and extensivity of root production or ectomycorrhizal and saprotrophic community may have been important differences in the experiment compared to the original Gadgil effect. A correlative study in a 33 year-old plantation of in *Pinus resinosa* (Aiton) had varied ectomycorrhizal ingrowth in 1 mm mesh bags that were incubated in a well-developed organic horizon comprising a pine needle litter, fermentation (F), and humified (H) layers on top of a sandy loam (Koide & Wu 2003). After 4 months, there was no difference in decomposition of the pine needle and F layer substrates across levels of ectomycorrhizal root tip density, and mass loss was likely only due to leaching and potentially decomposition of labile carbon from opportunists. After 1 year there was a clear negative correlation between the decomposition of pine needle and deeper F-layer substrates and ectomycorrhiza density in 1 mm mesh (Koide & Wu 2003).

In a study by Wardle and Zackrisson (2005), coniferous islands of different sizes represented a 5,000 year old chronosequence since fire, spanning from younger, more productive forests to older, more retrogressed forests (*i.e.* lower productivity). Here, the tree root exclusion experiment was initiated in August 1996. *Salix caprea* (willow) litter of the same quality (42% lignin) was incubated for two years in each of the plots across the island gradient. Control treatments led to higher decomposition than the tree root exclusion treatments, but this was only the case on the larger, more productive islands with a more recent history of fire (a stand age on average around 500 years). Otherwise, no significant differences in litter mass loss were found in the smaller islands. In the same chronosequence, almost two decades later, no Gadgil effect was found when bilberry litter was incubated for one year between trenched and control plots (Fanin et al. 2022). Differences in decomposition rates between the studies could possibly be associated with substrate quality and length of incubation, since bilberry litter has been shown to rapidly decompose within a few months (Johansson 1993). In comparison to other studies, it shows that the Gadgil effect is context dependent even in the boreal forest.

Substrate quality has been found to be an important factor in explaining the discrepancies in litter decomposition rates with or without ectomycorrhizal fungi (Smith & Wan 2019). The substrate quality mechanism specifies that in systems with lower litter quality (*i.e.* high C:N and high lignin : N ratios), saprotrophic fungal carbon-use efficiency (CUE) is significantly reduced because it takes more energy to assimilate low quality litter into fungal biomass (Sinsabaugh et al. 2016). Ectomycorrhizal fungi are not solely dependent on the litter substrate quality for nutrition since they derive carbon from the hosts. In this regard, they are hypothesized to inhibit saprotrophic fungi accession of nitrogen in low quality litter by mining and immobilizing organic nitrogen from soil organic matter. This difference in substrate preference is revealed in the realized niche differences between saprotrophic and ectomycorrhizal fungi in boreal forest soils; the carbon to nitrogen ratio decreases with depth in the boreal forest, and saprotrophs are found more abundantly in the upper part of the soil profile while mycorrhizal fungi are found in deeper organic and mineral horizons (Lindahl et al. 2007). These theoretical and experimental underpinnings support research showing that the Gadgil effect is most commonly demonstrated in high-latitude ecosystems with higher lignin:N and C:N ratios dominated by species in the genus *Pinus* (Fernandez & Kennedy 2016; Sterkenburg et al. 2018; Maaroufi et al. 2019; Smith & Wan 2019; Fernandez et al. 2020).

1.3.1 Digging deeper, the ‘mycorrhizal guild hypothesis’

The decomposition and accretion of organic matter in deeper mor in podzols with little vertical mixing is largely composed of decayed mycorrhizal mycelium and root inputs, which is considerably more important than the decay of surface litter in regulating soil organic carbon stocks in the boreal forest (Clemmensen et al. 2013; Kyaschenko et al. 2019).

Ectomycorrhizal fungi predominantly occur in the mor and mineral horizons (Lindahl et al. 2007) where belowground allocation by host trees may be up to 50% of recent photosynthates (Högberg et al. 2001). Though ectomycorrhizal fungi mediate a large proportion of carbon inputs belowground, soil organic carbon stocks have been found to increase from ectomycorrhizal dominated birch forests to less productive heathlands dominated by ericoid mycorrhizal dwarf shrubs (Hartley et al. 2012; Clemmensen et al. 2021). Over decades, planting birch and pine trees on an ericoid mycorrhizal heather moorland has been shown to lower soil organic carbon stocks (Friggens et al. 2020). The mass ratio hypothesis describes that functional diversity of the dominant plants (*i.e.* those that have the highest proportion of primary production) overwhelmingly have the largest effect or control on ecosystem processes (Grime 1998). In the boreal forest, understory shrubs can have proportional net primary production to canopy trees (Nilsson & Wardle 2005).

The '**mycorrhizal guild hypothesis**' posits that the contributions and interactions between the ericoid and ectomycorrhizal guilds are important for decomposition and accumulation of soil organic matter (Clemmensen et al. 2015). Labile organic nitrogen (e.g. protein and chitin) is abundant in forest soils (Fernandez & Koide 2012); however, organic nitrogen can be protected from decomposition through the complexation with polyphenolic compounds in organic horizons (Northrup et al. 1995). Ectomycorrhizal fungi have a competitive advantage over free-living heterotrophic fungi in substrates like humus that are depleted in metabolically valuable carbon (Lindahl et al. 2007), therefore, a higher abundance of ectomycorrhizal fungi (and less abundance of ericoid mycorrhizal fungi) would be expected to result in more mass loss in long-term carbon pools. In this case, ericoid mycorrhizal guilds may be involved more in the accumulation of organic matter, since they do not have the capacity to decompose with white rot mechanisms (Martino et al. 2018), and therefore can contribute to long-term organic pools (*i.e.* substrates with longer residence time, such as cellulose depleted humus or the 'mor' layer) because they are thought to be unable to decompose their own melanized necromass. This is in contrast to the prevailing understanding of ericoid mycorrhizal fungi are better decomposers because of the retained ability to degrade cellulose compared to ectomycorrhizal fungi, which lack this ability (Read et al., 2004, Kohler et al. 2015).

1.4 Mycorrhizal conservation values

The conservation of fungi in general has been long overlooked globally, and these organisms should be included in biodiversity targets (Goncalves et al. 2021), although it is not a simple task. Fungal conservation is far less studied compared to the conservation of plants and animals. The inclusion of fungi in national registries started to become prevalent in the late 1980s and early 1990s (Heilmann-Clausen & Vesterdal 2008) a few decades behind the red-listing for mammals and birds (Dahlberg & Mueller 2011). Fungal conservation was 'initiated' with the onset and recognition of global change factors, such as acid rain and pathogen attacks in central Europe (Arnolds 1991). As time went on, fertilization (Bååth et al. 1981; Arnebrant & Söderstrom 1992), nitrogen deposition (Lilleskov et al. 2002; Suz et al. 2015) and intensified land-use change, such as clear-cutting, (Dahlberg et al. 2010), have all been recognized as important drivers in the decline of fungal species of conservation concern. The effects of cutting trees on common ectomycorrhizal species, such as *Lactarius deterrimus*, had been realized even 100 years ago (Romell 1930).

Because of their unique physiological interdependence with a continuity of living trees, many ectomycorrhizal species are considered species of conservation concern. Certain ectomycorrhizal species are considered specialists on particular species of trees, while other ectomycorrhizal species are considered generalists due to their association with a wide range of tree species (Molina et al. 1992). When conservation values are accessed, ericoid mycorrhizal species are disregarded, and we essentially know very little in terms of conservation. Globally, some ericoid mycorrhizal fungal species have a very wide distribution range, while others are restricted to particular regions (Kohout 2018). In Sweden, plant understory coverage has been decreasing over the last decade in both productive forest land and formally protected areas (Kyaschenko et al. 2022), so perhaps some ericoid mycorrhizal populations are in decline as well.

Assessments of threatened fungal species are guided by all information possible about a species, in terms of expert knowledge on ecology, sporocarp records, habitat suitability, distribution, how fragmented sub-populations are, environmental DNA, and generation times of individuals *i.e.* the length of time for an individual to turnover. Mycologists have difficulties applying the IUCN criteria for fungi, particularly for the concept of the 'individual', the time it takes for a generation to turnover, and how uncertainty and absence of data should be handled (Heilmann-Clausen & Vesterdal 2008), but now general guidelines exist (Dahlberg & Mueller 2011). The number of globally red-listed fungi has increased dramatically from three (including two lichens) to around 600 fungal species in just over one decade (Mueller et al. 2022). In Sweden alone, nearly 300 ectomycorrhizal species are red-listed from near threatened (NT) to critically endangered (CR). Ultimately, retaining high species richness serves as an assurance that if some species are lost, then others may persist performing similar functions (Yachi & Loreau 1999).

On a stand level, a mycorrhizal fungus could be lowly abundant but frequently encountered; it could be very dominant in a few square meters, but only found in a few patches of an entire forest. At a regional or continental scale, populations of ectomycorrhizal species may also be threatened because suitable habitat is in decline. For example, *Phellodon secretus* (Niemelä & Kinnunen), the ‘taiga tooth fungus’ is a mycelial mat forming ectomycorrhizal fungus with basidiocarps found particularly under old dead and decayed wood in sandy pine forests (*Figure 5*). It is listed as vulnerable (VU) on the Swedish red-list because its extinction risk is high. The habitat of the fungus comprises old, somewhat frequently burned pine forests that have a long continuity of deadwood. The habitat has declined sharply over the past 50 years and this decline is expected to continue. Currently, there is a known population size of less than 1,000 individuals in Sweden, although the species was described only two decades ago (Artdatabanken 2020). In this case, *Phellodon secretus* fits into the category of being both patchily distributed at a local scale, and infrequently encountered across the boreal forest (*i.e.* basically only found under deadwood in pine forests with long continuity). Also, a species does not have to necessarily be rare to be threatened, but the population of a common species could be of conservation concern due to a rapid decline or predicted decline in the species population (Dahlberg & Mueller 2011).

Figure 5. The sandy pine forest habitat (>200 yrs) with a continuity of pine deadwood (**A**) along with a large display of the mycelial mat-forming *Phellodon secretus* (VU) under a carefully rolled up, well decomposed, deadwood log (**B**).

1.4.1 Tree-age and ectomycorrhizal diversity

The idea that community shifts of ectomycorrhizal fungi parallel changes in tree age was first postulated in the early 1980s where there appeared to be ‘early stage’ and ‘late stage’ fungi (Mason et al. 1982). Many studies have followed with varying degrees of agreement, although many lacking true replication (Johnson et al. 2005). Mason’s succession hypothesis was formulated while studying stands of aging birch trees (*Betula pendula*) on previously used cropland, and this is why it has been argued to only hold true for early succession tree species, such as birch (Molina et al. 1992). Furthermore, Molina and others (1992) go on to say that natural forest systems have a variety of more important factors that affect ectomycorrhizal succession, such as tree host identity. The terms ‘early stage’ and ‘late stage’ fungi are not aptly named, or only apply to forests regenerating after a severe disturbance, because it is possible to find late-stage fungi on young saplings and ‘early stage’ fungi on mature trees in forests with a continuous cover of trees (Jonsson et al. 1999). Either way, there are clear differences in ectomycorrhizal traits (e.g. spore-reactivity, or ability to form a spore bank or sclerotia) that will influence the ability of ectomycorrhizal fungi to disperse and re-establish after disturbances, such as clear-cut logging (Jones et al. 2003). Tree age may not necessarily be the best estimate of ectomycorrhizal community composition *per se*, but tree continuity of the forest is important to maintain many species ectomycorrhizal fungi with many life history strategies.

1.4.2 Conservation mycology with DNA-metabarcoding

Global metabarcoding studies have estimated that the distribution of ectomycorrhizal lineages contrasts with the general latitudinal trend of diversity for most other macro-organisms, as more lineages are endemic to temperate and boreal forest biomes than tropical forests (Tedersoo et al. 2010; Tedersoo et al. 2022). With rapidly advancing technology in sequencing and computational biology, genomic applications to conservation could be a promising (Lofgren & Stajich 2021). In general, metabarcoding studies also result in more fungal species observations on a stand level than sporocarp surveys (Kranabetter et al. 2005; Varenius et al. 2016). Comparable richness estimates have been made between sporocarp surveys by experts over multiple seasons and metabarcoding of soils for specific phylogenetic confined to Agaricomycetes and Agaricales (Frøslev et al. 2019). However, DNA-based high-throughput metabarcoding of fungal community composition in soils has a limited capacity to assess the presence of rare species in a forest stand because of the small fraction of the soil sampled (Sterkenburg et al. 2019). DNA-metabarcoding is not a replacement for inventorying threatened red-listed species (Varenius et al. 2017; Frøslev et al. 2019), although DNA-methods could be useful to detect rapid population declines of common taxa, add pertinent information about the distribution about data deficient species, and could be used to monitor in specific locations where species are known already to persist.

There are many different ways to be rare (Rabinowitz 1981). One potential use of DNA-based identification is that fungal species can be classified as endemic within a biogeographic context (Talbot et al. 2014; McPolin & Kranabetter 2020). Endemicity, *i.e.* whether a species is found exclusively in an area of interest with little representation elsewhere, is another way to narrow down potential fungal species of conservation concern and habitats or regions that are particularly unique hotspots for biodiversity. Endemicity can be assessed solely by sequence similarity to global databases, rather than by matching taxonomic nomenclature, which has not been nearly finalized across an estimated 3.8 million species in the fungal tree of life (Hawksworth & Lucking 2017). For example, a global assessment found more endemic ectomycorrhizal species in regions of temperate rainforests and moist and dry tropical forests than in any other regions (Tedersoo et al. 2022).

Globally, there are few soil organisms that are far more abundant than others, and most that are lowly abundant (Tedersoo et al. 2014; Delgado-Baquerizo et al. 2018). However, in soil sampling surveys, only a fraction of the soil is examined, which means that most of the species that are sampled are actually more likely to be common because they have genets larger than the distance between samples, and/or they are frequently distributed across sampled sites. Global surveys identify that soil fungi have relatively high insularity compared to other groups of organisms such as plants and animals (Tedersoo et al. 2022), although it should be taken into account that those species may not be very rare to begin with if only one 10,000th of the area in a hectare stand was sampled. The question remains whether DNA metabarcoding is a valid approach to identify species of conservation concern.

2. Objectives and Aims

The widespread presence and expansive diversity of saprotrophic, ericoid- and ectomycorrhizal fungi in boreal forests makes them an important conduit for soil nutrient cycling and carbon sequestration, and many ectomycorrhizal species are considered species of conservation concern; however, a lot of information on the diversity, functions, and interactions of these guilds are lacking.

Through my thesis, I examined both fundamental theories related to soil carbon sequestration and apply advanced techniques and understanding of mycorrhizal ecology to inform the current state of soil and biodiversity conservation in the boreal forest.

In **Paper I**, with experimental evidence derived from a long-term study, I assessed how saprotrophic, ecto- (pine) and ericoid (shrub) mycorrhizal guilds contribute to soil respiration on a stand scale in a manipulated forest experiment. Then in the same experiment for **Paper II**, I evaluated how the same three guilds decompose pine needle and humus substrates, specifically looking into how guild interactions effect short-term and long-term carbon pools.

Finally, in **Paper III**, I took an applied approach to examine how our current perceptions of forest conservation values reflect ectomycorrhizal diversity and species of conservation interest across 76 forests in Sweden.

In the midst of the Anthropocene, synergies and trade-offs between supporting species of conservation interest while also mitigating climate change need to be considered because they may not be mutually inclusive.

3. Guild Effects on Ecosystem Processes

3.1 The Jädraås Experiment: Study System & Design

Evaluating guild effects and interactions on the respiration and decomposition of boreal forest soils

Almost a century after Romell's trenching experiment, a randomized block forest experiment in Jädraås försökspark was setup in 2016 to assess fundamental theories in mycorrhizal ecology in **Papers I** and **II**. The forest, located at a well-studied research station 200 km north of Uppsala, has a homogenous stand of evenly aged and distributed trees canopy dominated by *Pinus sylvestris* L. (393 trees ha^{-1}) that regenerated naturally after charcoal and tar burning in the 1850s and was thinned in 1962 (Bråkenhielm & Persson 1980). Four ericaceous dwarf shrub species make up the understory with a large amount of *Vaccinium vitis-idaea*. L. (lingon) and *Calluna vulgaris* L. Hull (calluna), and with less cover of *Empetrum nigrum* L. (crowberry) and *Vaccinium myrtillus* L. (bilberry). The ground layer of mosses is mainly comprised of *Pleurozium schreberi* (Bridel) Mitten and *Cladonia rangiferina* (L.) F. H. Wigg. The vertical stratification of organic layers on top of the mineral soil consist of fallen needles and living moss on the top, to structurally intact dead moss, and then to a 2-10 cm humified organic horizon where the age of organic matter increases at depth (Lindahl et al. 2007).

In forests, plant-driven (autotrophic) and heterotrophic (free-living saprotrophic) contributions to soil respiration, decomposition and other soil processes have been estimated in various ways, from stem girdling to removals to trenching experiments where the belowground carbon flow is interrupted (Subke et al. 2006). However, few factorial shrub and pine root exclusion studies have been conducted. In our experiment, ericaceous dwarf shrubs were removed and we excluded carbon supplies to pine roots and ectomycorrhizal fungi with metal barriers inserted 70cm below the surface. The factorial experiment was set up and has been maintained since November 2016. There are 5 treatments with 8 replicates: root trenching (pine root exclusion), ericoid shrub removal, pine root exclusion and shrub removal, control (undisturbed forest conditions), and a disturbance control in which roots were initially trenched, but no barrier left (*Figure 6*).

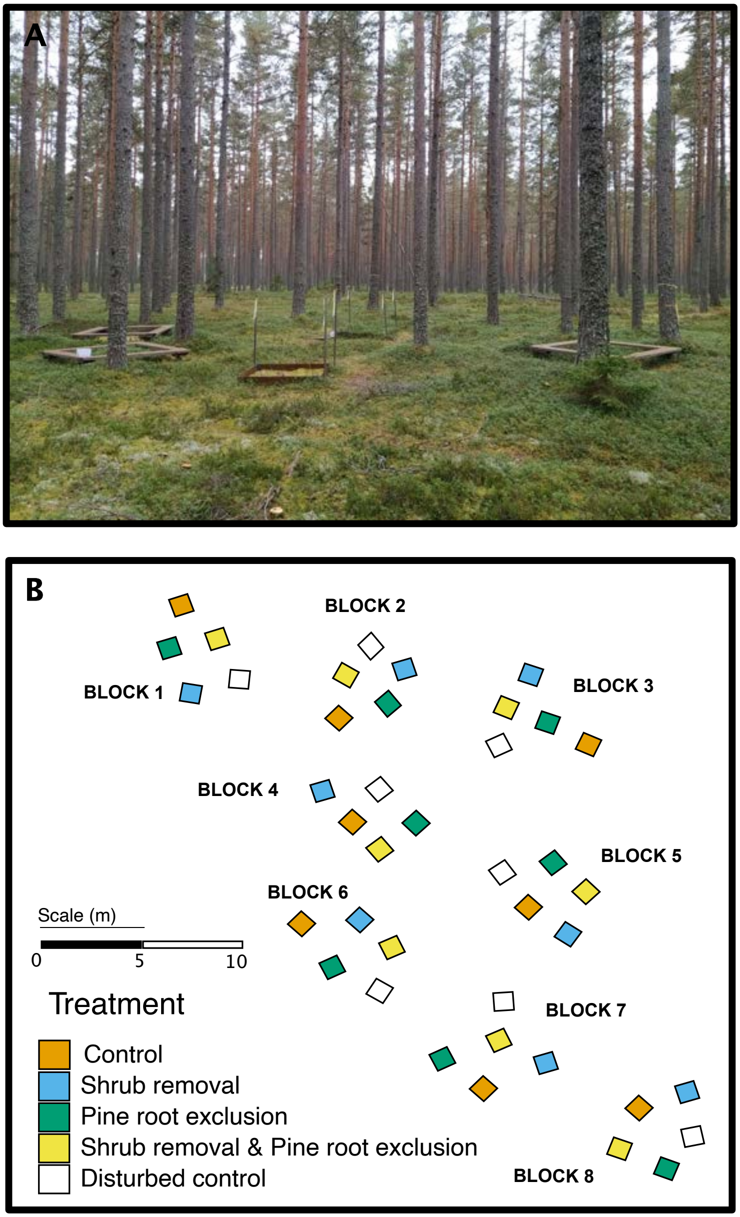

Figure 6. An example block (**A**) in the long-term trenching and shrub removal experiment. The pine forest is between 160-170 years old and located in Ivantjärnsheden V in Jädraås, Sweden. The overall randomized block experiment design is presented (**B**).

3.2 Hypotheses

Guild effects on soil respiration

In **Paper I**, we hypothesized that the contributions of ericoid shrubs to the total mycorrhizal guild (autotrophic) soil respiration would reflect their 30% share of root biomass, and consequently, pine roots and ectomycorrhizal fungi were expected to contribute 70% (*Figure 7*), reflecting their share of root biomass (Persson 1980).

Secondly, we expected the mycorrhizal and saprotrophic guilds would make up an equal proportion of respiration (*Figure 7*).

Finally, we hypothesized that there would be a 'competitive release' between the mycorrhizal guilds with the removal of each guild individually, and this would mean more soil respiration or activity from each guild in the absence of the other, and increased nitrogen availability only in the combined shrub removal and root exclusion treatment.

