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RESEARCH ARTICLE

Links between boreal forest management, soil fungal communities and below-ground carbon sequestration

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Abstract

1. Forest management has a potential to alter below-ground carbon storage. However, the underlying mechanisms, and the relative importance of carbon input and decomposition in regulation of soil carbon dynamics are poorly understood.
2. We examined whether interactive effects of forest fertilization and thinning on carbon stocks in the topsoil of boreal forests were linked to changes in fungal community composition, biomass and enzyme activities, in a long-term fertilization and thinning experiment distributed across 29 *Pinus sylvestris* forests along a 1,300 km latitudinal transect in Sweden.
3. Nitrogen fertilization increased fungal biomass, particularly towards the north and mainly by promoting root-associated Ascomycetes, but the response was moderated by thinning. Fungal biomass correlated positively with carbon stocks in the organic topsoil. However, ectomycorrhizal *Cortinarius* species were reduced in abundance by fertilization and correlated negatively with carbon stocks.
4. Plausibly, increased soil carbon stocks after fertilization are linked to increased input of carbon in the form of root-associated mycelium combined with the loss of ectomycorrhizal decomposers within the genus *Cortinarius*. These fungal responses to fertilization may mediate a natural climate solution by promoting carbon sequestration in the organic topsoil, but the effect of fertilization may also be undesired from a biodiversity perspective.

KEYWORDS
ectomycorrhiza, fertilization, forest management, fungal biomass, fungal community, thinning

1 | INTRODUCTION

Boreal forests cover large parts of the northern hemisphere and are persistent carbon (C) sinks of global importance (Pan et al., 2011). Two-thirds of the boreal forest area are managed in some way (Gauthier et al., 2015). Thinning is a common practice applied to increase revenue (Royal Swedish Academy of Agriculture and Forestry, 2015), and due to strong nutrient limitation, nitrogen (N) fertilization is also considered a cost-effective practice to increase forest yields (Nohrstedt, 2001; Saarsalmi & Mälkönen, 2001; Tamm, 1991). In

addition, fertilization can increase C stocks below-ground (Högberg et al., 2006; Janssens et al., 2010; Maaroufi et al., 2015), but the underlying mechanisms are still poorly understood (Mayer et al., 2020). In boreal forests, decomposing organic matter accumulates in a purely organic layer (equivalent to the 'mor layer' or O-horizon) on top of the mineral soil and accounts for a significant, and dynamic, fraction of below-ground C (Deluca & Boisvenue, 2012). The intuitive explanation that fertilization drives C storage in this organic topsoil by increasing plant litter input is poorly supported (Janssens et al., 2010; Jörgensen et al., 2021a; Kyaschenko et al., 2017b). Instead, increased C stocks following fertilization have primarily been attributed to decreased rates of CO_2 release from the soil (Janssens et al., 2010; Maaroufi et al., 2015; Zak et al., 2008).

Trees appear to regulate below-ground processes by altering root traits (Adamczyk et al., 2019; Sun et al., 2018), production of fine roots (Forsmark et al., 2021) and fungal associations (Clemmensen et al., 2013; Kyaschenko et al., 2019), suggesting that several root-related mechanisms interplay to promote C accumulation below-ground. Potentially, increased C stocks in fertilized stands can be explained by a higher C use efficiency of roots and micro-organisms (Forsmark et al., 2021; Manzoni et al., 2017, 2018), resulting in higher C allocation to biomass production and lower C losses from respiration. In boreal forests, soil fungi regulate C dynamics, both by providing source material for soil organic matter (Clemmensen et al., 2013) and as drivers of decomposition (Kyaschenko et al., 2017b; Lindahl et al., 2021). Increased C use efficiency of fungi may elevate mycelial growth and thereby input of fungal necromass into the soil organic C pool. Despite being comparably chemically labile, fungal necromass makes a significant contribution to soil C stocks (Clemmensen et al., 2013) through stabilizing interactions with root tannins (Adamczyk et al., 2019). Root-associated ascomycetes, including ericoid mycorrhizal fungi and root endophytes, but also a variety of other fungi with uncertain ecology, have been highlighted as important contributors of C to the organic topsoil (Clemmensen et al., 2015) and their proliferation would be expected to increase organic matter accumulation. Previous fertilization studies in Scandinavian coniferous forests have observed declines in fungal biomass (Demoling et al., 2008; Högberg, Blasko, et al., 2014), or reductions in fungal biomass only at high N input (Blasko et al., 2013; Maaroufi et al., 2019). However, fertilization stimulated fungal biomass production in strongly N limited, arctic ecosystems (Clemmensen et al., 2006). The response of fungal biomass to fertilization may, thus, vary depending on context and the initial nutrient status at the site.