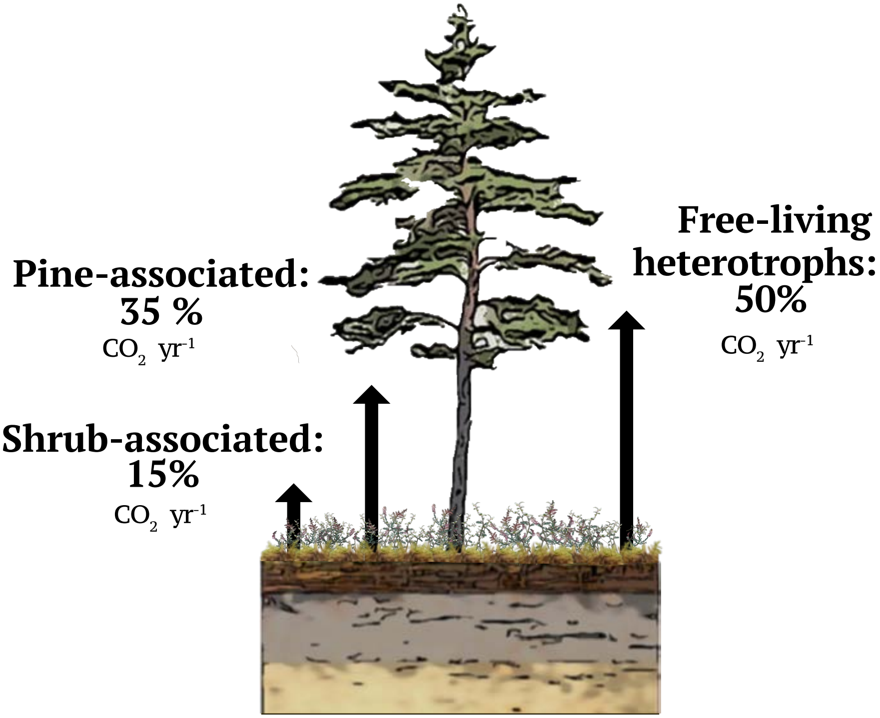

Figure 7. Hypothesized soil respiration contributions from the saprotrophic ('free-living heterotrophic'), ericoid (shrub-associated) and ectomycorrhizal (pine-associated) guilds based on root biomass estimates in the same forest.

Guild effects on decomposition

In **Paper II,** we hypothesized (H1a) that saprotrophic decomposition of pine needle litter would increase via a competitive release only when both mycorrhizal guilds were eliminated simultaneously, following the '**Gadgil effect**' as a response to the combined pine root exclusion and shrub removal (*Figure 8*). We further expected that any differences in litter decomposition would be related mainly to saprotrophic genera with a white rot capacity.

We further hypothesized (H1b) no increase in saprotrophic fungal abundance or decomposition of humus substrates *i.e.* that no Gadgil effect, would be present in the long-term, well-decomposed substrate.

Instead, we proposed the '**mycorrhizal guild hypothesis**' (H2), which posits that the presence of pine roots and ectomycorrhizal fungi would result in faster decomposition, while presence of ericoid mycorrhizal plants and fungi would slow decomposition. Thus, humus mass would be unaffected when both mycorrhizal guilds were present, or when they were both absent (*Figure 8*). We further expected that any augmented humus decomposition would be negatively related to abundance of ectomycorrhizal and/ or saprotrophic white rot fungi.

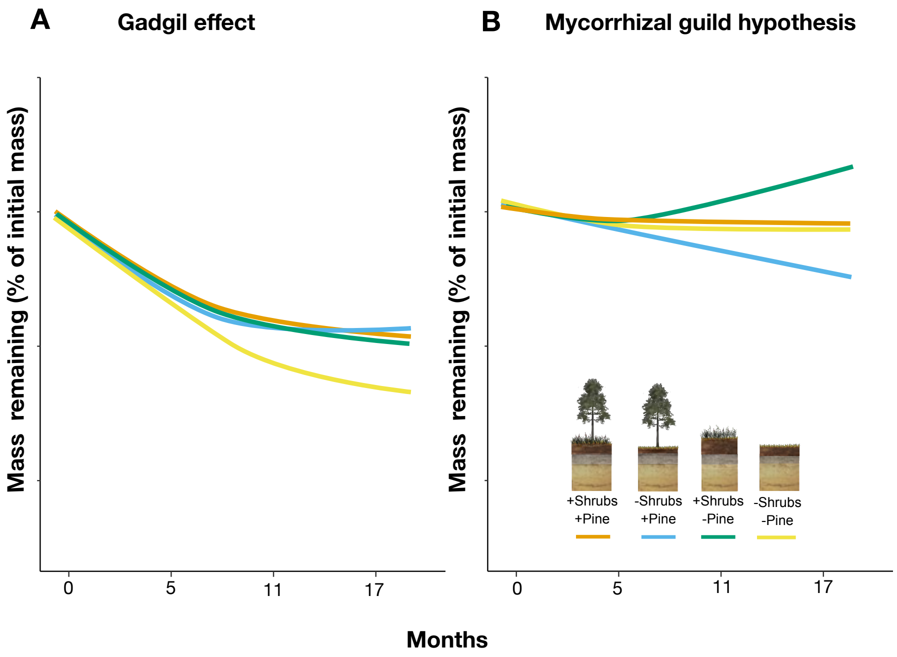

*Figure 8***.** Expected mass remaining of decomposing pine needle litter following the Gadgil effect hypothesis (**A**) and humus following the mycorrhizal guild hypothesis (**B**) over two growing seasons in response to the presence or absence of shrubs and pine roots and their associated mycorrhizal fungal communities in factorial design.

3.3 Methodological Notes

Monitoring soil respiration, moisture, temperature, nitrogen availability and decomposition

Soil moisture and temperature were monitored in hourly intervals at 5cm depth in each of the 40 plots. Soil respiration measurements were conducted every month before the onset of the growing season until late fall or early winter, to monitor the establishment of the experiment over three years, and confirm that belowground allocation of carbon was in fact cut-off. Soil respiration rates were integrated over each growing season (yr^{-1}). Resin strips were incubated in the first two growing seasons to assess nitrogen availability (NH_4^+), and lingon leaves were collected to assess nitrogen, carbon, and isotopic signatures with an isotope ratio mass spectrometer coupled to a carbon and nitrogen elemental analyzer. Mesh bags (50 µm-mesh) were filled with oven-dried pine needle litter or humus collected from the soil surface, or the fully organic layer, respectively, in the same forest to assess decomposition rates. In each plot, two litter bags were placed in the litter/moss layer, and two humus bags were placed at 5-10 cm depth in the mor layer at two time points; the first (set 1) in June 2017, and another (set 2) in June 2018. For both sets, one of the bags was incubated until November the same year (5 months) and one until November the following year (17 months). This resulted in 320 samples (2 substrates x 2 sets x 2 incubations x 5 treatments x 8 blocks). Mass remaining from both substrates were recorded after freeze-drying.

Fungal community composition and abundance

DNA extraction, PCR amplification of the ITS2 (Ihrmark et al. 2012), and Pac-Bio Sequel I sequencing of the organic substrates followed standardized methods found in (Clemmensen et al. 2016). Only humus mesh bags incubated from the 17-month incubation in the second set were used in **Paper I** to verify shrub root removal, and pine root exclusion of ectomycorrhizal fungi while all samples and percent mass remaining were included in **Paper II**. Over 1 million HiFi sequences were filtered and clustered using the SCATA pipeline (scata.mykopat.slu.se; Ihrmark et al. 2012). In total, 807 species level clusters (species) were formed from 639,923 sequences passing quality control, Further filtering narrowed the community to 190 fungal species at 98.5% similarity, whereby each species remaining had at least 1% relative abundance of the total fungal reads in at least one sample, and taxa were further manually curated and accessioned into guilds (e.g. ectomycorrhizal fungi, putative ericoid mycorrhizal fungi, putative dual ericoid/ectomycorrhizal fungi, saprotrophs, yeasts & molds, other root-associated fungi and fungi with unknown ecologies) using information in UNITE v.08 and FungalTraits (Nilsson et al. 2019, Polme et al. 2021). Genera with 'white rot' capacity were specified for ectomycorrhizal *Cortinarius* and *Hebeloma*, and saprotrophs, including *Trechispora*, *Mycena*, *Gymnopus*, *Galerina*, *Sistotremastrum*, based on genomic data (Kohler et al. 2015). As a representation of biomass of each fungal species or guild, their relative abundances (out of total fungi) were multiplied with total fungal ITS2 copy numbers, determined by qPCR, per substrate DW.

Statistical methods

Statistical tests and graphics were conducted in R using an array of methods. Linear mixed models were used to assess effects of pine, shrubs, soil moisture and temperature on changes in respiration rates and cumulative respiration in **Paper I**, while similar models assessed the effect of shrub and pine presence (and their interaction) on the abundances of different groups of fungi (**Papers I and II**), nitrogen availability (**Paper I**), and lingon leaf nutrient profiles (**Paper I**). Generalized additive models were used to visualize the responses of the different guilds to soil moisture and respiration in **Paper I**. Permutational analysis of variance was used to analyze shifts in the community composition across pine needle and humus substrates (**Paper II**), and Bayesian hierarchical models were incorporated to estimate rates of decomposition by different groups of fungi (**Paper II**).

3.4 Results & Discussion

Overall guild contributions to soil respiration

Terrestrial soil respiration dwarfs anthropogenic emissions by an order of magnitude; therefore, accounting for soil respiration in the delicate balance between total ecosystem respiration and gross primary productivity is necessary (Högberg et al. 2009). In **Paper I**, we demonstrated that the ericaceous dwarf shrub understory accounted for 11 ± 9% (g C yr^{-1}) of the total soil respiration and 22 ± 10% of the plant-driven (*i.e.* autotrophic) respiration over three growing seasons while the pine-associated respiration contributed 43 ± 1 % of total soil respiration and 78 ± 10% yr^{-1} of the total autotrophic respiration over the three growing seasons. This is direct evidence that ericoid shrubs do in fact contribute to total ecosystem respiration, and their activity (*i.e.* whether it decreases or increases) could have implications for the net ecosystem exchange within a coniferous boreal forest. Excluding the second year with drought, shrubs even make up to 30% of the plant-driven ('autotrophic') respiration, in concordance with the proportion of root biomass at the site (Persson et al., 1980a, b, 1983).

The drought in our experiment in 2018 reduced the difference in soil respiration in plots with and without the ericaceous dwarf shrubs, regardless of trenching (*Figure 9*). During drier and warmer conditions in the second year, shrubs most probably closed their stomata, while pine-driven and the free-living heterotrophic component contributed a higher proportion to soil respiration during that period. This could be detrimental to forests providing a carbon sink, since a portion of the autotrophic component or plant-driven part of soil respiration from shrubs are reduced while saprotrophs and ectomycorrhizal fungi continue, although more experimental evidence is needed combined with manipulated drought treatments and monitoring of guild contributions. Functional responses to temperature and moisture between ericoid- and ectomycorrhizal guilds are different, and this could have consequences on nutrient cycling in the context of a changing climate (**Paper I**). For example, the net balance of total ecosystem respiration and gross primary productivity determine whether a forest is a net sink or a net source of CO_2 (Chapin et al. 2011).

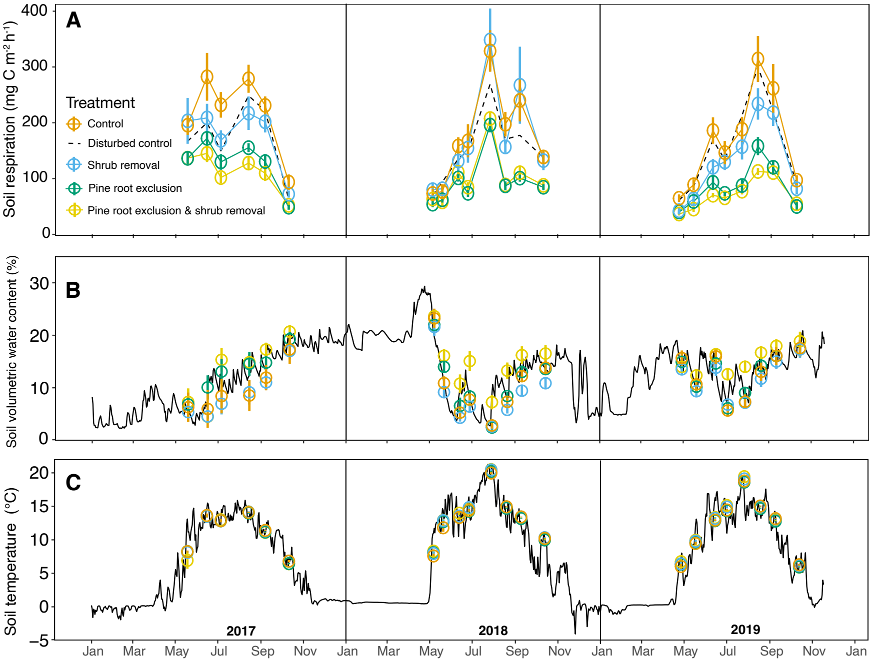

Figure 9. Growing season (**A**) soil respiration (mg C m^{-2} h^{-1}), (**B**) volumetric soil water content (%) and (**C**) soil temperature (°C) in control plots and in plots from which shrubs or pine roots or both had been removed. Respiration was measured monthly over the growing seasons of 2017 to 2019. Soil water content and temperature averaged per treatment across the 4 days preceding each respiration measurement are shown as colored circles, and the black trend lines represent daily averages across all treatments. Points are averages ±1 standard error (n = 7–8)

Key Results: Paper I

- The pine root exclusions and shrub removals effectively cut-off carbon allocation belowground from the respective guilds with little to no ectomycorrhizal or shrub root ingrowth into the humus.

- The ericoid guild accounted for 11 ± 9% yr^{-1} of the total soil respiration and 22 ± 10% of the plant-driven (*i.e.* autotrophic) respiration over three growing seasons while the ectomycorrhizal guild contributed 43 ± 1 % yr^{-1} on average over the three growing seasons and 78 ± 10% yr^{-1} of the total plant-driven respiration.

- There was a clear competitive release in terms of nitrogen, whereby inorganic nitrogen availability was significantly higher when both guilds were removed; however, there was no observed competitive release in terms of increased soil respiration in one mycorrhizal guild upon removal of the other mycorrhizal guild.

- Respiration responses attributed to the mycorrhizal guilds to temperature and soil moisture were different; ectomycorrhiza and saprotrophic were favored over the ericoid guild in warmer and drier conditions.

In northern Sweden, the net sink of a managed spruce production forest (*c.* 50 years) had shifted from a net sink to a net source because the amount of total ecosystem respiration had increased more than the relative amount of gross primary productivity (Hadden & Grelle 2016). Over a three year period, there was a documented annual loss of CO_2 to the atmosphere. Comparing different parts of ecosystem respiration, and their interactions, to the whole, especially over multiple years, is critical to understand why these changes occur. If shrub activity is reduced, then this could alter the overall balance in net primary productivity and total ecosystem respiration.

The ectomycorrhizal contributions to respiration

The drought period coincided with an increased proportion of pine-associated and saprotrophic respiration (**Paper I)**. The drought in the second summer also hampered fungal mycelial growth during the second growing season in our study, as demonstrated by the low biomass in the 17-month incubation from the first bag set and the 5-month incubation in the second bag set (*Figure 10*, **Paper II**). This suggests that respiration from pine roots and associated organisms continued, but likely not from recent mycorrhizal mycelial growth during the second season. Instead, persistent respiration could be due to maintenance respiration from roots, ectomycorrhizal fungi and other root-associated fungi (Romero-Olivares et al. 2019). The contributions of ectomycorrhizal mycelium to total soil respiration, in isolation, is far less studied even though 8000 m of ectomycorrhizal hyphae per meter of root have been estimated (Leake et al. 2004). The ectomycorrhizal activity is simply a part of the ‘autotrophic’ root respiration term in our study, even though it has been shown to account for 25% of soil respiration in a 15 year old *Pinus contorta* stand in England (Heinemeyer et al. 2007). Across a boreal pine forest chronosequence in Sweden, the respiratory contribution of mycorrhizal mycelium ranged from 14 to 26% of total soil respiration (Hagenbo et al. 2019), and contributions from ectomycorrhizal mycelium have also been shown to be as low as 4% (Rhyti et al., 2021). Ectomycorrhizal root tips may be a better buffer to drought and resource reservoir compared to extramatrical mycelium, so studying different tissues may be central to understanding differential responses of ectomycorrhizal fungi to drought across studies (Erlandson et al. 2021).

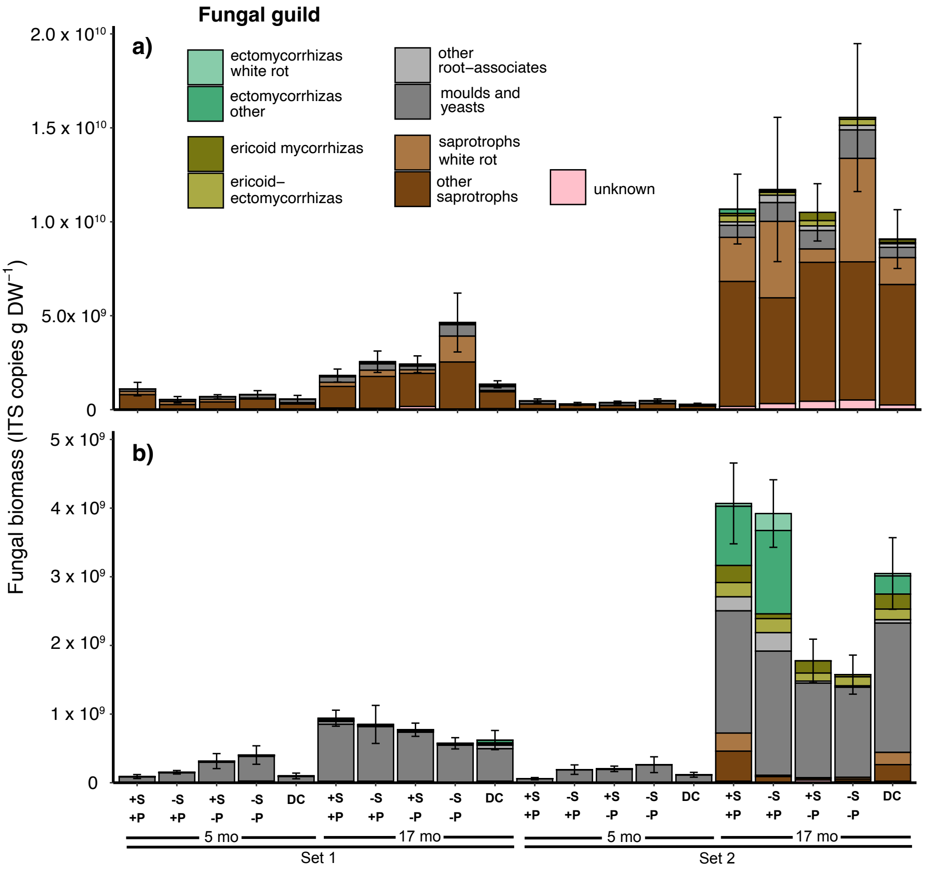

Figure 10. Total fungal (entire bars) and fungal guild (stacked bars) biomass (ITS2 copies g^{-1} dry weight (DW) substrate) in (**A**) decomposing pine needle litter and (**B**) humus incubated over 5 and 17 months (mo) in two decomposition bag sets initiated in June 2017 and June 2018. Total fungal biomass was estimated as copies of the fungal ITS2 region (quantitative PCR) and guild biomass was estimated by multiplying total fungal ITS2 copies with relative abundances of each guild derived from DNA sequencing of the ITS2 marker. Error bars are standard errors of the means of total fungal biomass (n=8, except n=7 for humus set 1 at 17 months for -S+P). See Supplementary Table S1 in **Paper II** for statistical tests of the 17 month incubations. Presence of pine roots indicated by (+P) and presence of shrubs are indicated by (+S). The disturbed control (DC) is displayed for comparison but was not statistically tested.

The Gadgil effect: pine needle decomposition

In **Paper II**, we found a 10% decrease in pine needle litter mass remaining, corroborating a Gadgil effect in pine litter substrates (*Figure 11*). Berg and Lindberg (1980) had reported the same phenomenon to occur after one year of incubation in the same forest as our trenching and shrub removal experiment. However, we had only seen a Gadgil effect in the first set after 17 months, which coincided with the severe summer drought. It is not entirely possible to compare results to the previous experiment almost four decades before, since it is unclear when the initiation of trenching and litter incubation was in the previous study (Berg & Lindberg 1980). A study by Koide and Wu (2003) demonstrated that soil moisture levels were significantly reduced as ectomycorrhizal myceliation of fine roots increased inside incubated mesh bags. In drier conditions, water could limit the activity of saprotrophic fungi in the litter layer, while in wet conditions, the uptake of water by ectomycorrhizal fungi and roots may have a little effect on decomposition by saprotrophic fungi in the litter layer (Bending 2003). Mechanisms for the Gadgil effect, such as nitrogen limitation and moisture limitation in the litter layer, may not be mutually exclusive (Fernandez & Kennedy 2016), since lower moisture may limit the breakdown of organic matter via the reduced activity of hydrolytic enzymes or just the mobility of essential nutrients, thereby reducing the amount of nitrogen accessible to litter saprotrophs. However, in our study, soil moisture availability was higher only in the combined shrub removal and pine root exclusion (**Paper I**), although the Gadgil effect was present in both pine root exclusion treatments (**Paper II**, *Figure 11*). Nitrogen additions *per se* have not been demonstrated to have a strong effect or interactive effect in either trenched or control plots, besides when large loads (50 kg N ha^{-1}) of nitrogen inhibited mass loss in trenched plots (Maaroufi et al. 2019). Also, it may be a matter of mutual invasibility, and not necessarily competition, between the saprotroph and ectomycorrhizal guilds that leads to the Gadgil effect (Simha et al. 2022). For instance, timing in mycelial production maybe be asynchronized after periods of drought between saprotrophs and ectomycorrhizal fungi, since ectomycorrhizal biomass will be dependent on a lagged carbon allocated belowground (Ekblad A 2005).

Both ericoid and ectomycorrhizal guilds reduced the availability of inorganic nitrogen in the soil in the fall the second growing season (2018; in a summer drought), and there was increased availability of soil nitrogen and water only when both pine and shrubs were eliminated (**Paper I**). Still, although not statistically significant, pine roots imposed a stronger sink for soil nitrogen than the shrubs, suggesting that a larger increase in available nitrogen after pine root removal may have supported higher saprotrophic fungal growth and decomposer activities (Sterkenburg et al. 2018). Otherwise, soil moisture conditions the following year could have alleviated any nutrient limitation from the Gadgil effect, since the third growing season of the experiment (2019) had higher precipitation than the first two years (365 mm yr^{-1}). The Gadgil effect was not observed after the 17-month harvest collected that third year (2019), indicating there could also be a transient effect with precipitation following drought that alleviates nitrogen limitation of saprotrophic fungi in pine needles even in the plots with ectomycorrhizal fungi and pine roots. There are often discrepancies in timescales of most experiments and theoretical investigations, therefore, more examinations of transient processes (*e.g.* different starting and progression of weather conditions) could support theoretical investigations into soil organic matter regulation (Hastings 2004).

The ericoid Gadgil effect

In **Paper II** we expected that the ericoid root ascomycetes would also inhibit saprotrophic decomposition in shrub leaf litter, similar to ectomycorrhizal fungi, because shrubs also act as a sink for available nitrogen (**Paper I**). However, we found did not find a shrub-mediated Gadgil effect, suggesting that the shrubs uptake of water and nitrogen were not drastic enough to limit the saprotrophic fungi. Shrubs even tended to have a negative effect on mass remaining in the second set, which is similar to Wardle and Zackrisson (2005), where shrub removal stimulated decomposition of willow litter in the more productive, larger islands compared to the retrogressed islands. In our case, we saw an increase in the ericoid mycorrhizal guild abundance in the pine needle litter in the presence of shrubs, suggesting that they support decomposition. In contrast, increases in soil inorganic nitrogen availability only by excluding both ericoid- and ectomycorrhizal guilds have benefitted saprotrophic decomposition of bilberry litter, supporting that guilds are still strong sinks for soil nitrogen in forests with higher productivity (Fanin et al. 2022). Differences between the studies could also be a matter of substrate quality, incubation depth (Bödeker et al. 2016), and changes in community composition of the fungi in the different litter types.

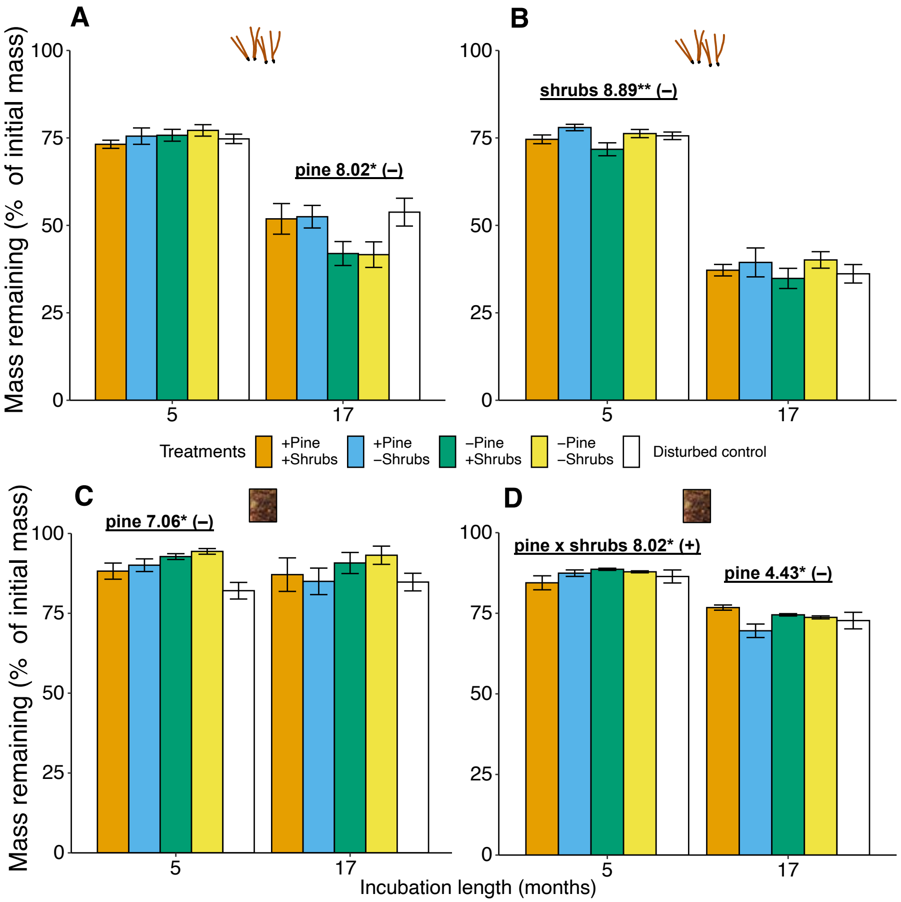

Figure 11. Mass remaining (% of initial mass) of two sets of Scots pine needle litter (**A** and **B**) and two sets of mor layer humus (**C** and **D**) decomposition bags, with each set consisting of 50 µm-mesh bags incubated for either 5 or 17 months in treatment plots in a mature boreal Scots pine forest (Fig. S1 **Paper II**). Treatments, indicated in color, involved factorial exclusion of pine roots and removal of shrubs. In disturbed control plots (white bars) pine roots were initially severed but were allowed to reestablish over the full duration of the experiment. Error bars are standard errors of the means (n=8, except n=7 for humus set 1 at 17 months in the +pine/-shrub treatment). Significant terms and their F-values ($df_{den} \sim 20$) based on linear mixed models encoding pine roots and shrubs as present (1) or absent (0) are shown for each incubation time (**, $P \leq 0.01$; *, $P \leq 0.05$), followed by the sign of the corresponding coefficient in brackets.

Contributions to soil respiration from the free-living heterotrophic guild remained just above 50% on average over the three years, meeting expectations. A higher proportion of saprotrophic contributions (63%) coincided with the Gadgil effect, however, the increased proportion of saprotrophs could have been due to the lack of a shrubs, which was seen with or without trenching. In many trenching experiments, cutting mycorrhizal roots by trenching could be deemed problematic in estimating heterotrophic and autotrophic components, since there is a release of excess carbon by the decomposition of roots and mycelial necromass that were cut off (Hanson et al. 2000). This has could be interpreted as a Gadgil effect (Rhyti et al., 2021), whereby the nitrogen immobilized in root and fungal necromass becomes accessible to nitrogen limited saprotrophs, increasing decomposition as seen in increases in respiration. Interpretations of the Gadgil effect may vary as strictly litter decomposition (Smith & Wan 2019) or even changes in bulk soil organic carbon (Harmer & Alexander 1985; Staaf 1988), which could also confound interpretations and inconsistencies in experimental results. In contrast, the rhizosphere is also an important component of 'priming' other free-living soil organisms with a consistent turnover of dead roots and mycelium, which we saw an overall lower amount of soil respiration in **Paper I** in the third year (2019) from the free-living heterotrophic component.

Based on our research in **Paper II**, fungi with white rot capacities will have a more positive effect on the rate of decomposition in pine needles than other saprotrophic fungi. Additionally, a root trenching experiment following days after trenching revealed that more moulds (with lower decomposition capacity) become dominant as opposed to other saprotrophic fungi with higher decomposition capacities (Lindahl et al 2010). High lignin : nitrogen ratios in pine roots also result in slower decomposition compared to other plant residues (See et al. 2019). Therefore, we suggested that root decomposition was slow in our plots, considering the negative effect of pine root removal on humus decomposition in **Paper II**. Additionally, the disturbed control allowed for the re-establishment of ectomycorrhizal fungi and pine roots, and the soil respiration never exceeded the control plots in three years. White rot saprotrophic fungi, largely dominated by the species *Mycena clavicularis* (Fr.) Gillet, had a positive effect on the decomposition rate of pine needle litter ($P(\beta W > 0) = 0.889$) compared to the inhibitory effect of other saprotrophic fungi ($P(\beta N < 0) = 0.987$). In the first set, white rot saprotrophs were stimulated in the combined shrub removal and pine root exclusion, although their abundance was not statistically different from the other pine needler litter bags incubated in the other plots after 17 months (*Figure 10*, **Paper II**). We also demonstrated that the pine root exclusion had little effect on the community composition in the litter (*Figure 11*). Therefore, it could be that the *Mycena* and white rot saprotrophs had reduced carbon use efficiency during the drought, and were aggravated even more by root uptake of water in untrenched plots, by allocating more carbon to maintenance respiration and secreting secondary metabolites rather than forming mycelia, although we cannot confirm that from our data. In the second set, *Mycena* may not have been limited by nitrogen or water, and decomposition progressed by increasing biomass and secreting lignolytic enzymes in both the controls and trenched plots.

Fungal Communities & Decomposition
in Pine Litter and Humus

Key Results: Paper II

- Total estimated fungal biomass (ITS2 copies g DW-1) in the litter and humus increased over the duration of incubation, particularly from 5 to 17 months, and fungal ingrowth was more evident in the second of two identical decomposition sets.

- There was a modest Gadgil effect (10% decrease in decomposition) in pine litter substrates, but only observed in the first of two identical consecutive decomposition set, coinciding with a summer drought. In the second set, the abundance of white rot saprotrophic fungi had a stimulating effect ($P(\beta W > 0) = 0.889$) while the abundance of other saprotrophic fungi had an inhibiting effect ($P(\beta N < 0) = 0.986$) on the decomposition parameter *k* in pine litter at the 17-month harvest.

- In the second humus set (and marginally in the first), the presence of shrubs particularly hampered white rot ectomycorrhizal fungi, such as *Cortinarius spp*. (in the presence of pine roots), and stimulated the abundance of ericoid mycorrhizal fungi, such as *Serendipita spp.*

- In concordance with the mycorrhizal guild hypothesis, pine roots significantly promoted humus mass loss, and and the decomposition model indicated that higher abundance of ectomycorrhizal fungi decreased humus mass remaining ($P(\beta Ecto < 0) = 0.773$). The humus decomposition model clearly related abundance of ericoid mycorrhizal fungi to larger mass remaining ($P(\beta N > 0) = 0.897$).

Intraguild turnover in ectomycorrhizal fungi with shrub removal

Between the incubation sets for humus, there was far more fungal biomass with longer incubations (17-months), and there were clear differences between the sets with overall higher amount of mycelial growth in the second set with the 17-month long incubation (*Figure 10*, **Paper II**). The pine root exclusion treatments were successful in eliminating ectomycorrhizal mycelial growth in humus substrates. The presence of pine roots promoted the longer-term colonization of humus by both of the ectomycorrhizal guilds, as well as of the dual ericoid-ectomycorrhizal guild and other root-associates (*Figure 10*, **Paper II**). No differences were found in the abundance of moulds & yeasts between the treatments. Fungal biomass in humus varied more with pine presence, and this is likely linked to variable ingrowth of different ectomycorrhizal fungi across treatments and individual bags (*Figures 10, 11*). On average, there was no difference of estimated biomass of fungi between the shrub removal and control treatments, however, there was a shift in community composition, as indicated in **Paper II**. As such, there was a clear dependence on the presence of shrubs of the fungi colonizing bags in presence of pine. In the presence of shrubs, *i.e.* in control and disturbed control plots, white-rot saprotrophic fungi were present, albeit infrequently, suggesting a dependence on both living pine roots and shrubs. The absence of shrubs, instead stimulated colonization of humus by white rot ectomycorrhizal fungi, mainly species of *Cortinarius*, *e.g. C.* aff. *obtusus*, *C. ominosus*, *C. caperatus* and *C. biformis*. Other ectomycorrhizal species, largely composed of *Piloderma sphaerosporum*, did not change abundance in the absence of shrubs, although there were exceptions, such as *Tretomyces lutescens*, which had an affinity to the shrub removal treatments. Many *Cortinarius* species were also stimulated by a long-term shrub removal experiment in northern boreal forest, meaning this was not a site-specific response and can be seen in mesh bags or in full soil profiles (Fanin et al. 2022).

Digging deeper: the mycorrhizal guild hypothesis

Across all bags in the second harvest of the second humus set, the shrub removal had the most mass loss (<75% in total after 17 months; *Figure 11*). The ectomycorrhizal guild was therefore critical for decomposition when the shrubs were removed. This was in agreement with the mycorrhizal guild hypothesis, although we did not see an increase in organic matter in the bags with the shrub only treatments. Instead, we saw an inhibition of mass loss (*i.e.* increased mass remaining) in the controls and in the pine root exclusion. This suggests that either synergistic interactions are at play between both ecto- and ericoid mycorrhizal fungi and shrub roots that build organic matter, or there are antagonistic effects that slow decomposition. These also may not be mutually exclusive. This may indicate that co-existence of the mycorrhizal guilds and intraguild turnover in ectomycorrhizal fungi facilitates organic matter accumulation. Potentially there is a mechanism through the chemical complexation of exudates and residues derived from pine and shrub roots as well as mycorrhizal necromass (Adamczyk 2019) in combination with suppression of the most efficient ectomycorrhizal decomposers by ericoid plants and mycorrhizal fungi. Furthermore, ericoid mycorrhizal fungi, do not have the capacity to recycle their own melanized necromass. In pine forest ecosystems, this could be a crucial interaction that is important for the decomposition and accumulation of soil organic matter. In the past decade there has been a decrease in shrub cover in production and protected coniferous forests across Sweden (Kyaschenko et al., 2022), which warrants further investigation into how that may affect carbon balances in boreal forest soils.

Our decomposition model indicated that higher abundance of the ectomycorrhizal fungal guild, as a whole, was related to decreased humus mass in **Paper II**. When we further tested whether the white rot guilds were related to decomposition, neither white rot saprotrophs (mainly *Mycena clavicularis*) nor white rot ectomycorrhizal fungi (mainly *Cortinarius*) were related to mass loss. This should be further investigated as it could be confounded by simultaneous *ingrowth* of mycelial mass into the bags. The incubation time may be too short and the time points may be aggregating uneven dynamics of ectomycorrhizal ingrowth and decomposition. Also, there is a lack of sufficient replication to capture the inherent stochasticity in ectomycorrhizal mycelial colonization of soil (Sterkenburg et al. 2019). Either way, we find that a group-level relationship does not automatically characterize the relationship at the level of the individual.

Shrub removal reduced ericoid mycorrhizal colonization of the bags, and this was most evident with the genus *Serendipita*. There appeared to be some exceptions where shrub removal did not reduce certain putative ericoid mycorrhizal fungi (*e.g. Hyalscypha* aff. *hepaticicola*) with or without pine roots excluded. Also, it should be noted that shrub roots could colonize the bags, adding to an increase in mass in the humus. We were unfortauntely unable to discriminate how much biomass of shrub roots entered the bags. Either way, during the second year, ericoid mycorrhizal growth was basically non-existent in the humus (*Figure 10*, **Paper II**), and this coincided with the complete reduction in soil respiration of the ericoid shrubs during periods in the second summer (**Paper I**). Potentially, ericoid-mycorrhizal fungi that are associated with the shrubs have a versatile capacity to decompose cellulose for this reason: they can't always depend on their host shrubs to reallocate carbon belowground.

In **Paper I,** we expected to see a competitive release, or an increase in respiration, and uptake of nitrogen by the remaining guild, upon the exclusion of the other mycorrhizal guild. There was clear indication of competition for nitrogen, as nitrogen availability was reduced in the presence of both shrubs and pine roots individually or combined (as compared to the heterotrophic plots), and lingon leaves had increased the amount of nitrogen in their leaves with trenching (**Paper I**). However, the respiration between the guilds remained additive, and there was no interaction found over the cumulative respiration over three growing seasons. In fact, the difference in soil respiration between shrub removals in the presence of pine roots tended to be higher than in the trenched plots with and without shrubs (**Paper I**). Likewise, a synergism between mycorrhizal guilds on soil respiration has been demonstrated in the southern Finnish boreal forest potentially due to shared mycelial connections between shrubs and ectomycorrhizal roots (Rhyti et al., 2021).

Concluding remarks on guild effects on respiration and decomposition

What we found is that activities among mycorrhizal guilds and saprotrophs could also shift with warmer and drier conditions, reducing soil respiration of the shrub roots and associated organisms compared to ectomycorrhizal fungi and free-living decomposers. When there are beneficial growing conditions for mycelial production, more mass loss was evident in the humus, and differences between the guilds effects strengthened. Shrubs contributed more to slowing decomposition in the presence of pine roots, and ectomycorrhizal fungi contribute to more decomposition with the absence of shrubs. Saprotrophs have little effect in the cellulose depleted humus, but increased decomposition of pine needles persisting through drought conditions. Overall, shifts in guild contributions could have effects on soil carbon accumulation and decomposition, and a mycorrhizal guild interactions are a more important research priority in long term carbon pools, than ectomycorrhizal-saprotrophic interactions.

4. Ectomycorrhizal Conservation Values

Assessing the relationship between perceived forest conservation values and ectomycorrhizal diversity

4.1 The Forest Conservation Gradient: Study System & Design

Forestry has accelerated the transformation of boreal forests from long tree continuity towards younger, even-aged monocultures, and poses a serious threat to forest biodiversity. So far, conservation assessments have focused mainly on aboveground biota, while effects on ectomycorrhizal fungal species – dependent on photo-assimilated carbon from living trees – have received much less attention. With the advent of DNA metabarcoding, promising approaches could complement conservation efforts because the sampling does not require a limited set of field experts, and the phenological conditions for sporocarp surveys do not necessarily need to be met (*i.e.* timing surveys in the fall, and beneficial amounts of precipitation for sporocarps). However, it remains to be tested if this is a viable option.

For **Paper III**, production forests and voluntary set-asides or 'woodland key habitats' were selected from a public Swedish database, and the forests formed a conservation gradient across mature and old-growth coniferous forest stands (all with productivity of >1 m^3 timber ha^{-1} yr^{-1}) in two regions of the Swedish boreal forest (*Figure 12*). According to the developers (Drakenberg & Lindhe 1999), the conservation assessment score can be used to measure biodiversity potential of different forests, and modified versions of the survey have been used in many different countries around the world. The survey is divided into six sections where points are given for up to 12-15 values in each; general site characteristics, dynamics, microhabitats, trees, structures, and deadwood. In our study, the inventory was done in a 2 ha. area within each forest stand. The presence of all values were registered and summed to give the score of the stand, which is referred to as conservation assessment score or 'conservation value' in our case, which is typically no more than 30 points.

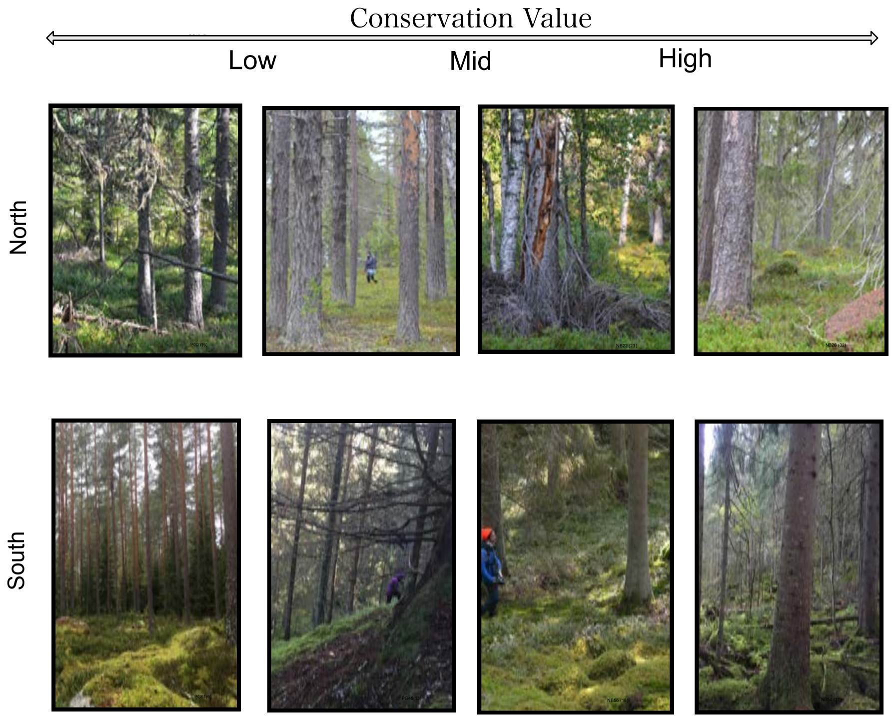

Figure 12. Example forest stands from low to high conservation assessment scores or 'conservation values' in the northern and southern regions of the forest conservation gradient, respectively.

4.2 Hypotheses

Given the importance of tree productivity in supporting ectomycorrhizal fungi (Lindahl & Clemmensen 2017), we hypothesized: (H1), that ectomycorrhizal fungal abundance and species richness would be higher in southern compared to northern boreal forest, but

(H2), that the conservation assessment score had no explanatory power of patterns in total ectomycorrhizal communities or of ectomycorrhizal species of conservation interest because it mainly reflects aboveground forest structures and species groups.

Instead, soil fertility was hypothesized (H3a) to be positively related to ectomycorrhizal fungal abundance and richness, assuming that more ectomycorrhizal species could persist with higher resource availability, given the limited nitrogen deposition in the boreal forest (Clemmensen et al. 2015; Sterkenburg et al. 2015). Furthermore, we hypothesized (H3b), that ectomycorrhizal fungal richness would positively relate to the stand-level tree continuity, due to accumulated richness over time. Finally, we expected (H3c), that a lower proportion of forest with long continuity in the landscape would relate to lower richness of ectomycorrhizal fungi due to dispersal limitation.

These hypotheses were tested both for total ectomycorrhizal communities and for a more restricted group of ectomycorrhizal fungi, previously only found in Europe, assuming that these would be more representative of species of conservational interest.

4.3 Methodological Notes

Soil sampling and processing for high-throughput sequencing

60 soil cores (3cm diameter and <30 cm deep) were pooled and frozen from each of the 76 forests (*Figure 13*), and later homogenized in a frozen mill. Subsamples were taken and finely milled to assess organic matter content and pH. DNA was extracted from a 50-450 mg freeze-dried and milled subsample, depending on organic matter content (from 12-96%). A similar procedure was used to amplify PCR products and sequence fungal communities as in **Papers I** and **II**, although 4 SMRT cells were used for one sequencing pool in this study, aiming for a deeper sequencing to capture more complete fungal communities in the pooled samples. A total of 1,019,166 HiFi sequences were filtered in a similar manner to **Papers I** and **II**. One sample was lost in the sequencing and/or filtering process, and the resulting 75 samples were each represented by 5,030 sequence reads on average. For over 500 ectomycorrhizal fungal species, taxonomy, endemicity status, and functions were assigned manually, guided by UNITE, NCBI, FungalTraits and further probing into literature of type specimens. Species were classified as endemic to Europe based on current information on earlier geographical distribution of species hypotheses (based on the ITS marker) in UNITE (v.08) (McPolin & Kranabetter 2020; Tedersoo et al. 2022). Red-listed species were included as those that are defined as near-threatened, as data deficient (examined by experts to have too little information available for an assessment), or vulnerable in Sweden (Artdatabanken 2020).

Statistical methods

Statistical tests and graphics were conducted in R using an array of methods. Correlation matrices were used to evaluate collinearity among explanatory variables. Generalized linear models and Mantel tests were used to evaluate alpha and beta diversity metrics and relative abundance of the different ectomycorrhizal groups across explanatory variables, such as conservation value, soil fertility, tree continuity, and tree basal area. Ordination methods were used to formulate a soil fertility index (PCA) and to analyze shifts in the community composition (CCA and DCA) across the forest conservation gradient.