Being biotrophically dependent on products of photosynthesis, the abundance of root-associated fungi should reflect the above-ground tree biomass (Sterkenburg et al., 2019), and thinning would be expected to reduce C input by roots and associated fungi. However, knowledge about how fungal communities respond to thinning is scarce. In a Mediterranean forest system, fungal biomass decreased after thinning (Collado et al., 2020), whereas another study observed no effects on fungal community composition (Castaño et al., 2018). In a temperate pine stand, fungal biomass 5 years after thinning did not differ from non-thinned stands (Maassen et al., 2006). In boreal *Pinus contorta* forests, thinning alone did not affect root colonization rates or fungal community composition, but reduced the impact of N fertilization on the composition of the fungal community (Teste et al., 2012).

Soil C stocks may also be regulated by losses via decomposition. Additions of easily available N through fertilization can alleviate nutritional constraints on saprotrophic litter decomposers, and thereby increase decomposition rates, since higher N availability would increase fungal growth and associated C demand (Boberg et al., 2008). Stimulated decomposition should then increase microbial respiration and decrease C stocks after fertilization. However, field experiments commonly report decreased decomposition following N addition (Janssens et al., 2010). This contradiction may relate to different responses of hydrolytic and oxidative mechanisms of decomposition. In temperate broad-leaf forests, N additions, even at low rates, increased activities of enzymes involved in the hydrolysis of cellulose but decreased activities of oxidative, ligninolytic enzymes (Carreiro et al., 2000; Sinsabaugh et al., 2002). This shift was attributed to a loss of ligninolytic saprotrophs, which have a high potential to oxidize non-hydrolysable and presumably recalcitrant organic matter (Entwistle et al., 2017). Similar shifts may also occur in boreal forests, where additions of N seem to hamper oxidative decomposition of organic matter (Bödeker et al., 2014) and favour cellulolytic fungi relative to ligninolytic species (Talbot & Treseder, 2012). Carbon stocks in the organic topsoil of boreal forests have been found to correlate negatively with the activity of fungal manganese peroxidases (Kyaschenko et al., 2017b) as well as with manganese availability (Stendahl et al., 2017). Organic topsoils consist to a great extent of decomposed and chemically complex organic matter that may primarily serve as a source of N rather than C for decomposers. It is, thus, possible that 'N-miners' with oxidative capacity are disfavoured when N-limitation is alleviated by fertilization, and this may, indirectly, hamper C turnover (Craine et al., 2007).

In boreal forests, however, potent saprotrophic decomposers are mainly confined to the uppermost litter layer, especially in nutrient-poor soils, while most of the organic (and mineral) soil is dominated by ectomycorrhizal fungi (Clemmensen et al., 2013; Kyaschenko et al., 2017b; Lindahl et al., 2007). Ectomycorrhizal fungi are considered to be sensitive to N additions (Bahr et al., 2013; Högberg, Yarwood, et al., 2014; Nilsson & Wallander, 2003), possibly due to downregulated C allocation from trees to mycorrhizal symbiosis when inorganic N becomes easily available (Franklin et al., 2014; Högberg et al., 2010; Lilleskov et al., 2019). During their evolution from saprotrophic ancestors, some ectomycorrhizal genera have retained mechanisms for oxidative decomposition and may, thus, be considered as symbiotic N-miners with a capacity to access nutrients in persistent, root-derived organic compounds (Bödeker et al., 2014; Lindahl & Tunlid, 2015; Sterkenburg et al., 2018). By releasing organically bound nutrients and share them with their host trees, such 'ectomycorrhizal decomposers' may counteract nutrient retention and maintain tree growth, despite low rates of nutrient mineralization (Baskaran et al., 2017). Notably, species of the genus *Cortinarius* seem to be central ectomycorrhizal decomposers that, through their production of manganese peroxidases, appear to be involved in the