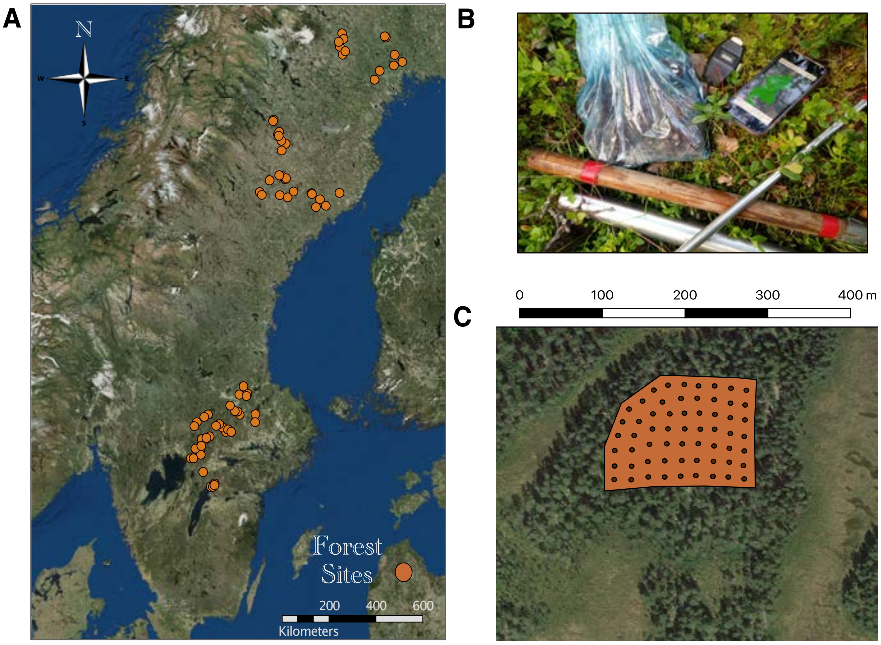

Figure 13. Map including the approximate locations of 76 forest stands situated in northern and southern regions of the boreal forest in Sweden (**A**). Sampling tools used for collecting soils including a 3 cm diameter soil corer, GPS with the shapefile of the site, clicker counter, and pooled 60 core sample (**B**). An example of a 2 ha forest stand with the systematic sampling spots of 60 cores taken at least 10 meters apart (**C**).

4.4 Results & Discussion

Do conservation values reflect ectomycorrhizal diversity?

In this study, spanning most of the latitudinal extent of boreal forest in Sweden, the southern forests generally had higher abundance and richness of ectomycorrhizal fungi than the northern forests (*Figure 14*), both in terms of species endemic to Europe and of the more widespread ectomycorrhizal fungi ('non-endemic' species). This is in concordance with the latitudinal species hypothesis (and our H1), which predicts that more southern regions with milder and more stable climates, higher productivity, and a longer growing season will lead to a greater abundance and richness of species than northern regions (Pianka 1966; van der Linde et al. 2018).

In total we found 529 ectomycorrhizal species, of which 130 species had distributions confined to Europe (*Figure 15*). We found 24 ectomycorrhizal fungal species that matched to the Swedish red-list (18 species) or signal species list (6 species). *Cortinarius* was the most species rich genus with 140 observed species while *Cenococcum geophilum* coll. was the most abundant species. *Cortinarius* was clearly the most species rich ectomycorrhizal genus, largely composed of *Cortinarius* subg. *Telamonia*. The most abundant and frequently encountered species in our sites, *Cenococcum geophilum* coll., has also been commonly found dominating the ectomycorrhizal community less than one decade after clear-cutting in pine chronosequences (Kyaschenko et al. 2017a; Hasby 2022). The genera *Russula, Elaphomyces,* and *Piloderma* were more abundant in the south compared to the north, potentially indicating a warmer climatic niche.

The relationship between species richness and conservation value was different between the north and south. Species richness was unrelated to conservation values in the north, and only positively related in the south (*Figure 14*). Conservation values were largely assessed based on presence of aboveground forest structures (*i.e.* deadwood), but they also reflected the status of common ectomycorrhizal fungi in the south (partial rejection of H2). This could indicate that intensive forest management in the south has had a negative impact on ectomycorrhizal fungal species richness on a stand level. In essence, the stands with lower conservation values hosted fewer

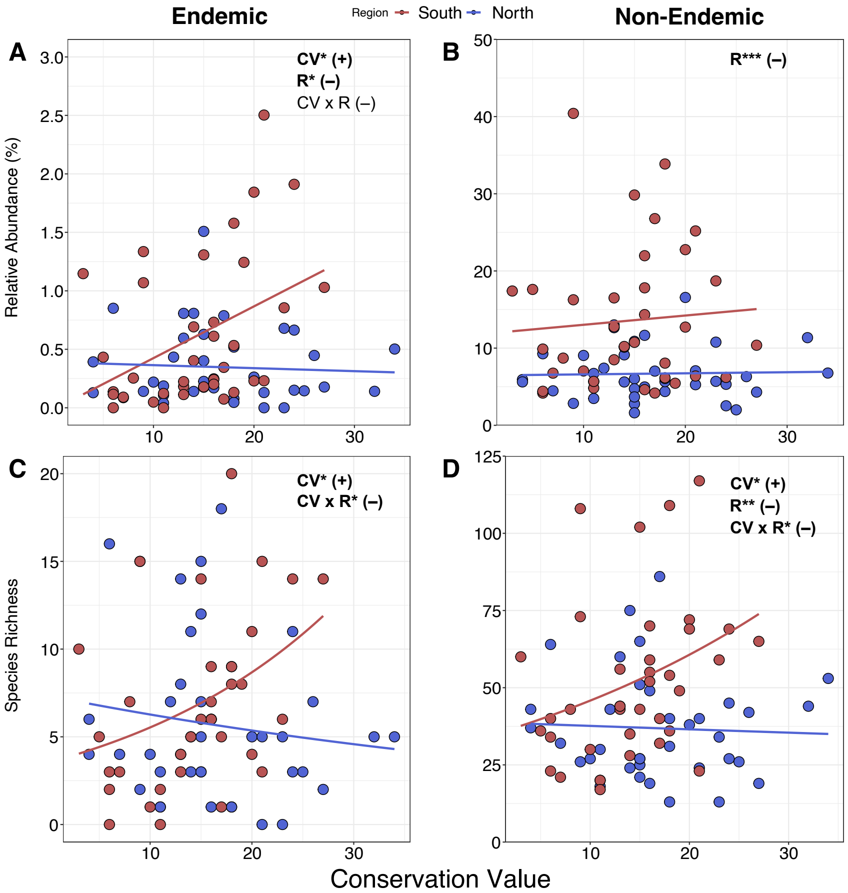

Figure 14. Abundance and species richness metrics of endemic (confined to Europe) and non-endemic ectomycorrhizal fungal communities in relation to conservational value in 2 ha. stands (each dot) in southern (red, n = 34) and northern (blue, n = 38) regions of boreal forest in Sweden. Ectomycorrhizal fungal responses are represented by relative abundance (% of total fungal abundance in DNA amplicons) (**A**,**B**) and species richness (**C**,**D**) of endemic and non-endemic ectomycorrhizal fungi, respectively. Generalized linear models tested effects conservation value (CV), Region (R), and their interaction. Significant terms were bolded (***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$), or not bolded if $P \leq 0.10$.

ectomycorrhizal species in the south. Potentially, no relationship between ectomycorrhizal species richness and conservation value in the north is due to the shorter history of intensive forestry (Esseen et al. 1997; Svensson et al. 2019). In terms of species turnover (*i.e.* pairwise community dissimilarity) among sites, the conservation value had little relationship, and comparing sites pairwise across the conservation gradient – from low to high conservation values – does not result in a significantly larger turnover in the ectomycorrhizal community with higher conservational values.

Key Results: The Forest Conservation Gradient

Paper III

- Higher ectomycorrhizal species richness was found in the southern sites (59 ± 30) compared to the northern sites (42 ± 21) on average.
- Conservation value was not related to ectomycorrhizal species richness or turnover in the northern sites; however, there was a positive relationship in the south, although tree continuity was not strongly related to ectomycorrhizal species diversity metrics.
- Soil fertility was positively related with ectomycorrhizal species richness and community turnover and was the most important factor underlying ectomycorrhizal community composition in a forward selection CCA for both the north and south.

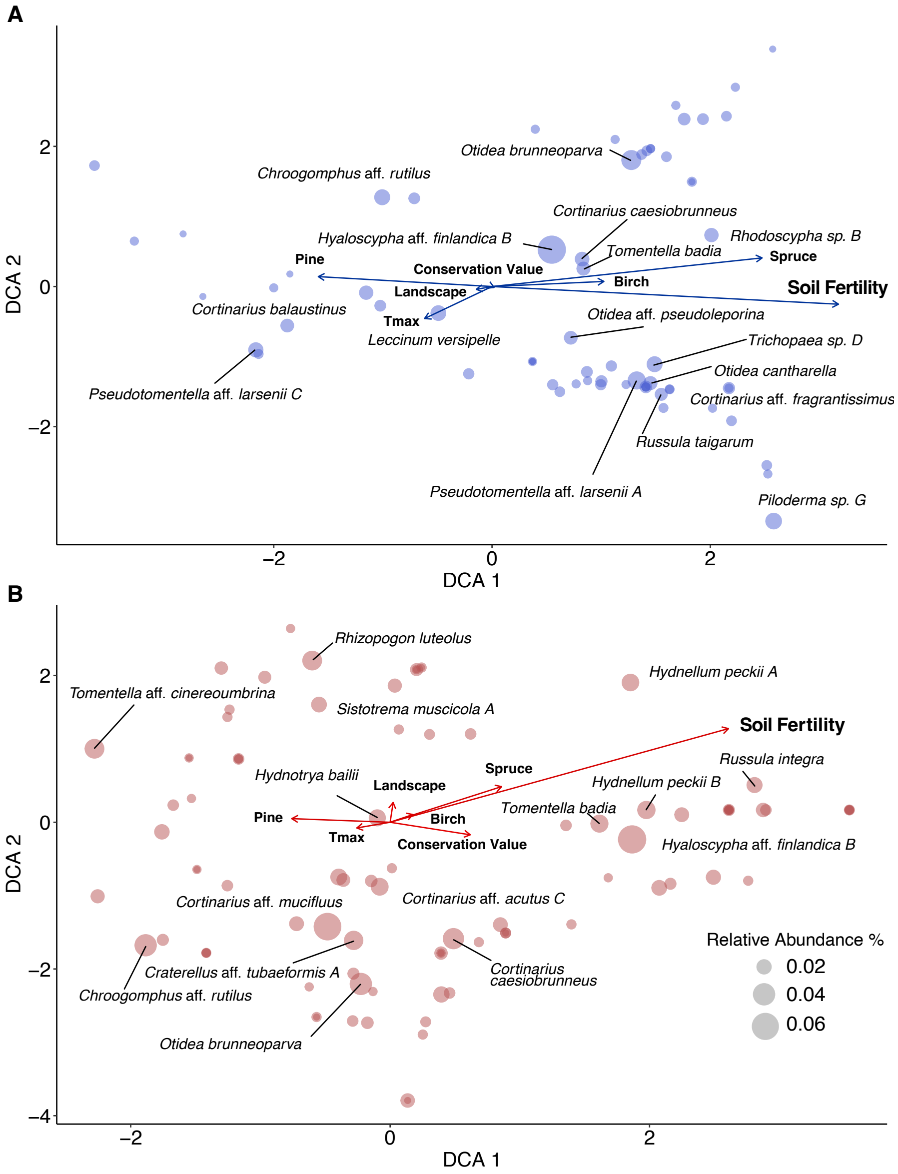

Figure 15. Species plots of detrended correspondence analyses (DCA) of all ectomycorrhizal fungi in northern (**A**) and south-central (**B**) Sweden, but only the endemic species (with a European distribution) are displayed with the top 15 most abundant endemic species named. Fungal communities were analyzed in composite 60 soil core samples by DNA-sequencing of amplified ITS2 markers. Vector lengths indicate the degree of correlation between the DCA axes and stand-level characteristics.

Soil fertility, tree basal area and ectomycorrhizal diversity

Our data overall supported the hypothesis (H3a), that ectomycorrhizal soil fertility was positively related to ectomycorrhizal fungal diversity and abundance, although with some exceptions (*Figure 16*). In the northern region, the relative abundance, richness, and diversity of both endemic and non-endemic ectomycorrhizal fungal communities increased toward sites with higher soil fertility. This is in agreement with a study demonstrating increased biomass and relative abundance of ectomycorrhizal fungi (out of total fungi) towards naturally more fertile old-growth boreal forests exposed to limited N-deposition (Sterkenburg et al. 2015). Similarly, among continuity forests in the north (>60 degree latitude), the relative abundance of ectomycorrhizal fungi out of total fungi was positively correlated with tree biomass (Lindahl et al. 2021). An additional reason for lower relative abundance of ectomycorrhizal fungi at lower fertility could be that non-ectomycorrhizal root-associated ascomycetes made up a larger proportion of the soil fungal community in retrogressed forests (*i.e.* forests at later successional stages with reduced productivity). Such low-fertility and poorly-productive forests are characterized by a dense understory of ericaceous dwarf shrubs, which host a high diversity of root-associated fungi, that limit productivity, and thereby constrain ectomycorrhizal mycelial production, shifting the fungal community towards lower ectomycorrhizal relative abundance (Clemmensen et al. 2015). We also found that effects of soil fertility are tightly linked to shifts in basal area of the main ectomycorrhizal trees, which is commonly found in the boreal forest (Sterkenburg et al. 2015; Kyaschenko et al. 2017b). The increase in fertility paralleled an increase in birch basal area in the northern sites, leading to an increase in endemic species richness and relative abundance, but there was no effect in the southern sites with lower birch basal area. Birch does not associate with as many ectomycorrhizal species as pine and spruce, and shares fewer species in common (Otsing et al. 2021), but still trees, such as birch, with specialized mycorrhizal associations, especially endemic species, require focused conservation measures (Tedersoo et al. 2022). Ectomycorrhizal community composition clearly shifted along the soil fertility gradient and was the most important component in describing fungal community composition in both regions, which corroborates previous observations at a regional scale (Sterkenburg et al. 2015).

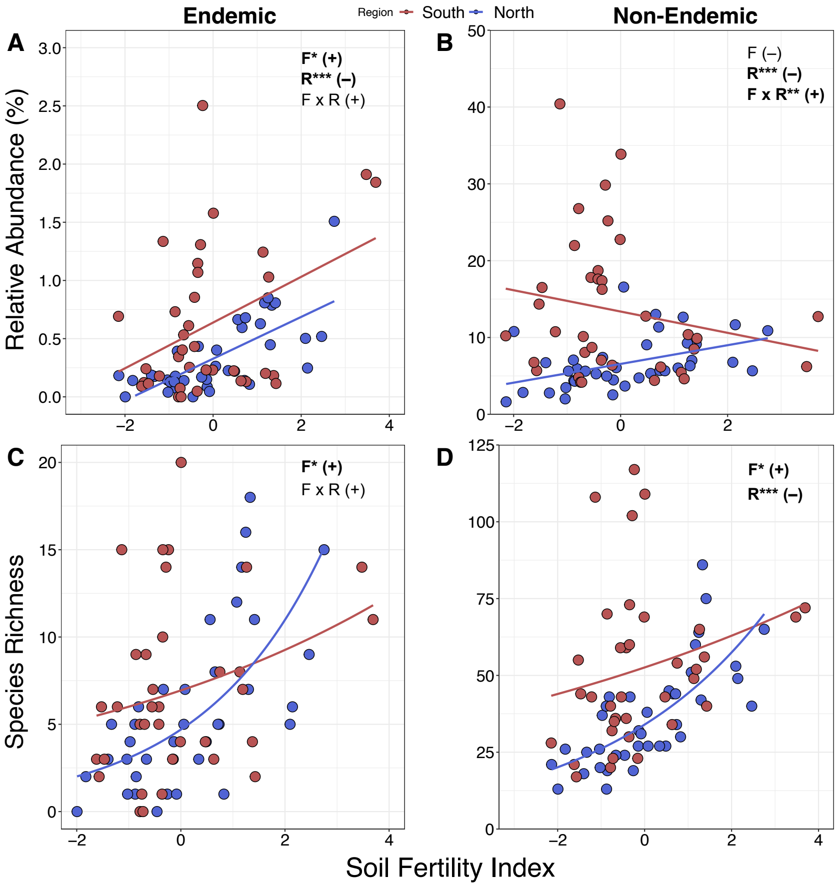

Figure 16. Abundance and species richness metrics of endemic (confined to Europe) and non-endemic ectomycorrhizal fungal communities in relation to soil fertility in the southern and northern regions of the Swedish boreal forest. Ectomycorrhizal fungal responses are represented by relative abundance (% of total fungal abundance in DNA amplicons) (**A,B**) and species richness (**C,D**) of endemic and non-endemic ectomycorrhizal fungi, respectively. Generalized linear models tested effects of region (R), fertility (F), and their interaction. Significant terms were bolded (***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$), or not bolded if $P \leq 0.10$.

Relationships to local and landscape forest continuity

Our study is one of the few investigations of effects of local and landscape-level forest continuity on ectomycorrhizal fungal community composition. We did not detect any relationships between oldest trees in our stands and ectomycorrhizal richness, even for endemic species (rejecting hypothesis H3b; *Figure 17*). Also, in contrast to expectations in (H3c), landscape forest continuity was negatively associated with ectomycorrhizal species richness, diversity and relative abundance the northern sites. This potentially suggests little dispersal limitation of commonly found ectomycorrhizal species within the 2 km buffer zone in which forest continuity was assessed, which is corroborated by little insularity (*i.e.* endemism) of soil fungi globally through metabarcoding efforts (Tedersoo et al. 2022). But perhaps we generally find few landscape effects because species found through soil DNA metabarcoding are generally the species that are likely to be frequently and abundantly encountered; so a circular argument. The negative effect of old-growth forest in the landscape could be an example of extinction debt where an excess of species in fragmented forests persists after the surrounding landscape has been clear-cut (Tilman et al. 1994), such as for epiphytic lichens (Berglund & Jonsson 2005). Forests that are particularly species rich with a high relative abundance of fungi in the north (*i.e.* with higher fertility) may have been relatively more fragmented since industrialized clear-cutting began, and the effects of clear-cutting on forests with a long tree continuity may not yet be realized. Therefore, the ectomycorrhizal community composition and maturity of forests in the landscape may be more crucial for younger forests and forests approaching harvest age with lower species diversity (*i.e.* forests at canopy closure), than for old-growth forests, although this remains to be tested. Multiple studies suggested that ectomycorrhizal diversity remains low, even as the stand approaches canopy closure at 30-40 years, with simultaneous increases in soil organic matter and ectomycorrhizal biomass (Wallander et al. 2010; Kyaschenko et al. 2017a; Hasby 2022). However, we cannot yet say how long time it takes for diversity to return to pre-clear-cutting levels, since there is no study looking explicitly at this in the boreal forest. Further studies are needed to integrate how mycorrhizal fungal spore dispersal and mycorrhization dynamics depend on stand development and management for species of conservation interest.

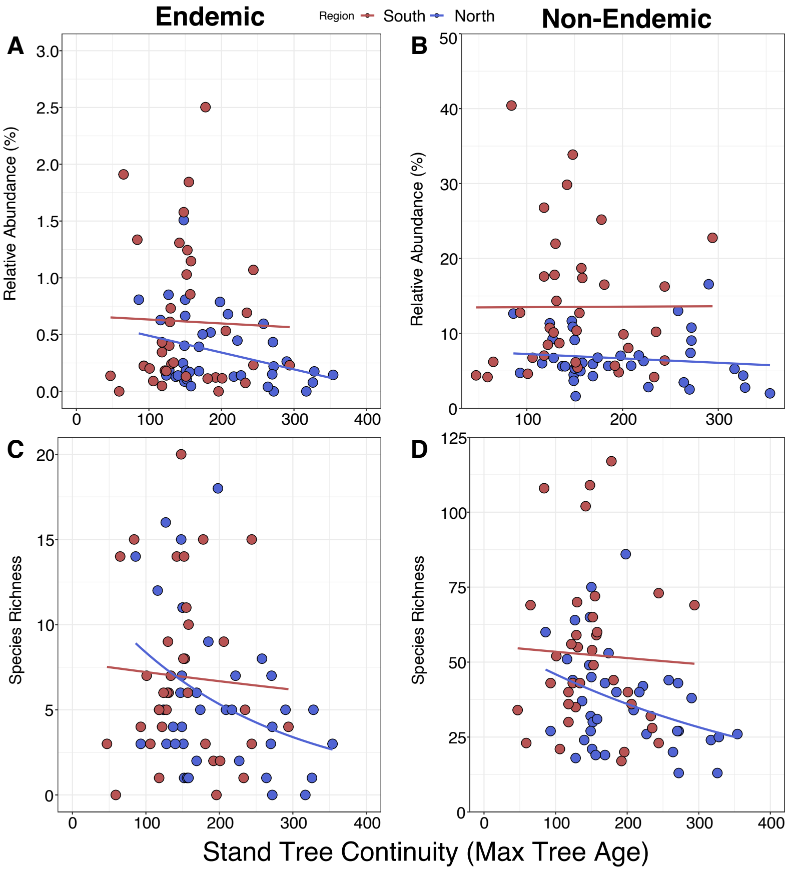

Figure 17. Abundance and species richness metrics of endemic (confined to Europe) and non-endemic ectomycorrhizal fungal communities in relation to stand tree continuity (max tree age in each stand) in the southern and northern regions of the Swedish boreal forest. Ectomycorrhizal fungal responses are represented by relative abundance (% of total fungal abundance in DNA amplicons) (**A,B**) and species richness (**C,D**) of endemic and non-endemic ectomycorrhizal fungi, respectively. Generalized linear models tested effects of region (R), Tmax (T) and their interaction. Significant terms were bolded (***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$), or not bolded if $P \leq 0.10$.

Detection of ectomycorrhizal species of conservation interest

One critical finding in **Paper III** is that even with 60 pooled soil samples from each of 76 smaller forest stands, we detected only 19 of the 154 ectomycorrhizal fungi that are red-listed (data deficient, near threatened, or vulnerable) in coniferous forests of Sweden. This is not surprising and rather expected due to two main reasons:

(a) red-listed species are listed because they are likely declining (*i.e.* due to forestry in this case) and are also often rather rare in the habitat and conditions they occur, and secondly,

(b) the extremely small part of the soil that can be analysed only allows for frequently encountered and abundant taxa with large mycelia to be appropriately analyzed; in this sense, we may only pick up red-listed taxa that are more common than previously thought based on fruit body surveys, or red-listed taxa with larger or more persistent mycelia.

Table 1. All Swedish red-listed fungi found across the forest conservation gradient (data-deficient (DD), vulnerable (VU) and near threatened (NT) and signal (S) species. Other information includes their closest UNITE species hypothesis, whether their distribution, based on sequences with 99% similarity, was confined to Europe, information on exploration type and sporocarp morphology as found in FungalTraits, and any notes on reference sequences or identification. Some species hypotheses may indicate species complexes or are unable to be resolved at the species level with the ITS2. NE refers to not examined by the Swedish Red-List.

Taxa	UNITE Reference	Europe	Exploration Type	Sporocarp Morphology	Swedish Red List	Notes
Sistotrema aff. *alboluteum*	SH1665340.08FU	N	medium-distance fringe	corticioid	DD	
Sistotrema alboluteum A	SH1665343.08FU	N	medium-distance fringe	corticioid	DD	Nilsson et al., 2006
Sistotrema alboluteum B	SH1665338.08FU	N	medium-distance fringe	corticioid	DD	Nilsson et al., 2006
Phlegmacium serarium	SH1503953.08FU	Y	medium-distance fringe	agaricoid	VU	Liimatainen et al., 2022, syn. *Cortinarius serarius*
Cortinarius ionophyllus/ultimiionophyllus	SH1710164.08FU	N	medium-distance fringe	agaricoid	NT/NE	Liimatainen et al., 2020
Thaxterogaster pinophilus	SH1503841.08FU	N	medium-distance fringe	agaricoid	NT	Liimatainen et al., 2022, syn. *Cortinarius pinophilus*
Elaphomyces leveillei	SH1702445.08FU	Y	short-distance delicate	gasteroid-hypogeous	NT	
Elaphomyces s. l. anthracinus	SH1611782.08FU	Y	short-distance delicate	gasteroid-hypogeous	VU	Confined to Scandanavia
Hydnellum peckii A	SH1644050.08FU	Y	mat	agaricoid	S	OP150200
Hydnellum peckii B	SH1644050.08FU	Y	mat	agaricoid	S	MK602733 Larsson et al 2019
Hydnellum aurantiacum	SH1730119.08FU	N	mat	agaricoid	S	
Sarcodon s. str. imbricatus	SH2479265.08FU	Y	mat	agaricoid	S	Larsson et al. 2019
Hydnotrya aff. *michaelis*	NA	Y	contact	gasteroid-hypogeous	VU	SI18-2161 Lindahl et al., 2021
Otidea brunneoparva	SH1664063.08FU	Y	short-distance coarse	apothecium	DD	Type KM010026 Confined to Scandanavia
Otidea mirabilis	SH1798965.08FU	Y	short-distance coarse	apothecium	DD	Holotype GMFN:1951
Lyophyllum semitale/deliberatum	SH1840402.08FU	N	medium-distance fringe	agaricoid	NT/S	Yamada et al., 2001
Phellodon melaleucus	SH1835627.08FU	N	mat	agaricoid	NT	*Phellodon connatus* coll.
Phellodon niger coll.	SH1707141.08FU	N	mat	agaricoid	NT	
Tretomyces aff. *lutescens A*	SH1669516.08FU	N	medium-distance fringe	corticioid	VU	
Tretomyces aff. *lutescens B*	SH1669517.08FU	Y	medium-distance fringe	corticioid	VU	
Tretomyces aff. *lutescens C*	SH1669515.08FU	N	medium-distance fringe	corticioid	VU	
Polyozellus alobatus	SH1829418.08FU	N	medium-distance smooth	corticioid	DD	Svantesson et al 2021, syn. *Pseudotomentella alobata*
Polyozellus pinophilus	SH2976204.08FU	N	medium-distance smooth	clavarioid	DD	Svantesson et al 2021, syn. *Pseudotomentella pinophila*
Polyozellus tristis	SH1829404.08FU	N	medium-distance smooth	corticioid	DD	Svantesson et al 2021, syn. *Pseudotomentella tristis*
Tricholoma fucatum	SH2137796.08FU	Y	medium-distance fringe	agaricoid	LC/S	neotype LT000170.1
Tricholoma c.f. viridilutescens	SH2569757.08FU	Y	medium-distance fringe	agaricoid	LC/S	Heilmann-Clausen et al. 2017, *T. viridilutescens* type II

We did not analyze how many of the sporocarp-based, least concern (LC) species were found in our metabarcoding dataset. Potentially, the lack of certain LC species could be an indication of rarity or small genet size, given they are found in public sequence databases. Mismatches in taxonomy and naming could have complicated comparisons from our DNA-based taxa and those listed in the Swedish red-list, and warrants further investigation. In the study by Frøslev et al. (2019), they looked for 502 of the 656 Danish red listed species that were present with sequence data in UNITE (excluding wood-inhabiting fungi and other non-soil substrates), and the soil DNA metabarcoding approach performed almost as good as sporocarp surveys, although they picked up different species.

If ectomycorrhizal species of conservation concern are considered in conservation assessments (*i.e.* before development or clear-felling), then at this point, extensive sporocarp searches should be done over multiple years when conditions are good. In this way, all sporocarps of red-listed and signal species and their mycelia can be identified (by skilled mycologists). The likelihood of DNA metabarcoding to depict a species with one or a few mycelia, or specialized and rare species irregularly extending in one or maybe a few square meters within 2 hectare stands (20,000 m^2), is unlikely. Red-listed species assessed in Frøslev et al. (2019) were quite comparable between soil sporocarp surveys over multiple years by experts (100 species, before removing mismatches to UNITE) and DNA soil metabarcoding (80 species). The plots were 40 x 40 m with soil cores taken every 4 m in Fröslev et al. (2019). If this would have been at a 2 ha scale like in our study, then it would require 1,000 pooled soil cores from each of our stands. Furthermore, computationally pooling samples would provide higher sampling effort (i.e. detect more species) than physically pooling (Song et al. 2015), although there would be many more consumables and a longer processing time.

Metabarcoding of soil fungal communities may be more sensitive than sporocarp surveys to identify certain species of conservation interest, either due to lack of easy sporocarp identification and/or their hidden lifestyle. For example, *Polyozellus spp.,* many of which are previously known as *Pseudotomentella* (Svantesson et al. 2021) are red-listed as data deficient (DD), and also had distributions of environmental DNA confined to Europe based on the current global sampling effort (Nilsson et al. 2019). Since these species are detected in our study, this may also indicate that either these particular species (*P. tristis*, *P. pinophilus*, *P. alobatus*) have large mycelia, or they have many individuals across a forest stand; however, we cannot say for sure. A one-off sampling effort, such as our study is also static, and can only supplement red-list efforts, since there is no description of population trends or reproductive fitness from our observations; such trends are critical for red-listing for the IUCN classifications (Mueller et al. 2022).

Species in the family *Bankeraceae*, or *Ramaria spp.* were found sparsely in our metabarcoding study with far less species richness compared to sporocarp inventories in the same 2 ha forest stands (Hekkala et al. unpublished). This is likely due to the ease of basidiocarp identification, persistence of sporocarps, and the patterns of mycelial growth with few big patches vs. many small genets widely distributed within forest stands as common for most other species (van der Linde et al. 2012; Sterkenburg et al. 2019). Based on the same forest stands, the southern forests, on average, had lower richness of species of conservation concern, including some macro-fungi, than northern stands, possibly due to the longer legacy of selective logging and clear-cut forestry in the south (Hekkala et al., unpublished).

Are ectomycorrhizal species restricted to Europe of conservation interest?

I analyzed what environmental factors that determined diversity and community composition of ectomycorrhizal fungi in the Swedish boreal forest. We addressed the importance of both abiotic and biotic factors, and if composition of ectomycorrhizal fungi were related to conservation values assessed based on aboveground structural diversity. Here, I focused on species confined to Europe (termed ‘endemic’ in a broad sense), in order to focus on species that may be particularly vulnerable to land-use change occurring in Europe. We defined endemic species by a restricted geographical detection of their DNA barcode in earlier published data. This method is, thus, independent of correct taxonomic information, and can be applied to undescribed species as well. However, endemism to Europe is very broad, and may not be the best way to describe high mycorrhizal fungal conservation values. Certain species may still be extremely common, albeit with a narrow distribution within Europe. For example, should a species with an affinity to *Craterellus tubaeformis* be considered a fungus of high conservation value just because it is endemic (in this broad sense), but commonly found across Scandinavia? On the other hand, species in the genus *Otidea* may be considered as vulnerable (VU) species given the high proportion of endemic species that are infrequently encountered only in mature forest stands within a limited range in Scandanavia. Because our sampling is restricted to a single country, then we may have an artificially larger ITS2 barcoding gap (*i.e.* DNA-sequence-based dissimilarity between species) without having representation from sister species in other regions. In contrast, we may be sampling some of the most closely related species, so the barcoding gap will be much smaller (Garnica et al. 2016). This could affect our clustering levels, *i.e.* whether we may have over- or under-clustered some species groups, and therefore our species concept. Even with caveats, metabarcoding provides complementary data to make a more informed approach to the conservation of common ectomycorrhizal fungi, and is useful in guiding assessments of data deficient species or least concern species. DNA metabarcoding does not, however, replace expert field mycologists collecting information about the reproductive fitness of species of conservation interest that occupy only a few square meters, unless we were to homogenize all of the soil in a forest stand and extract it all, which would not be advisable.

Concluding remarks on the forest conservation gradient

The assessed conservation value of forest stands was not a better indicator of ectomycorrhizal diversity overall, but there were differences between regions. Soil fertility, corresponding with tree species composition, was the strongest indicator of ectomycorrhizal abundance, diversity and ectomycorrhizal community composition in both regions. The basal area of birch was correlated with endemic species richness in the north, and higher pine basal area and soil fertility were important indicators for species turnover across stands in the south. Assessed conservation value of forest stands was a better indicator of the ectomycorrhizal species richness in the south; however, it also indicated that the longer history of intensive forest management in the south has decreased the relative abundance and richness of putative endemic ectomycorrhizal fungi. The high species turnover in the ectomycorrhizal community, and overall non-nestedness among forest stands suggests that if conservation of all ectomycorrhizal diversity is a prioritized target, it is better to preserve communities in a large number of stands, rather than just stands with higher conservation assessment scores. Using molecular methods for assessing mycorrhizal species of conservation concern are, in fact, limited. The reproductive success of fungi is considered to be very important for the overall maintenance of the population, and identifying whether the fungus has the potential to persist in the environment (i.e. presence of its DNA) is also a feature that is only supplemental for assessing the status of common species. Overall, forests with higher fertility and high basal area of different tree species should be prioritized not only for timber production, but also for conservation efforts of ectomycorrhizal fungi that are exempt from clear-cut felling.

5. Mycorrhizal Values and Soil Carbon Sequestration

Synthesis & Future perspectives

In a flat, sandy pine stand, soil organic carbon is somewhat more evenly distributed, as one square meter basically holds an equivalent amount of soil organic carbon as another square meter. However, in terms of fungal biodiversity, one square meter may hold a completely different fungal community than another. Making efficient compromises for both carbon sequestration and biodiversity conservation can only be achieved when both are included in decision making processes (Thomas et al. 2013). In this thesis, my aim was to examine both aspects of fungal diversity and carbon sequestration, and look at the underlying differences in decomposition and respiration in these small patches of forest, depending on the different abundances of saprotrophic, ericoid and ectomycorrhizal fungal guilds.

In northern forests, a high proportion of birch usually indicates a history with recent fires (Essen et al. 1997). In our study in **Paper III**, the data suggest that a conservation strategy targeting species-rich spruce and birch stands in the north with higher soil fertility may be a strategy to conserve endemic species; therefore, relating to an island chronosequence with increasing durations without fire (Wardle & Zackrisson 2005), burns may counteract retrogression (*i.e.* poorly productive forests) and favour ectomycorrhizal diversity. Such low-fertility and poorly-productive forests are characterized by a dense understory of ericaceous dwarf shrubs, which host a high diversity of root-associated fungi – that limit productivity – and thereby constrain ectomycorrhizal mycelial production. In effect, this shifts the fungal community towards a 'less ectomycorrhizal' forest with increase in soil organic matter stocks (Clemmensen et al. 2015). It is unclear which mechanisms are the most responsible for this and the further accumulation of organic matter, but based on **Paper II**, the decelerating decomposition in the humus was due to some interactions between the ericoid and ectomycorrhizal guild. This could be a synergism, with increasing connections among mycelium, shrubs, and pine roots, or it could be an antagonism (*i.e.* inhibiting decomposition of white rot decomposers by spatial exclusion or chemical inhibition). Both mechanisms could contribute to increased allocation of carbon into pools that are produced faster than they turnover.

The forest conservation gradient in **Paper III** included low fertility pine forests with soils containing high C:N ratios, to mixed coniferous to higher fertility spruce stands with increasing soil fertility and pH. The Jädraås experiment in **Papers I** and **II**, a sandy pine forest, would be quite similar to a few of the older sandy, low fertile, pine forests I encountered in the north. The Jädraås forest is 160-170 years old, and was regenerated after charcoal burning and clear-felling in the 1800s. In our northern region, forest stands of high conservation value dominated by pine were underrepresented, and old-growth sand pine forests are threatened by clear-cut forestry. This potentially demonstrates that pine forest stands in the north have been over-exploited because of their accessibility (Nitare 2019) and/or succeed into spruce forests without low-intensity fire intervals (Wardle et al. 2012), which warrants further investigation.

In the southern region, there were positive, although weaker, relations between most ectomycorrhizal fungal alpha-diversity metrics and fertility, but the relative abundance of more widespread ectomycorrhizal fungi decreased with higher soil fertility. This unexpected negative relationship was driven by high relative abundances species in the family *Cortinariaceae* and *Cenococcum geophilum* coll. at lower fertility (with a slight shift to pine dominated forests). A higher relative abundance of saprotrophic fungi in soils has been shown to increase with increasing soil fertility along a small-scale gradient (Kyaschenko et al. 2017b). Therefore, with increasing mineralization and available nitrogen, saprotrophic fungi may be at a competitive advantage over ectomycorrhizal fungi (Lindahl et al., 2021), although we have not tested this yet.

Additionally, we assume that our highly composite soil samples adequately reflect a representative subset of stand-level ectomycorrhizal fungal communities, and that overall fungal biomass did not vary substantially across forests, thus allowing us to directly compare relative abundances of ectomycorrhizal communities out of total fungal communities as a representation of absolute abundances. It would be beneficial to add a marker for biomass to be more confident in these proportions. Ultimately, studies including the relative abundances fungal guilds in ratios could help unravel large-scale patterns in belowground organic matter accumulation and potential correlations or trade-offs with belowground fungal community values, both in terms of conservation of rare biodiversity and sustaining important functionality provided by fungi. Furthermore, it would will be really interesting to test whether organic matter stocks increase over the forest conservation gradient with an increasing dominance of ericoid mycorrhizal fungi, and whether there would be any synergy or trade-off with forest conservation values.

Overall, interactions between fungal guilds matter for ecosystem-level process rates, and guild interactions could also be affected in the context of climate change. Forest conservation values across the boreal forest are not optimally related to ectomycorrhizal species diversity. Ericoid- and ectomycorrhizal guilds may be underappreciated and understudied in terms of conservation, but their importance in the accumulation of soil organic matter in long term carbon pools of boreal forest soils emphasizes that it is important to understand the many dimensions of fungal conservation in boreal forests.

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Popular science summary

The soil is both a home – and a grave – to millions of species. There is a higher proportion of fungal biomass or 'living fungal tissues' in boreal forests than most other ecosystems on the planet. At the same time, the boreal forest soils also contain more carbon than plants and atmosphere combined. The amount of carbon released from land is far greater than the carbon footprint of people, so the rate at which organisms store, decompose, and respire carbon in boreal forest soils is an important part in stabilizing Earth's climate. The capacities of different organisms to contribute to these processes may also differ, but we are really lacking the basic information on how different organisms, such as fungi, contribute ecosystem processes.

In my thesis, I examine three specific groups of fungi because they are dominant in the boreal forest. Ectomycorrhizal fungi, which act as an extension of the root system, exchange nutrients and water in the soil for carbon photosynthesized from trees. Like a threadbare sock, they fit on the small root tips of trees, making them important channels for carbon sequestration belowground; however, many ectomycorrhizal species are also considered species of conservation concern, but information taking into account both biodiversity of fungi and their role in carbon sequestration is lacking. Ericoid mycorrhizal fungi have a similar symbiosis or mutualism, but they associate with dwarf shrubs like blueberry and have a more recently developed symbiosis in an evolutionary perspective. Saprotrophic fungi are free-living decomposers that get energy by decaying organic carbon compounds from dead and decaying material. To look at the effects and interactions of these different groups of fungi, we established a long-term experiment in a sandy pine forest where we had four specific treatments: 1) we made a treatment where dwarf shrubs were removed by gently pulling them out, 2) we made another treatment where we cut off pine roots by inserting metal barriers (this cuts off the carbon supply belowground to the roots and ectomycorrhizal fungi), 3) there was a combined treatment where both shrubs were removed and pine roots were excluded, and then 4) we left plots alone as they are naturally as a control. Then we looked at how each group contributes to soil respiration. This is basically the breath of the soil released as carbon dioxide. For example, the difference between the control and the shrub removal represented the contribution of the ericoid guild to soil respiration. In this case, shrubs contributed (11 ± 9%) of growing season respiration on average, but they were sensitive to drought compared to pine-associated (43 ± 1%) and saprotrophic respiration (53 ± 5). While respiration measurements were monitored over three growing seasons, we also assessed decomposition rates by measuring the mass remaining from bags of pine needles and humus. Humus is a deeper, older and organic part of the boreal forest soils. Both substrates were incubated in multiple rounds over two growing seasons. What we found is that when ectomycorrhizal fungi are present, they suppressed the decomposition ability of the saprotrophic fungi in pine needles by 10%; however, that was only the case during a drought, and did not happen the following growing season. Ectomycorrhizal mycelial production was extremely reduced during drought, and it suggests that respiration is coming from overall root and mycelial maintenance rather than new growth. Overall, interactions between fungal guilds matter for ecosystem-level process rates, and guild interactions could also be affected in the context of a changing climate. With warmer and drier conditions, there may be less of an effect of ericoid mycorrhizal fungi and shrubs on accumulation carbon in the soil but more of an effect of saprotrophic fungi with greater decomposition capacity. We also found that the ericoid and ectomycorrhizal guilds together slow decomposition in the deeper, older humus in boreal forest soils, and this interaction persisted even during drought although there was much less mass loss. Saprotrophs alone do not seem to have much of an effect on decomposition in the deeper humus layer. When removing shrubs, we found more mass loss in the incubated bags of humus, and also the abundance of certain fungi increased, such as *Cortinarius*, which are very common and diverse mushroom forming fungi.

Because ectomycorrhizal fungi form a tight relationship with trees, many are also species of conservation concern due to the clear-cutting of forests. When the trees disappear, the ectomycorrhizal fungi also disappear. It is important to consider fungi in conservation assessments because they hold such an incredible amount of diversity in the boreal forest – far more than the amount of plants aboveground. However, they are often not adequately included in surveys, but instead, indirect assessments of the stand age and structure are used to estimate the biodiversity potential of a forest. Therefore, a more applied part of my thesis was to assess whether the values we put on forests are good are aligned with ectomycorrhizal diversity. We did this by selecting forests across southern and northern regions of the boreal forest to represent stands of high and low conservation values. Then I set out to collect soil samples from each of the forest stands and assess the fungal community composition through DNA-metabarcoding. DNA sequences present in each soil sample are then determined and grouped into species based on their similarity. A representative sequence is used to match to existing database where different physical specimens of fungi have already been sequenced and annotated. As a result, we found over 500 ectomycorrhizal fungi in our dataset across the forest conservation gradient. More ectomycorrhizal species were found in the south, compared to the north, which may likely be due to the climatic limits on tree productivity in the north, since ectomycorrhizal fungi are dependent on carbon from the trees. Also, there may likely be more of other groups of fungi in the north, such as ericoid mycorrhizal fungi that are more suited to harsher conditions in the north, but it remains to be tested. Overall, we did not see any clear relationship between ectomycorrhizal species diversity and conservation value in a broad sense, but we did find lower number of ectomycorrhizal fungi that have a distribution confined to Europe in the south with lower conservation values. This positive relationship with ectomycorrhizal species richness and conservation values in the south could indicate that longer more intense forest history in the southern boreal forest compared to the northern boreal forest has reduced the viable amount of less widespread ectomycorrhizal species. Overall, the conservation values that we use to indirectly assess the diversity of ectomycorrhizal fungi are not optimal, especially for the north. Instead, ectomycorrhizal fungal diversity-metrics were positively related to soil fertility – from low fertility soils in pine stands to higher fertile stands with birch and spruce. Mycorrhizal guilds may be underappreciated and understudied in terms of biodiversity conservation but their importance in the accumulation of soil organic matter in long term carbon pools of boreal forest soils emphasizes that it is important to consider the many aspects of conservation, from soil carbon sequestration to biodiversity protection, in boreal forest soils.

Populärvetenskaplig sammanfattning

Jorden i marken är både ett hem och en grav för miljontals olika arter. I den boreala barrskogen finns mer levande svampbiomassa än i något annat ekosystem på jorden, samtidigt som mer kol lagras där än i växterna och atmosfären tillsammans. Hur snabbt markorganismerna bryter ner, frisätter och lagrar in organiskt material som innehåller kol i skogsmarken har stora konsekvenser för jordens klimat, eftersom inlagrat kol ofta ligger kvar under mycket lång tid. Mängden kol som ligger fast i skogsmark är mycket större än den andel som människan släpper ut.