regulation of C stocks in organic topsoils of boreal forests (Bödeker et al., 2014; Lindahl et al., 2021). The genus *Cortinarius* is considered to be particularly sensitive to N additions (Arnolds, 1991; Lilleskov et al., 2019; Lindahl et al., 2021; Strengbom et al., 2001; van der Linde et al., 2018). While an overall decline in the production of ectomycorrhizal fungal mycelium may reduce below-ground C input, a specific loss of these potent oxidizers may also contribute to increased C stocks in the organic topsoil following N additions.

Effects of phosphorus (P) fertilization on fungal communities and nutrient cycling in boreal forests are not well understood, but alleviated N limitation under elevated N input may shift systems towards P deficiency, with subsequent effects on the structure and functioning of the fungal community. In a *Picea abies* forest in southern Sweden, subjected to moderate levels of N deposition, both P and N + P fertilization shifted the composition of the fungal community and both fungi and trees showed signs of P limitation (Almeida et al., 2019). In addition, increased levels of extracellular acid phosphatases after N addition have been observed in temperate coniferous forests (Forstner et al., 2019).

We investigated the interactive effects of fertilization and thinning on C sequestration in both standing biomass and the organic topsoil in *Pinus sylvestris* forests planted along a latitudinal transect across Sweden and found that N fertilization had a positive effect on C storage both above- and below-ground (Jörgensen et al., 2021a). Carbon sequestration in the organic topsoil was stimulated even further when P was added in combination with N, despite that P addition did not affect tree growth above-ground. The positive effect of N fertilization on C storage was greater at higher latitudes, presumably due to stronger N limitation. Interestingly, reduction of tree biomass and associated litter input by thinning decreased organic topsoil C stocks, but only in N fertilized stands, suggesting that N fertilization increased the importance of litter input relative to decomposition in the regulation of soil C stocks. We also observed declines in soil respiration after N fertilization. Here, we address whether these previously described responses were related to treatment-induced effects on the soil fungal community. We hypothesized that increased C stock following fertilization would be linked to (1a) higher mycelial biomass, an effect that (1b) would be reduced by thinning and/or (2a) decreased abundance of ectomycorrhizal fungi, particularly *Cortinarius* species and (2b) decreased activity of decomposer enzymes, especially fungal peroxidases. Hypothesis (1) assumes that increased C stock is an effect of increased input of persistent litter (here mycelial necromass), whereas hypothesis (2) assumes that increased C stock primarily is due to down-regulated decomposition linked to reduced ectomycorrhizal N-mining.

2 | MATERIALS AND METHODS

2.1 | Experimental setup

We used a long-term thinning and fertilization experiment at 29 sites distributed across a 1,300 km latitudinal transect (56–67°N) in boreal Scots pine *Pinus sylvestris* L. dominated forests in Sweden (median *Pinus sylvestris* proportion of stem volume: 100%, min: 84%). The forests were established by direct seeding or planting, and the experiment was established between 1969 and 1982 when the trees were between 32 and 54 years old. At each site, four different treatments were applied on 0.1 ha plots: 'No thinning', 'Thinning', 'Thinning + N' and 'Thinning + NP'. At nine of the sites, an additional treatment of 'No thinning + N' was implemented. The understorey vegetation depended on the treatments but was dominated by mosses such as *Pleurozium schreberi* and *Hylocomium splendens*, and low herbs such as *Vaccinium vitis-idaea* and *Vaccinium myrtillus*. No permissions were needed for the field work. For more information about vegetation, see Strengbom et al. (2018). All soils were podzols with distinct organic horizons ranging from 2.5 to 11.7 cm (average: 5.9) in thickness, with C:N ratios of 25–63 (average: 41). The pH ranged from 3.3 to 4.6 with an average of 3.7 was slightly higher in the 'Thinning + NP' treatment, and increased with latitude in all treatments (Figure S1; Table S1).