I min avhandling undersöker jag tre vanligt förkommande svampgrupper i boreal barrskog. *Ektomkyorrhizasvampar* är en grupp svampar som lever i samspel med trädens rötter och får kol i utbyte mot näringsämnen. *Ljungassocierad mykorrhiza* är en grupp svampar som växer tillsammans med olika buskar på marken, tex blåbär och ljung. *Saprotrofa svampar* är frilevande nedbrytarsvampar som lever av att bryta ner dött organiskt material. För att undersöka deras funktion anlades ett fältexperiment i en tallsandskog med fyra olika behandlingar: 1) borttagning av buskar och därmed ljungassocierad mykorrhiza, 2) avskärning av trädrötter genom att metallramar slogs ner i marken, vilket tar bort ektomykorrhizasvamparna, 3) en kombination av de två tidigare behandlingarna, vilket gör att enbart nedbrytarsvampar finns kvar i marken, och 4) kontroller där ingenting gjordes. Sedan tittade vi på hur varje svampgrupp bidrar till markrespirationen (det motsvarar ungefär markens andning där koldioxid släpps ut) genom att beräkna skillnader mellan de olika behandlingarna. Till exempel skillnaden mellan kontrollerna (där alla svampgrupper finns) och borttagning av buskar (och därmed också ljungassocierad mykorrhiza) visar hur mycket de ljungassocierade mykorrhizasvamparna bidrar. Vi fann att buskarna och ljungassocierade svampar bidrar med ca 10% av markrespirationen under växtsäsongen. Dessa svampar är även känsligare för torka än ektomykorrhizan som växer med trädrötterna eller nedbrytarsvamparna. Markrespirationen mättes under tre växtsäsonger, och vi studerade även nedbrytning genom att se hur mycket av tallbarr och humus (djupare, nedbrutet organiskt material) som bröts ned över tid. När ektomykorrhiza är närvarande minskar de nedbrytarsvamparnas förmåga att bryta ner tallbarren. Detta skedde främst under en torr växtsäsong, och kunde inte observeras året efter. Vi fann även att ljungassocierad mykorrhiza och ektomykorrhiza som växer tillsammans saktar ner nedbrytningen i humus från djupare jordlager i boreal skogsmark.

Eftersom ektomykorrhizasvampar formar ett nära samarbete med träden finns det även många arter som har naturvårdsvärden och som påverkas av tex kalhuggning. När träden försvinner så försvinner även ektomykorrhizan. När man planerar för naturhänsyn är det viktigt att även ta hänsyn till svamparna som är otroligt artrika – det finns många fler svampar än växter. Svamparna inkluderas oftast inte på ett bra sätt i inventeringar. Ofta sker bara indirekta utvärderingar av skogsbeståndets ålder och struktur för att uppskatta biodiversitetspotentialen hos skogen. Mitt andra mål med avhandlingen var därför att undersöka om metoden för att bedöma skyddsvärden i skog överensstämmer med ektomykorrhizasvampars biologiska mångfald. För att undersöka marksvampsamhällenas artsammansättning använde vi DNA-baserad mass-sekvensering. Efter detta analyseras DNA-sekvenserna och grupperas i artgrupper beroende på hur lika de är. En representativ sekvens används för att match varje artgrupp mot en databas med kända svampar. Vi hittade mer än 500 arter av ektomykorrhizasvampar i vår studie, men över skogsgradienten av skogar med olika bevarandenivå hittade vi inget tydligt samband. Slutsatsen blev därför att våra nuvarande metoder för att avgöra artrikedom hos ektomykorrhizasvampar inte fungerar. Istället fann vi att artrikedomen samvarierar med markens bördighet, från låg bördighet i tallskogar till högre bördighet i gran- och björkskogar. De olika trädslagen påverkar svamparnas sammansättning. Mykorrhizasvampar som funktionella grupper må inte vara väl studerade eller representerade i bevarandearbetet, men deras betydelse för uppbyggnad av organiskt material och kolinlagring i boreal skogsmark visar på att det är mycket viktigt att öka förståelsen för svamparnas roll.

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RESEARCH ARTICLE

Ericaceous dwarf shrubs contribute a significant but drought-sensitive fraction of soil respiration in a boreal pine forest

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Abstract

1. Boreal forests often have a dense understorey of ericaceous dwarf shrubs with ecological adaptations that contrast those of the canopy-forming trees. It is therefore important to quantify contributions by understorey shrubs to ecosystem processes and disentangle shrub- and tree-driven responses to climatic factors.
2. We quantified soil respiration driven by the pine canopy and the ericaceous shrub understorey over 3 years, using a factorial pine root exclusion and shrub removal experiment in a mature *Pinus sylvestris* forest. Soil temperature and moisture-related responses of respiration attributed to autotrophs (shrubs, pine roots) and heterotrophs were compared. Additionally, we assessed effects of interactions between these functional groups on soil nitrogen availability and respiration.
3. Understorey shrubs accounted for 22% ± 10% of total autotrophic respiration, reflecting the ericaceous proportion of fine root production in the ecosystem. Heterotrophic respiration constituted about half of total soil respiration. Shrub-driven respiration was more susceptible to drought than heterotrophic- and pine-driven respiration. While the respiration attributed to canopy and understorey remained additive, indicating no competitive release, the plant guilds competed for soil N.
4. *Synthesis*. Ericaceous understorey shrubs accounted for a small, yet significant, share of total growing season soil respiration. Overlooking understorey respiration may lead to erroneous partitioning and modelling of soil respiration mediated by functional guilds with contrasting responses to soil temperature and moisture. A larger contribution by heterotrophs and pine root-associated organisms to soil respiration under drought conditions could have important implications for soil organic matter accumulation and decomposition as the climate changes in boreal forests.

KEYWORDS
autotrophic respiration, ericaceous dwarf shrubs, Gadgil effect, mycorrhiza, nitrogen availability, *Pinus sylvestris*, root trenching, understorey

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1 | INTRODUCTION

Much of the boreal forest is a continuous expanse of ectomycorrhizal trees and ericoid mycorrhizal understorey shrubs (Romell, 1938, Lahti & Väisänen, 1987, Read et al., 2004, Kron & Lutyen, 2005), yet the potentially contrasting and interactive effects of these functional groups on below-ground processes are often overlooked. Multiple, global-scale analyses indicate that higher latitude ecosystems dominated by ectomycorrhizal trees accumulate more soil organic matter than lower latitude arbuscular mycorrhizal ecosystems (Averill et al., 2014; Read, 1991; Soudzilovskaia et al., 2019; Steidinger et al., 2019). However, within high latitude systems, there are multiple studies pointing to an increase in soil organic matter stocks along gradients from ectomycorrhizal to ericoid mycorrhizal dominance (Clemmensen et al., 2013, 2021; Friggens et al., 2020; Hartley et al., 2012; Read, 1991; Ward et al., 2021). In much of the boreal forest, ericaceous dwarf shrubs and trees coexist and have spatially overlapping root systems. Ericaceous roots are located largely in the organic horizon (mor layer; Persson, 1983) and their hair roots are typically colonized by a range of ascomycetous fungi including ericoid mycorrhizal species (Kohout et al., 2011; Lindahl et al., 2007). Tree fine roots are more extensively distributed across soil horizons, but typically with highest density at the interface between mineral and organic layers (Persson, 1983; Rosling et al., 2003). The ectomycorrhizal fungi associated with tree roots are primarily basidiomycetes with prominent mycelia extending into the surrounding soil (Rosling et al., 2003). Trees and ericaceous shrubs with their root-associated fungal communities have contrasting ecological niches, and appear to affect soil C and N dynamics in contrasting ways on both small and large scales (Clemmensen et al., 2015; Sietiö et al., 2018; Ward et al., 2021).

Soil respiration is the most important biological process that leads to losses of soil organic carbon from terrestrial ecosystems, and consequently, knowledge of how soil respiration is partitioned into autotrophic and heterotrophic components is vital to understand soil respiration dynamics and ecosystem carbon budgets (Chapin III et al., 2009; Högberg et al., 2001). An autotrophic component in this case, is defined as the respiration that directly depends on plant carbon allocation to roots, mycorrhizal fungi and other closely associated organisms. Heterotrophic respiration is defined as the respiration that is independent of recent below-ground plant allocation, and instead depends on the decomposition of organic matter by free-living organisms. In forests, plant-driven contributions to soil respiration have been estimated in experiments where the below-ground carbon flow was interrupted by stem girdling or root exclusion (i.e. trenching; Subke et al., 2006). One girdling study in a boreal forest demonstrated a decrease in soil respiration of 54% (within 1–2 months) with a steep decrease just 5 days after below-ground inputs were disrupted (Högberg et al., 2001). A meta-analysis of 25 independent experiments in boreal coniferous forests found, on average, higher heterotrophic (65%) than autotrophic (35%) contributions to soil respiration (Subke et al., 2006). However, in all these studies, understorey shrubs were removed (see Bond-Lamberty et al., 2004; Haynes & Gower, 1995; Vogel et al., 2005), absent or ignored (Lavigne et al., 2003; O'Connell et al., 2003), despite their generally high abundance in boreal forests (Kron & Lutyen, 2005; Nilsson & Wardle, 2005; Romell, 1938).

Only a few studies have estimated contributions of ericaceous dwarf shrubs to soil respiration, and they have provided a wide range of results from almost no effect (Friggens et al., 2020; Kritzler et al., 2016) to contributions between 8% and 55% (Kopittke et al., 2013; Ryhti et al., 2021). Unaccounted respiration by dwarf shrubs could lead to an overestimation of the heterotrophic contribution in tree root exclusion studies, especially in boreal ecosystems where net primary productivity of the understorey may be comparable to trees (Nilsson & Wardle, 2005; Wardle et al., 2012). Furthermore, soil respiration mediated by saprotrophs, pine roots and ericaceous dwarf shrubs may elicit different responses to variation in soil moisture and temperature, since they occupy different parts of the soil profile (Lindahl et al., 2007; Persson, 1983), and harbour fungi with different ecological strategies (Clemmensen et al., 2015; Sietiö et al., 2018).

Nitrogen limitation is the primary constraint on plant growth in boreal forests (Tamm, 1991) and both ecto- and ericoid mycorrhizal symbioses have likely evolved to overcome low nutrient availability caused by retention of nutrients in the organic topsoil (Read & Perez-Moreno, 2003). Ericoid mycorrhizal fungi have been found to mobilize N from organic sources such as ^{15}N-enriched ectomycorrhizal fungal necromass, both in laboratory microcosms (Kerley & Read, 1997) and after canopy girdling, indicating that relaxed competition for N benefitted the shrubs (Bhupinderpal Singh et al., 2003). More recently, it has been recognized that some boreal ectomycorrhizal fungi may be even more efficient in accessing organic N sources than ericoid mycorrhizal fungi, through their production of oxidative enzymes and Fenton reaction mechanisms (Bödeker et al., 2014; Lindahl & Tunlid, 2015; Rineau et al., 2012). Heterotrophic contributions however tend to be over-estimated in root exclusion and girdling experiments, as saprotrophic growth, decomposition and respiration are stimulated by the flush of recently cut mycorrhizal roots and mycelium (Comstedt et al., 2011; Hanson et al., 2000; Lindahl et al., 2010; Savage et al., 2018). A 'Gadgil effect' could additionally increase the activity of free-living decomposers because of a competitive release when ectomycorrhizal roots are excluded (Berg & Lindberg, 1980; Fernandez & Kennedy, 2016; Gadgil & Gadgil, 1971; Sterkenburg et al., 2018). Such a competitive release could be linked to increased soil moisture (Comstedt et al., 2011; Koide & Wu, 2003) and/or soil N availability (Fernandez & Kennedy, 2016). Thus, both free-living decomposers and mycorrhizal fungal guilds may experience a competitive release if N availability is increased after pine root exclusion or shrub removal.

Multi-year, in situ estimates of the contribution of understorey ericaceous dwarf shrubs to soil respiration in forested ecosystems, contextualized with soil N availability and microclimate dependencies, are still lacking. We therefore conducted a factorial root exclusion and dwarf shrub removal experiment to assess the contributions and interactions among three respiration sources: pine roots, dwarf shrubs and heterotrophs, to total soil respiration over three growing seasons in an old-growth boreal pine forest. We hypothesized that dwarf shrubs and pine roots contributed to autotrophic respiration in proportion to their relative fine root production at the same site; 30% and 70% for shrubs and trees, respectively (Persson, 1983). Second, we hypothesized that excluding a plant guild would result in increased root-associated respiration and N uptake by the remaining guild, indicating a competitive release. Third, we expected that heterotrophic respiration would contribute, on average, <50% of soil respiration when also accounting for the understorey. Furthermore, we explored how respiration attributed to each of the three sources, that is, heterotrophs, tree and shrub-associated communities, responded to variation in soil moisture and temperature. Disturbed control plots were used to monitor treatment side effects and ectomycorrhizal root and mycelial re-establishment.

2 | MATERIALS AND METHODS

2.1 | Mature Scots pine forest

A 0.5-hectare study site was established in a well-documented forest in central Sweden, Jädraås, Ivantjärnsheden V (60°48′56.6″N, 16°30′43.2″E) at 185 m above mean sea level (Persson, 1983; Figure S1a). The forest of *Pinus sylvestris* L. (393 trees ha^{-1}) regenerated naturally after charcoal and tar burning in the 1850s and was thinned in 1962 (Bråkenhielm & Persson, 1980), resulting in a homogenous stand of evenly aged and distributed trees. The understorey (65% ground cover) consists mainly of the ericaceous dwarf shrubs *Vaccinium vitis-idaea* L. and *Calluna vulgaris* (L.) Hull, with a lower cover of *Empetrum nigrum* L. and *Vaccinium myrtillus* L. (Bråkenhielm & Persson, 1980). The bottom layer of mosses (c. 90% ground cover) and lichens (<10% ground cover) is mainly comprised of *Pleurozium schreberi* (Bridel) Mitten and *Cladonia rangiferina* (L.) F. H. Wigg. For this study, we avoided areas dominated by lichens. No grasses or herbs, but a few small spruces, were present in the study site. Earlier, 313 ± 15 g m^{-2} of pine roots, 158 ± 11 g m^{-2} of *Calluna* and 158 ± 6 *Vaccinium vitis-idaea* roots <10 mm in diameter were observed across mineral and organic horizons down to 30 cm in the same site (Persson, 1983). The soil profile consists of a glacifluvial sandy podzol with a 10–15 cm thick mor layer (pH 3.0), and a pale eluvial horizon followed by a rust-red illuvial horizon of the mineral soil (pH 4.4–4.8; Bringmark, 1980). The bedrock is composed of older granites, sediments and volcanic rocks, which are widespread in Fennoscandia. The mean annual air temperature during the study period (2017–2019) was 4.8°C with an average daily maximum of 24.4°C in July 2018 and a minimum of −20.7°C in January 2017. The mean growing season precipitation was 300 mm, with 335, 227 and 364 mm in the three study years respectively. The approximate duration of snow cover is from late November to late April. The growing season, defined as the period during which the daily average temperature was above 5°C for five consecutive days, typically lasts from the beginning of May to mid-October. In our study this accounted for 150, 161 and 171 days in 2017, 2018 and 2019 respectively.

2.2 | Experimental design

At the end of November 2016 during a period of light frost, we initiated a fully factorial, randomized-block experiment to distinguish respiration fluxes driven by dwarf shrubs, pine trees and free-living decomposers (Wardle & Zackrisson, 2005). The experiment included factorial combinations of pine root exclusion by trenching and ericaceous shrub removal. Eight replicate blocks with five treatment plots in each (1.2 m × 1.2 m, spaced by 5–10 m) were set up, giving a total of 40 plots (Figure S1). The five treatments encompassed a control (representing the sum of all soil respiration fluxes), shrub removal, pine root exclusion, combined pine root exclusion and shrub removal (presented as 'heterotrophic' respiration), and a disturbed control in which pine roots and associated mycorrhizal mycelium were severed but allowed to re-establish in the plot from the edges. The shrub removal was initiated in November 2016 and any re-sprouting shoots were removed regularly during the following growing seasons. This was done by gently pulling out rhizomes attached to above-ground shoots in the plots and in a 20 cm zone outside untrenched plots to inhibit any horizontal ingrowth from dwarf shrubs. Most rhizomes could be removed initially, without major disturbance in the lightly frozen organic soil, however, deeper rhizomes were not removed. The initially removed shrub biomass was split into species and further cut into above- and below-ground components at the uppermost lateral root and dried. The pine root exclusion was achieved by inserting open-ended, steel boxes with sharpened lower edges to 70 cm below the surface around each plot, using a backhoe to push the boxes into place with minimal disturbance incurred. Given the even tree distribution at the site, several trees were within 10 m of all plots, suggesting that several pine root systems overlapped in all plots. The efficacy of the barriers and potential side effects were monitored using a 'disturbed control' by cutting to 30 cm depth around the plots with a spade well below the densest part of the rooting zone in the organic mor layer (Persson, 1983). No permanent barrier was left in place in the disturbed control, enabling the tracking of re-establishment of roots and mycelium. Thus, we would expect to see similar respiration in the disturbed control as in the pine root exclusion plots directly after the treatments, but convergence to the control levels as roots and ectomycorrhizal mycelium re-established. Any large side effects caused by decomposition of severed roots and mycelium should result in respiration levels in the disturbed control that are elevated above the control levels. All plots without barriers were surrounded by boardwalks with an inner size of 1.4 × 1.4 m and height of 25 cm in May 2018 to minimize impact of trampling. Only the inner 1 × 1 m of each plot was used for sampling to minimize any edge effects.

2.3 | Soil respiration measurements

Soil respiration was measured at 3–5-week intervals (eight to nine times) throughout the growing season, over three consecutive years after the field manipulations had been implemented. Measurements were made with a portable, infrared gas analyser coupled to a 1296 cm^3 dark chamber in a closed air circuit (EgM-4 with SRC-1 probe type; PP Systems). The chamber was pressed firmly to the soil surface and into the moss layer between any vascular plants in the same 10 cm diameter sampling area on each sampling occasion. This procedure ensured a tight seal and minimal below-ground disturbance with the intention that the measurements should reflect the plot-level treatments. Respiration was monitored between 11:00 and 18:00 CET once for 120 s per plot and calculated as a function of linear increases in CO_2 concentration in the chamber. The sampling was alternated block-wise each monitoring day to minimize temporal sampling bias. Measurement quality was ensured by automatic baseline calibration by the 'auto-zero' option at least every 20 min and by keeping the start CO_2 concentration in the chamber at ambient level (Parker et al., 2020). No measurements were made under high wind speeds, and a subset of plots was re-measured to ensure reproducibility during the same day. Cumulative growing season respiration was estimated by multiplying the average respiration rate of two adjacent measurements by the time passed, and these values were summed to obtain cumulative values for each growing season (Bhupinderpal Singh et al., 2003). This integration procedure assumes negligible effect of the shorter-term fluctuations in respiration, for example, those linked to short-term temperature and moisture fluctuations, on the overall average for each period.

2.4 | Soil moisture, temperature and nitrogen availability

Hourly measurements of soil volumetric water content (VWC) and temperature were monitored with a EM50 data logger (METER Group) in each block and Decagon 5TM sensors placed at 5 cm depth next to the respiration sampling area in each plot. Loggers were in place in blocks 2, 5 and 7 from December 2016, in blocks 3 and 8 from the middle of June 2017, and in the remaining three blocks from the middle of August 2017. Data were manually checked, and errors were removed, based on technical advice from the manufacturer.

We used ion-membrane exchange strips to capture changes in soil nitrogen availability. Blank anion and cation membrane sheets (Maltz Sales) were cut into 2×3 cm strips and charged in 0.5 M $NaHCO_3$ with a minimum of 10 ml solution per strip for 1 h. In May 2017, four charged strips (two anion, two cation) were inserted at approximately 5–10 cm depth in each plot. In July, the strips were collected and replaced with a new set, incubated until September. In 2018, two similar sets were incubated from July to September and from September to November. Upon collection, each set of four strips from a plot was pooled, rinsed in deionized H_2O and transferred to a 50 ml centrifugation tube. The adsorbed N was extracted by vertical shaking for 4 h in 36 ml of 2 M KCl (Shaw & DeForest, 2013). Extracts were frozen until analysed for NH_4-N and NO_3-N using a flow injection analyser (FIAstar TM, FOSS Tecator). Values below the detection limit (0.02 mg NH_4-N or NO_3-NL^{-1}) were treated as zero values.

We also sampled four to five healthy, current-year *V. vitis-idaea* leaves per plot in October 2017 as an integrative indicator of N availability (Bhupinderpal Singh et al., 2003). When present, re-sprouted shoots in the shrub removals were included in the analysis. The leaves were dried (70°C, 24 h) and ground to a fine powder in a ball mill. Leaf C and N concentrations and isotopic ^{15}N:^{14}N ratios were analysed on an isotope ratio mass spectrometer (IRMS; Isoprime, Micromass-GV Instruments) coupled to a Eurovector CN elemental analyser.

2.5 | Verification of treatment efficacy

To verify that the shrub removal and tree root exclusion efficiently terminated below-ground autotrophic carbon flows, we incubated humus-filled mesh bags to monitor ericaceous root and ectomycorrhizal mycelial production in all plots over 17 months. Since ectomycorrhizal fungi depend on host trees for carbon, they should be sensitive indicators of below-ground carbon allocation. Mesh bags (2×8 cm) were constructed of 50 μm-mesh allowing the ingrowth of fungal mycelia and ericaceous fine roots, and filled with homogenized, oven-dried (40°C) humus from the organic layer. Bags were incubated at 5–10 cm depth of each plot from June 2018 until the end of November 2019. Upon collection, the 40 bags were kept intact at −20°C until the content was freeze-dried and ball milled. DNA was extracted from 50 mg of organic material using the NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany). Three samples from the original humus material were included to assess background levels of DNA. ITS2 rDNA markers were amplified by 18–30 cycles of duplicate polymerase chain reactions using the gITS7 and ITS4 primers elongated with sample-unique identification tags (Castaño et al., 2020; Clemmensen et al., 2016; Ihrmark et al., 2012). These primers were designed to target fungi, but they also match all four of the ericaceous plant species in our system. Adaptor ligation and sequencing on a Pacific Biosciences Sequel I platform were performed by SciLifeLab (NGI). Sequences shorter than 150 bp and with a mean quality score lower than 20, individual bases with a quality score lower than 10, or missing 3′ or 5′ tags were removed. Sequences were screened for primers by requiring a minimum match of 90%, and reverse complemented if necessary. This quality filtering removed 57.3% of the total sequences and an additional 10% unique genotypes were removed to reduce the incidence of sequencing errors. To obtain species level clusters, remaining sequences were run through pairwise comparisons with USEARCH (Edgar, 2010) followed by single linkage clustering with the minimum similarity to the closest neighbour set at 98.5% (Kõljalg et al., 2013). In total, 349 species level clusters (hereafter species) were formed, based on the total of 60,974 sequences passing quality control. The most abundant sequence from each species was selected as a representative and identified by BLAST comparisons with the UNITE (Kõljalg et al., 2013) and NCBI databases. Species occurring in more than 1% of any sample were manually annotated for ectomycorrhizal status. Dual ectomycorrhizal and ericoid mycorrhizal fungi were not included because of their uncertain association with dwarf shrubs or pine roots. After removal of non-fungal sequences (<3% of all sequences), the summed relative abundance of ectomycorrhizal fungi out of all fungal sequences was calculated for each sample. Plant species (2.5% of all sequences) were almost entirely composed of the four ericaceous shrubs present at our site, and their relative abundance was calculated as a percentage out of all sequences per sample.

2.6 | Statistical analyses

The soil respiration, integrated across each growing season, was tested for the effects of pine root presence, shrub presence and year using a linear mixed effects model in the NLME package in R (Pinheiro et al., 2019). Pine root and shrub presence were included as binary fixed factors. The random effect of plot, nested within block, accounted for the spatial dependency of the randomized block design. A log transformation was needed to obtain homoscedasticity of respiration data. We tested whether there was an interaction effect between pine root and shrub presence, with reduced respiration of either component in the presence of the other, which would be indicative of antagonistic suppression. The effects of year, pine roots and shrubs on growing season averages of soil moisture and temperature were tested separately with the same model setup.

The monthly soil respiration measurements were tested for effects of pine root presence, shrub presence, soil moisture, temperature and their interactions, with linear mixed effects models. Again, pine root and shrub presence were included as binary, fixed factors. Soil moisture and temperature were included in this model to account for differences in environmental conditions across plots and time. For this, soil moisture and temperature measurements were averaged over 4 days preceding each respiration measurement per plot to account for a lagged transfer of photosynthates to manifest (Ekblad et al., 2005). To obtain data corresponding to all respiration measurements, missing moisture and temperature plot level data in 2017 were gap-filled with treatment averages for each date, as moisture clearly depended on treatment. We regard this the best solution to avoid disregarding collected respiration data in the model. To account for potential dependencies across space and time, an error correlation matrix with decimal date was specified, and plot, nested by block, was included as a random effect. The final model excluded non-significant ($p > 0.2$) three- and four-way interaction terms by stepwise removal of the least significant term. Linear mixed models were also used to test the effects of pine roots, shrubs and year on the soil moisture and temperature measurements averaged over the 4 days preceding each respiration measurement (excluding gap-filled data).

The response of respiration to moisture and temperature was visualized for each respiration component using the GAMM4 package in R (Wood, 2017) based on data from the second and third study years. In these years, we assumed a negligible disturbance-related decomposition. Pine-associated respiration was calculated as the difference in respiration between control and pine root exclusion plots, while shrub-associated respiration was the difference in respiration between control and shrub removal plots (all calculated per block). Heterotrophic respiration was assessed as respiration in plots with pine roots excluded and shrubs removed. Mosses were not removed from the experiment and were assumed to have stable contributions in all treatments; thus, they were included in the heterotrophic respiration component and cancelled out in the estimate of pine- and shrub-associated respiration responses to soil moisture and temperature. Standardization rescaled the data to have a mean of zero and a standard deviation of one to visualize differences in environmental responses among functional groups. Soil moisture and temperature data were averaged across the eight control plots per time point to reflect the ambient conditions of the site as a basis for shrub and pine root-associated 'gamm4' visualizations.

3 | RESULTS

3.1 | Verifying pine root and shrub removals

The biomass of ericaceous shrubs removed during the initial set-up of the experiment amounted to 144 ± 10 g m^{-2} above-ground and 47 ± 7 g m^{-2} below-ground, with *Vaccinium vitis-idaea* contributing 60%, *Calluna vulgaris* 23%, *V. myrtillus* 9% and *Empetrum nigrum* 8% above-ground. After the 17 months of incubation, the ingrowth bags designed to capture new shrub root and ectomycorrhizal growth showed that the shrub removal efficiently reduced relative abundance of ericaceous DNA from 6% ± 2% in the control to 0.5% ± 0.3% after shrub removal ($p < 0.001$; Figure S2). Root trenching reduced the relative abundance of ectomycorrhizal fungal DNA in the ingrowth bags from 24% ± 4% in the control to 0.6% ± 0.2% after trenching ($p < 0.001$; Figure S2).

3.2 | Contributions by shrubs, pine roots and saprotrophs to soil respiration

The soil respiration integrated over each growing season was overall highest in the first year and lowest in the third year (Figure 1; Table 1). Pine root exclusion significantly reduced growing season soil respiration and shrub removal tended to lower soil respiration, but this depended on the year. Over the 3 years, we estimated that heterotrophic respiration (in combined pine root exclusion and shrub removal plots) contributed 53% ± 5% of the total soil respiration in control plots (Figure 1; Table 1). Pine root-associated respiration (the difference between controls and pine root exclusions) contributed 41%–45% and shrub-associated respiration (the difference between

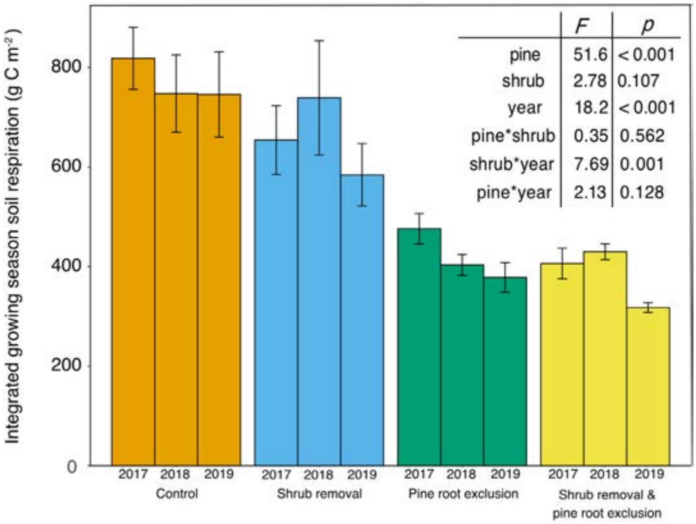


FIGURE 1 Soil respiration integrated across each growing season (g C m^{-2}) for control plots or for plots from which understorey shrubs, pine roots or both had been removed. Here, we used a linear mixed effects model to test the fixed and interactive effects of year, pine root and shrub presence on soil respiration, including plot nested within block as random factors (df = 82). Insignificant ($p > 0.2$) three-way interaction were removed. Bars are averages ±1 standard error ($n = 8$)

TABLE 1 Soil respiration attributed to shrubs, pine roots and saprotrophs in (a) absolute and (b) relative terms, as well as (c) the relative contributions of shrub and pine root guilds to autotrophic respiration, integrated over each growing season. Heterotrophic respiration is assessed as respiration after combined pine root exclusion and shrub removal. Pine-associated respiration is estimated as the difference between control and pine root exclusion plots, while shrub-associated respiration is estimated as the difference between the control and shrub removals. Average contributions to soil respiration (±*SE*) for each year are calculated across blocks ($n = 8$) while the average (±*SE*) across the 3 years is also given ($n = 3$)

(a) Soil respiration (g C m^{-2})	Shrub associated	Pine associated	Heterotrophic
2017	164 ± 84	342 ± 49	406 ± 31
2018	8.5 ± 140	344 ± 86	429 ± 16
2019	162 ± 113	367 ± 92	317 ± 10
Average	111 ± 51	351 ± 8	384 ± 59
(b) Soil respiration (% of control)	**Shrub associated**	**Pine associated**	**Heterotrophic**
2017	17 ± 9	41 ± 3	51 ± 5
2018	0 ± 19[a]	42 ± 6	63 ± 8
2019	15 ± 11	45 ± 7	46 ± 5
Average	11 ± 9	43 ± 1	53 ± 5
(c) Guild respiration (% of total autotrophic)	**Shrub associated**	**Pine associated**	
2017	31	68	
2018	2	98	
2019	31	69	
Average	22 ± 10	78 ± 10	

[a] The shrub removals in some cases had higher respiration than their corresponding controls, so the average shrub-associated respiration was set to 0%.

controls and shrub removals) accounted for 0% to 17% of the total growing season soil respiration (Table 1). Thus, pine roots and shrubs contributed 78% ± 10% and 22% ± 10% of the total root-associated (i.e. autotrophic) respiration respectively (Table 1). There was no significant interactive effect of pine and shrubs on soil respiration (Figure 1).

3.3 | Variation in soil temperature and moisture

The daily average soil temperature at 5 cm depth ranged between −4.1 and 21.4°C over the 3 years, with an overall mean of 6.2°C (Figure 2c). The average growing season soil temperature varied among years ($p < 0.001$) with the highest average of 12.6°C in the second year compared to 10.8 and 11.5°C for the first and third years respectively (Figure S3; Table S1). There was no effect of treatments on temperature (Figure 2c; Figure S3; Table S1).

Daily average soil moisture ranged from 1.9% to 29% (volumetric water content) with a mean of 13% over the 3 years (Figure 2b). The average growing season soil moisture was higher in the third year than previous years, and in 2018 (the second year) soil moisture was significantly higher in the combined pine root exclusion and shrub removal compared to the controls (Figure S3; Table S1). Living pine roots also decreased moisture levels across individual growing season point measurements, but the combined pine root exclusion and shrub removal increased soil water content disproportionately to single guild removals, resulting in a significant interaction term (Figure 2b; Table 2b). Shrubs had no main effect on soil moisture, but shrub removal (in the presence of pine roots) even seemed to reduce soil moisture levels in the second and third years (Figure 2b).

3.4 | Functional group responses to soil moisture and temperature

The effects of pine roots and shrubs on soil respiration were tested in a model that also included soil temperature and moisture as predictors across all measurements (Figure 2a). Soil temperature correlated positively, while soil moisture correlated negatively, with soil respiration (Table 2a). Pine roots increased soil respiration, and more so under warmer conditions, resulting in a synergistic interaction between pine presence and temperature. Shrubs did not affect respiration significantly but instead tended to interact negatively with temperature (Table 2a). There was no significant interaction between pine roots and shrubs on soil respiration (Table 2a), although the effects of both plant guilds on respiration tended to depend on the interaction between soil moisture and temperature (see three-way interactions in Table 2a).

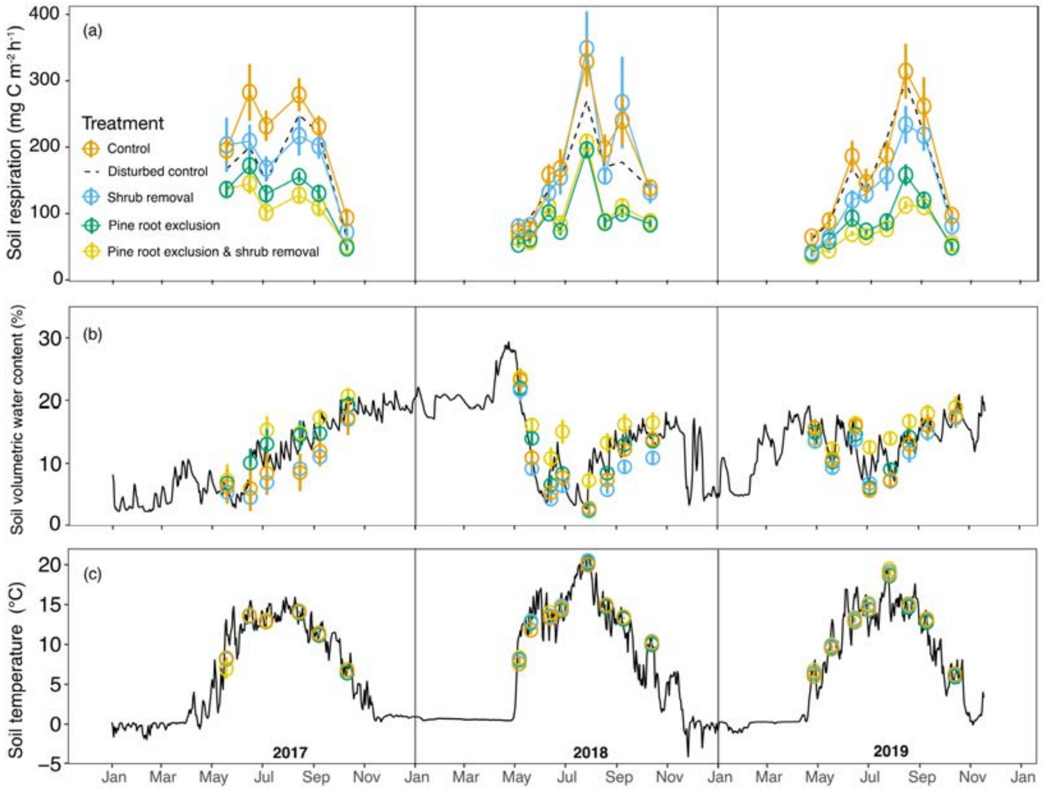


FIGURE 2 Growing season (a) soil respiration (mg C m^{-2} h^{-1}), (b) volumetric soil water content (%) and (c) soil temperature (°C) in control plots and in plots from which shrubs or pine roots or both had been removed. Respiration was measured monthly over the growing seasons of 2017 to 2019. Soil water content and temperature averaged per treatment across the 4 days preceding each respiration measurement are shown as coloured circles, and the black trend lines represent daily averages across all treatments. Points are averages ±1 standard error (n = 7–8)

TABLE 2 Linear mixed effects models of (a) soil respiration and (b) soil moisture (volumetric water content) and temperature point measurements. The individual and interactive effects of shrubs and pine roots, soil moisture and temperature on monthly measurements are shown. Pine root and shrub presence were coded as binary factors. Potential dependencies across space and time were accounted for and data from the disturbed control were not included. Insignificant ($p > 0.20$) three-way interaction terms were removed stepwise from the models. Values in bold ($p < 0.05$) have coefficient signs indicating positive or negative effects. Coefficient signs in parentheses indicate the direction of tendencies ($p < 0.10$)

	df	F	p	Coefficient sign
(a) Respiration (g C m^{-2} h^{-1})				
Pine	(1,21)	**47.5**	**<0.001**	+
Shrub	(1,21)	2.22	0.151	
Moisture	(1652)	**111**	**<0.001**	–
Temp	(1652)	**197**	**<0.001**	+
Pine × shrub	(1,21)	1.28	0.270	
Pine × moisture	(1652)	1.40	0.237	
Shrub × moisture	(1652)	0.08	0.772	
Pine × temp	(1652)	**9.47**	**0.002**	+
Shrub × temp	(1652)	3.50	0.062	(–)
Moisture × temp	(1652)	3.04	0.082	(–)
Pine × moisture × temp	(1652)	2.84	0.092	(+)
Shrub × moisture × temp	(1652)	3.27	0.071	(+)
(b) Soil moisture (%)				
Shrub	(1,21)	1.29	0.269	
Pine	(1,21)	**11.5**	**0.003**	–
Year	(2670)	**6.86**	**0.001**	
Pine × shrub	(1,21)	**5.79**	**0.025**	+
(c) Soil temperature (°C)				
Shrub	(1,21)	0.086	0.772	
Pine	(1,21)	0.033	0.858	
Year	(2670)	**10.7**	**<0.001**	
Pine × shrub	(1,21)	0.003	0.954	

Generalized additive models described how respiration attributed to each guild had contrasting dependencies on soil moisture and temperature (Figure 3). Pine root-associated respiration increased with warmer conditions and was less sensitive to soil moisture (*adjusted* (*adj.*) $R^2 = 0.174$, $p < 0.001$; Figure 3a). Shrub-associated respiration, in contrast, was favoured by mid-range conditions and tended to decrease during wet and cold conditions and during drought conditions, but this was not well explained by the model (*adj.* $R^2 = 0.000$, $p = 0.085$; Figure 3b). Heterotrophic respiration varied the most across the soil moisture and temperature conditions experienced during the experiment and their GAMM models had the best fit (*adj.* $R^2 = 0.518$, $p < 0.001$), with particularly high respiration in warm and dry periods (Figure 3c).

3.5 | Soil nitrogen availability

Soil ammonium availability increased significantly in the absence of pine roots in the summer of 2017 and in both summer and fall periods of 2018 (Figure 4a). There was a significant interaction between the pine root and shrub presence in the second year with a large increase in N availability in the combined removals (Figure 4a). Nitrate availability remained below detection levels in all treatments (data not shown). Nitrogen concentration of *V. vitis-idaea* leaves, used as an additional proxy for N availability during the first year, showed additive effects of plant groups; N concentrations were lowest when both plant groups were present, intermediate in the individual removals and highest in the combined removal (Figure 4b). There were no differences in leaf δ^{15}N between the control and any of the removal or exclusion plots, however, the disturbed control had significantly lower δ^{15}N values than the shrub removal while all other treatments were intermediary (Figure S4).

4 | DISCUSSION

4.1 | Soil respiration contributions by understorey shrubs

Better estimates of the contribution by ericaceous dwarf shrub understories to soil respiration, relative to canopy-forming trees, should advance our understanding of environmental dependencies of soil respiration at the ecosystem level and why more soil

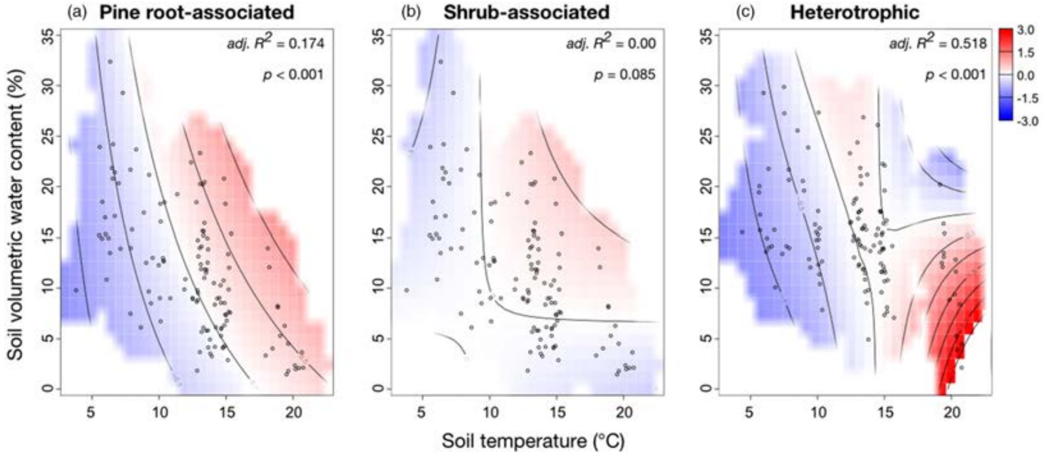
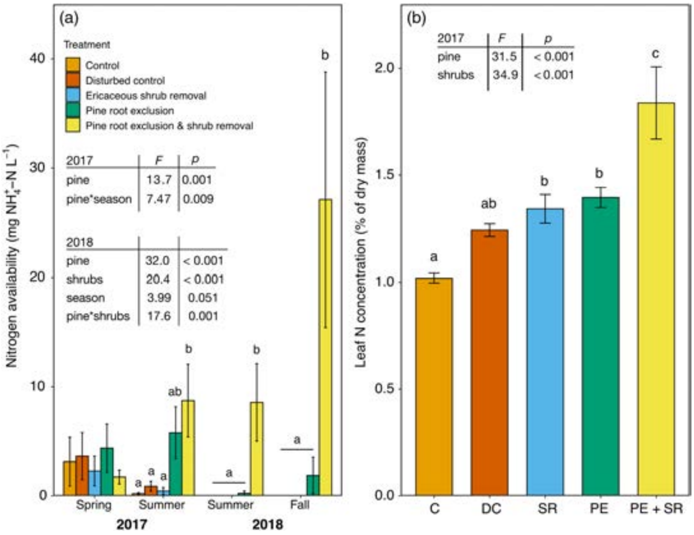


FIGURE 3 Generalized additive models of soil respiration (from blue = low to red = high) associated with (a) pine, (b) shrub and (c) heterotrophic sources in response to soil temperature and moisture (indicated by points) across the second and third growing seasons (n = 112). The respiration associated with each guild was rescaled to have a mean of zero and a standard deviation of one. Plot grid pixels that are extrapolated too far from the measured explanatory soil moisture and temperature data were excluded from the plot. Pine-associated respiration (a) is the difference between control and pine root removal plots, while shrub-associated respiration (b) is the difference between the control and shrub removals, all calculated per block and sampling date. Heterotrophic respiration (c) is assessed as respiration in the combined pine root exclusion and shrub removal treatment. Soil moisture and temperature data from the control plots were used for both pine root- and shrub-associated respiration. The zero-contour lines (the line between blue and red) represents the standardized mean for each of the three respiration sources

organic matter develops in ericoid dominated habitats (Clemmensen et al., 2015; Hartley et al., 2012; Tedersoo et al., 2020). We found that the ericaceous dwarf shrub understorey, on average, over 3 years, contributed 11% of the total soil respiration and 22% of autotrophic respiration. We hypothesized that the contribution of dwarf shrubs to autotrophic respiration would reflect their 30% share of fine root biomass (Persson, 1983), which closely matched our estimate of 31% if excluding the second growing season with drought conditions. Earlier studies in the boreal forest have estimated that autotrophic respiration contributes 35%–55% of total soil respiration (Comstedt et al., 2011; Hanson et al., 2000; Högberg et al., 2001; Subke et al., 2006), but only a few studies have monitored understorey contributions with girdling or trenching in forested ecosystems, and they provide conflicting evidence (Friggens et al., 2020; Grau-Andrés et al., 2020; Parker et al., 2020; Ryhti et al., 2021). In a southern Finnish boreal forest, dwarf shrubs and pine roots were estimated to contribute 8% and 48%, respectively, of total soil respiration over 3 years (Ryhti et al., 2021). These contributions are half of our estimate for shrubs in non-drought summer seasons (16%) and slightly higher than our pine root-associated contributions (43%). The currently available, albeit scarce, data suggest that shrubs can make a significant contribution to soil respiration, likely reflecting their contribution to primary productivity (Kulmala et al., 2019; Nilsson & Wardle, 2005), particularly below-ground. However, one potential process underlying ericaceous shrub accretion of soil organic matter may be a lower metabolic quotient (respiration per unit biomass) compared to pine roots, as demonstrated in a microcosm experiment (Kulmala et al., 2018). This remains to be further tested in situ.

4.2 | Large, drought-related variation in shrub-associated respiration

The proportion of autotrophic respiration attributed to dwarf shrubs varied dramatically from 2% to 31% among growing seasons. The removal of ericaceous shrubs had only a marginal effect in the second year of the experiment, coinciding with a severe summer drought in 2018. In northern Sweden, a synchronized lack of response to shrub removals was found in an experiment where shrubs were removed continuously during 22 years in advance of the respiration measurements (Grau-Andrés et al., 2020). This suggests a direct effect of drought on shrub performance, rather than the influence of experimental side effects such as elevated heterotrophic decomposition of severed roots and mycelia, to have caused this lack of response to shrub removal. Plausibly, ericaceous dwarf shrubs closed their stomata completely to cope with the low moisture conditions in the upper soil horizons, where most of their roots are found (Persson, 1983). In addition, ericoid mycorrhizal fungi have maintained genetic capacities similar to free-living saprotrophs (Martino et al., 2018) and this apparent versatile ecology may be an adaptation to cope with unreliable below-ground allocation of photosynthates by shrubs. Pines, in contrast, have around half of their root biomass in deeper mineral soil horizons at our site (Persson, 1983). Accordingly, the pine-mediated respiration



2017	F	p
pine	13.7	0.001
pine*season	7.47	0.009

2018	F	p
pine	32.0	< 0.001
shrubs	20.4	< 0.001
season	3.99	0.051
pine*shrubs	17.6	0.001

2017	F	p
pine	31.5	< 0.001
shrubs	34.9	<0.001

FIGURE 4 Nitrogen availability in (a) soil solution and (b) assessed by *V. vitis-idaea* leaf N concentration, as affected by pine root exclusion and understorey shrub removal. In (a), soil ammonium availability during the first and second years of treatments was based on early and late incubation periods of ion exchange strips. Here, we analyzed the effect of pine and/or shrub presence (binary) and season as fixed factors in a linear mixed model, including plot nested in block as random factors. In (b), leaf N concentrations (% of dry mass) of *V. vitis-idaea* were analysed in the fall of the first year to indicate plant available N ($n = 7$–8, except the combined shrub removal and pine root exclusion for which $n = 3$). Treatments are indicated by C = control, DC = disturbed control, SR = shrub removal, PE = pine exclusion, PE + SR = combined pine root exclusion and shrub removal. Again, we tested pine and shrub effects on leaf N concentrations in a linear mixed model, including plot nested within block as random factors. Letters above bars indicate significant differences between treatments within each sampling period ($\alpha = 0.05$)

was much more consistent across years (41%–45%) and less affected by drought than the shrub-driven respiration. A study of pine root growth in southern Finland during the same drought season of 2018 found that the growth of pioneer pine roots was unaffected, while the growth of the fibrous, mycorrhizal side-roots ceased temporarily during the drought (Ding et al., 2020). Subarctic Scots pine stands have also been found to maintain higher respiration rates through extreme weather events, particularly dry and hot spells, than Norway spruce stands (Matkala et al., 2021), and the interannual variation in photosynthesis of the trees seemed to be more stable from year to year than understorey shrubs in an ecosystem respiration budget (Kulmala et al., 2019). Further, diurnal fluctuations in respiration by the understorey have not been well assessed and contrasted to those of canopies, although such differences may be important, especially during drought. Overall, the contrasting responses to soil temperature and moisture between autotrophic guilds signifies that there is a strong dependence on phenological attributes (Makita et al., 2018), and potentially different activity thresholds for the understorey and canopy.

4.3 | Competition or coexistence between dwarf shrubs and pine roots

Our second hypothesis predicted that dwarf shrubs and pines would compete for scarce resources in this ecosystem, and that the exclusion of both functional groups would lead to a larger increase in soil N availability than predicted from the single plant type eliminations. The N concentration of *V. vitis-idaea* leaves, however, increased additively from single to the combined exclusion in the first year. This was congruent with soil ammonium availability over the summer period of the first year, where treatments may have led to increased N mineralization from decomposing roots and mycelium (Hanson et al., 2000). As a reflection of this, we expected that the $\delta^{15}N$ signatures in the leaves would increase, as mycorrhizal mycelium is enriched in ^{15}N compared to plant pools (Bhupinderpal Singh et al., 2003). Instead, we found little effect of the pine root exclusion relative to the controls and a clear increase in the shrub removal treatment relative to the disturbed control. While most of the dwarf shrub rhizomes were pulled out of the soil, many fine roots harbouring the root-associated fungi were likely left to decompose in the plot, and ericoid mycorrhizal fungi strongly fractionate stable N isotopes by keeping more ^{15}N in their mycelium during transfer of N to their plant partner (Emmerton et al., 2001). Consequently, the N pool that became available after shrub removals may have been a particularly ^{15}N-enriched pool in our system, leading to the elevated leaf $\delta^{15}N$ signatures. In the second year, there was a large and non-additive increase in soil ammonium availability in the combined pine root exclusion and shrub removal treatment, indicating that low N availability persisted with only one remaining plant group present because of increased N uptake after a competitive release. Uptake by plants and mycorrhizal fungal associates thus represented a major sink for soil N in the summer and fall. This evidence for strong N limitation and competition between pine and shrub roots was, however, not strongly tied to increased below-ground respiratory activity, which remained additive for the two plant groups throughout the experiment. Potentially, roots may have increased their carbon-use efficiency with higher N availability in this N-limited system (Forsmark et al., 2021; Robinson et al., 1999). Remaining plants could thus have increased root N uptake and growth without a corresponding increase in respiration.

4.4 | Heterotrophic respiration and experimental side effects

We expected that saprotrophs would contribute less than half of soil respiration when accounting for both autotrophic sources; however, heterotrophic contributions, on average, remained around 50% of total soil respiration. Even so, as visualized in the response functions, heterotrophic respiration varied the most across the soil moisture and temperature conditions during the experiment, with particularly high values in warm and dry periods. As a result, the cumulative heterotrophic respiration was higher and contributed more to total soil respiration (63%) in the second drought year, when understorey shrubs were hampered. It is quite surprising that free-living decomposers seemed unabated by low moisture, but a meta-analysis of warming experiments also indicated a stronger positive dependency of heterotrophic respiration on temperature than on water content, especially in northern forested ecosystems (Wang et al., 2014). Furthermore, mosses may have increased their respiration differentially across the treatments, which we are unable to evaluate in the present experiment.

The permanent barriers succeeded in excluding pine roots and ectomycorrhizal fungi. The respiration in the disturbed controls rebounded from trenched to control respiration levels in the second growing season. This indicates that C flow to roots, ectomycorrhizal mycelium and other root-associated organisms had, at least partially, been re-established in the disturbed controls. In permanently trenched plots, soil respiration remained at low levels throughout the 3 years, and no ectomycorrhizal DNA was found in the humus-filled mesh bags, confirming that pine below-ground C allocation did not re-establish in the plots with barriers.

Treatment side effects, such as increased soil moisture and inputs of necromass from severed roots and mycorrhizal mycelium, may boost saprotrophic activities (e.g. respiration, decomposition and mineralization) in trenching experiments (Hanson et al., 2000). In our forest site, 313 ± 15 g m^{-2} of living pine roots <10 mm in diameter have been quantified down to 30 cm (Persson, 1983). Dead pine roots (1–10 mm diameter) have been shown to lose 10%–20% of mass over one growing season when the most labile compounds were degraded, and close to 40% mass loss over three growing seasons in our forest (Berg, 1984). Patterns of ^{14}C distribution in soil profiles of nutrient poor pine forest indicated that 10% of root-derived C was lost during a year, and 60% was lost during a 10-year period (Kyaschenko et al., 2019). There is likely to be 15–50 g m^{-2} of ectomycorrhizal mycelium in coniferous boreal forest soils (Finlay & Clemmensen, 2017), which would mostly turn over during 1 year in an older pine forest (Hagenbo et al., 2016), besides a smaller recalcitrant fraction. Based on these estimates, decomposition of severed roots and ectomycorrhizal mycelium during the first year corresponded to 44–83 g C m^{-2} which may have inflated the heterotrophic respiration (of 406 ± 31 g C m^{-2}) by up to 10%–20% over the first growing season. However, because of the timing of trenching, mycelial decomposition could have happened before respiration measurements began. For example, mycelium has been shown to disappear within 5 days under optimal growing conditions (Finlay & Read, 1986), and more than 50% mass loss was observed after 7 days under field conditions (Ryan et al., 2020). Further, the respiration in the disturbed controls never exceeded that in control plots in the second or third years where pine roots and ectomycorrhizal mycelium re-established. Thus, the flux from severed roots and mycelium in any of our trenched plots was likely not very large compared to the estimated autotrophic and heterotrophic fluxes. Additionally, the treatments with severed below-ground pine inputs had lower cumulative respiration in the third year than in the first year. This could be linked to the induced flush from severed pine roots and mycelium in the first year, which was sequentially exhausted, but the reduction in respiration over time could also be exaggerated by ceased rhizosphere inputs (via a decreased 'priming effect') from mycelial and root production. We suggest that facilitation and interdependence among saprotrophs and root-driven inputs are likely to be important and should be emphasized in future experiments.

5 | CONCLUSIONS

Taken together, over 3 years, we estimated that autotrophic and heterotrophic respiration each contributed around half of total soil respiration in an old-growth pine forest. Shrub-associated respiration was about a third of total autotrophic respiration under non-drought conditions, reflecting their share of fine root production. We found evidence of competition for N between pine roots and shrubs, but contrary to our expectations, tree root- and shrub-associated respiration showed no clear sign of a competitive release (i.e. they remained additive throughout the 3 years). Pine root-associated and heterotrophic respiration persisted, and even increased in relative terms, through periods of drought. In contrast, shrub-associated respiration almost ceased during drought, indicating that relative contributions of ericaceous dwarf shrubs to soil respiration may shift dramatically with changing environmental conditions. It remains to be investigated whether the simultaneous inactivity of dwarf shrubs and augmented activity of free-living saprotrophs and pine roots during drought have important consequences for soil organic matter accumulation in the long term. Disparate trade-offs in respiratory versus growth responses between trees and understorey shrubs should be a future research priority.

AUTHORS' CONTRIBUTIONS

The field experiment was initiated and designed by K.E.C., B.D.L., R.D.F. and A.E. and the specific study was conceived by all authors. The field and laboratory work was performed by L.A.M. and K.E.C. Isotope ratio mass spectroscopy was performed by A.E. Data were analysed by L.A.M. The first draft of the manuscript was written by L.A.M. and all authors contributed to the data interpretation and revisions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest associated with this work.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

All data are available in Dryad Digital Repository https://doi.org/10.5061/dryad.1ns1rn8wt (Mielke et al., 2022), apart from raw sequence data, which can be found under the Sequence Read Archive (SRA) with BioProject accession number PRJNA834027.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Supplementary Information

Figure S1. The experiment location **(a)**, and **(b)** design and **(c)** an example block (5) with the five treatments: control (orange), ericaceous shrub removal (blue), pine root exclusion without shrub removal (green), combined pine root exclusion and shrub removal (yellow) and the disturbed control (red).

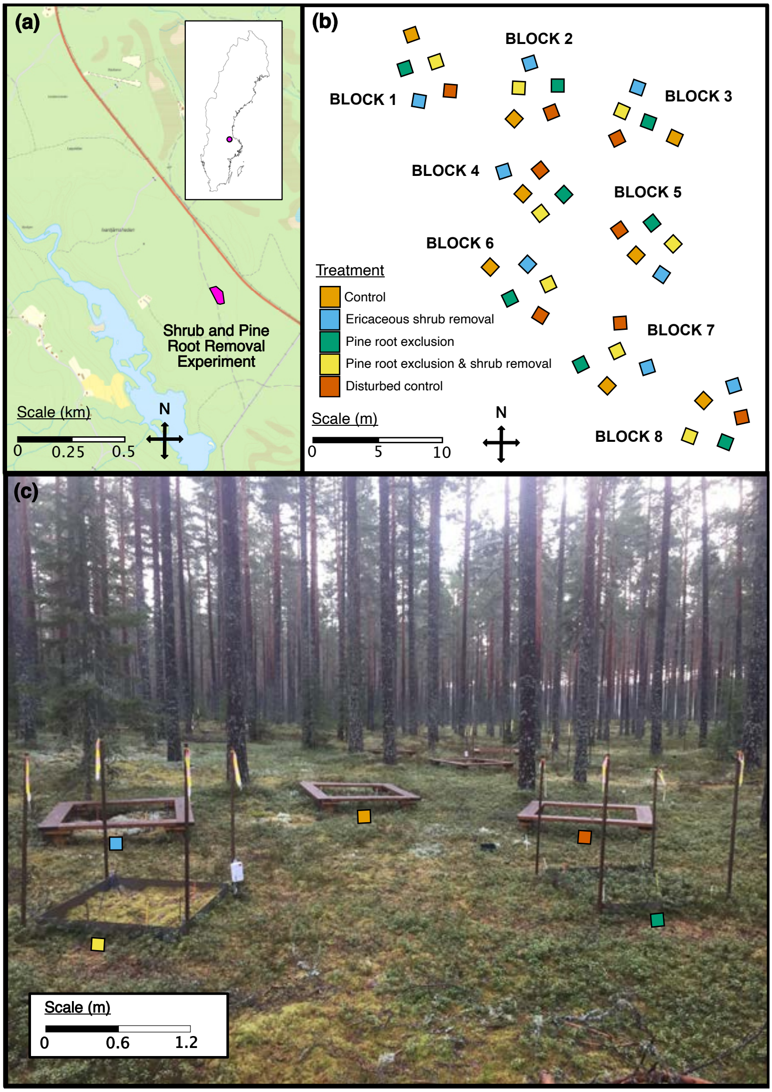

Figure S2. Proportion of the ITS2 copies from **(a)** ericaceous shrubs out of total ITS2 reads and **(b)** ectomycorrhizal fungi out of total fungal ITS2 reads in 50 µm-mesh bags filled with organic soil and incubated for 17 months from June 2018 to November 2019. Significant differences are indicated with different letters (α = 0.05) among treatments in a linear mixed model with plot nested in block as random effects. Treatments are indicated by C = control, DC = disturbed control, SR = shrub removal, PE = pine exclusion, PE + SR = combined pine root exclusion and shrub removal. Means ± standard errors (n=8, except PE where n=7).

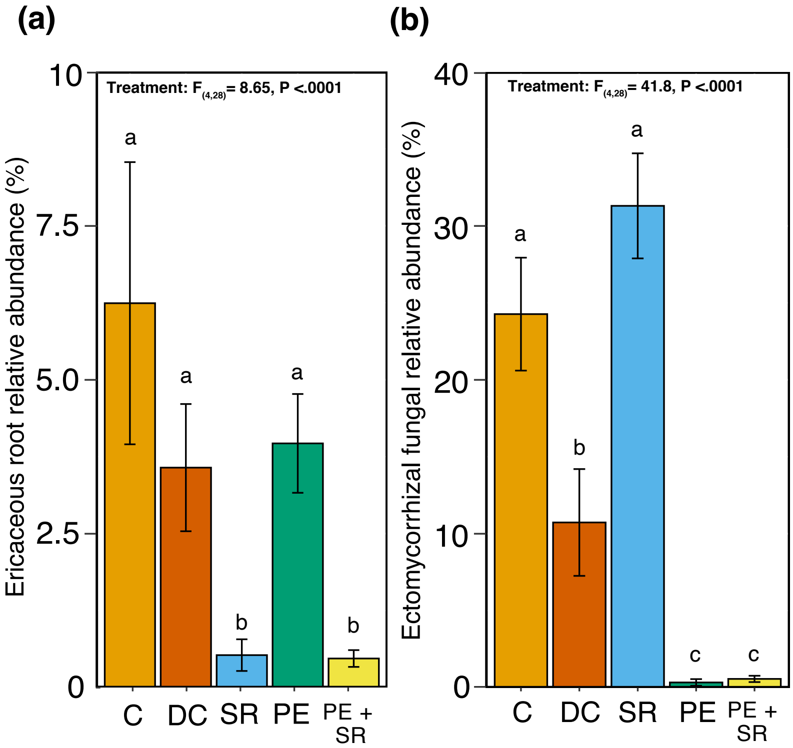

Figure S3. Soil **(a)** temperature (°C) and **(b)** volumetric water content (%) at 5 cm depth averaged over each growing season from 2017 to 2019. There was no data for the disturbed controls in 2017 and one replicate for the combined pine root exclusion and shrub removal due to incomplete installation of data loggers. Otherwise, averages ±1 standard error are shown per treatment and year (n=3 for 2017, n=7-8 for 2018 and 2019). Significant differences in treatments in a linear mixed model per year are indicated with different letters ($\alpha = 0.05$) including plot nested within block as random effects.

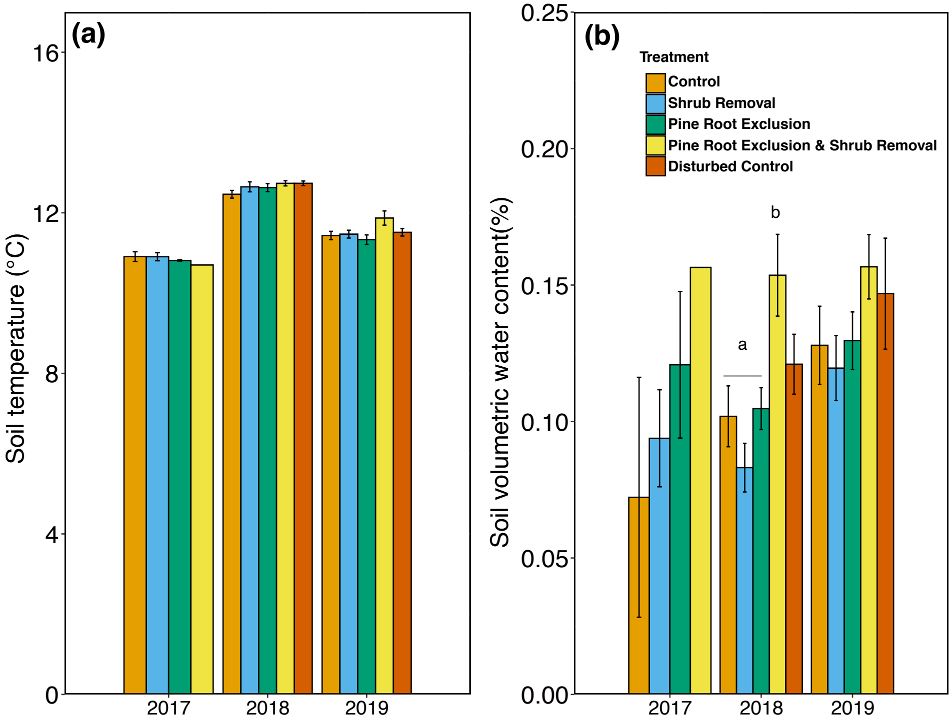

Figure S4. The current year's leaf C:N ratio **(a)** and δ^{15}N **(b)** of understory lingonberry (*Vaccinium vitis-idaea* L.) collected in September 2017. Points are averages ± 1 standard error (n = 7-8; except for PE +SR where n=3). Significant differences among treatments are indicated with different letters (α = 0.05) from a linear mixed model with treatment as a main fixed effect and plot nested in block as random effects. Treatments are indicated by C = control, DC = disturbed control, SR = shrub removal, PE = pine exclusion, PE + SR = combined pine root exclusion and shrub removal.

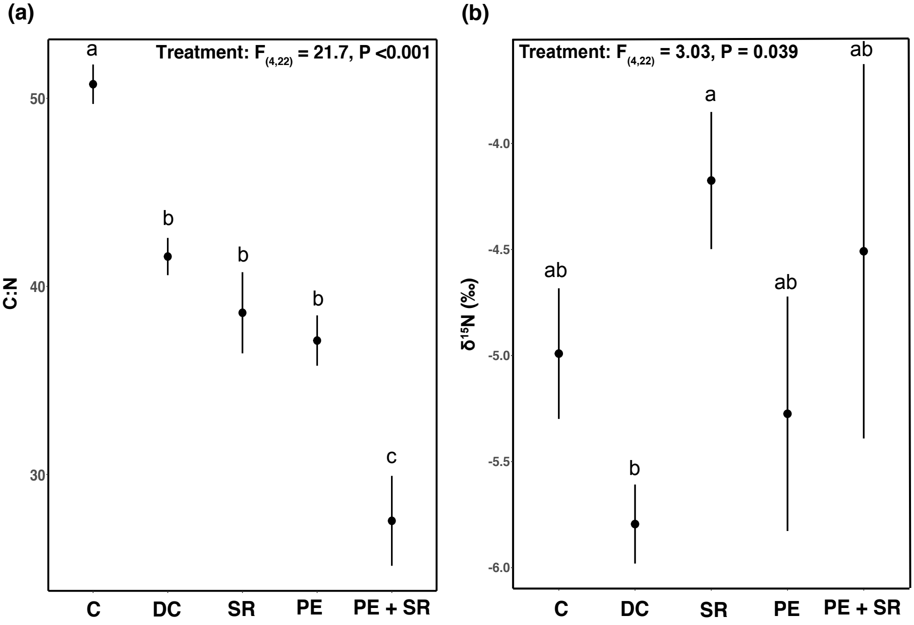

Table S1. Effects of experimental treatments on growing season averages of soil temperature (°C) and volumetric water content (%) at 5 cm depth in 2018 and 2019 were tested in linear mixed models with nested plot by block for the random effects term (n = 7, 8). Disturbed controls were not used in this analysis. Significant values are included in bold.

		(numDF, denDF)	*F*	*P*
Soil temperature (°C)				
2018	Treatment	(3,25)	2.23	0.109
2019	Treatment	(3,26)	2.25	0.107
Soil water content (%)				
2018	Treatment	(3,25)	7.81	0.001
2019	Treatment	(3,26)	1.89	0.156

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The immense diversity and biomass of fungi in boreal forests are essential for soil organic matter dynamics, and many ectomycorrhizal fungi are considered species of conservation concern. In a long-term experiment, ericoid and ectomycorrhizal fungal guild interactions were determined to be more important for late-stage organic matter balance in boreal forest soils than saprotrophic and ectomycorrhizal interactions. By characterizing fungal communities through DNA-metabarcoding, we found that prevailing forest conservation values were not related to ectomycorrhizal fungal diversity across the boreal forest.

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