The thinned plots were thinned one to four times during 1974–2015 to maintain a constant basal area of around 18 m^2/ha, corresponding to a 20%–25% reduction compared to un-thinned stands. At each thinning, stems were removed while branches and stumps were left in the plot. In the un-thinned stands, trees that had died on site were left. Nitrogen was added as ammonium nitrate (NH_4NO_3) to fertilized plots at a rate of 100–150 kg/ha every 5th year for the first 25 years and thereafter every 7th year. Phosphorus in the form of superphosphate ($CaSO_4 + Ca(H_2PO_4)_3$) was added at a rate of 100 kg/ha at the start of the experiment and thereafter every 20–21 years. More information about nutrient loads and background N deposition levels are presented in Table S2. The experimental setup is described in more detail in Nilsson et al. (2010).

2.2 | Soil sampling, processing and pH

The study used the samples described in Jörgensen et al. (2021a). In the summer of 2016, the organic topsoil was sampled in all 125 plots by pooling 25 soil cores (3 cm in diameter) taken in a grid pattern across each 0.1 ha plot. Soil samples extended down to the organic–mineral soil transition. Living mosses, grasses and roots coarser than 2 mm were removed before pooling. The samples were frozen within 48 hr of sampling and kept at −20°C until analysis. Frozen samples were homogenized, and subsamples were freeze-dried, and ball-milled. Organic topsoil pH was measured on a PHM 93 Reference pH meter (Radiometer, Copenhagen) in a 1:3 weight ratio of fresh soil and H_2O.

2.3 | Ergosterol extractions and enzyme assays

Fungal biomass was estimated by ergosterol extraction (Nylund & Wallander, 1992) from 0.2 g freeze-dried, finely ground soil. Ergosterol was extracted in 10% KOH dissolved in methanol and

transferred to a cyclohexane phase. The cyclohexane was evaporated under N_2 gas flow and the ergosterol was resuspended in methanol, after which the extracts were filtered through a 0.45 μm Teflon syringe filter (Millex LCR-4; Millipore) before analysed using high-performance liquid chromatography (HPLC) according to the protocol of Hagenbo et al. (2017). Activities of the hydrolytic enzymes β-1,4-glucosidase, β-1,4-N-acetylglucosaminidase and acid phosphatase were assessed using fluorogenic methylumbelliferyl substrates (Saiya-Cork et al., 2002) in dilute soil suspensions at a concentration of 1 g (fresh weight) soil/L (Kyaschenko et al., 2017a). β-1,4-glucosidases release glucose from oligosaccharides, and play a central function in cellulose decomposition. β-1,4-N-acetylglucosaminidase plays a corresponding role in decomposition of chitin, and is thought to be important in mobilization of organically bound N in boreal forest soils. Acid phosphatases release organically bound phosphate from monoesters (Sinsabaugh et al., 2008). Fluorescence was measured on a luminescence/fluorescence spectrophotometer (LS 50B, PerkinElmer Inc.). Manganese peroxidase (MnP) activity was assayed on soil extracts of 10 g (fresh weight) soil/L using the colorimetric MBTH-DMAB method (Daniel et al., 1994) as described in Kyaschenko et al. (2017b). Absorbance was measured on a SpectraMax Plus 384 microplate reader (Molecular Devices).

2.4 | DNA extraction and characterization of the fungal community

DNA was extracted from 2 g of freeze-dried soil with 10 ml 3% CTAB (Clemmensen et al., 2016), precipitated with isopropanol, resuspended in 700 μl SL1 buffer from the NucleoSpin Soil kit (Macherey-Nagel) and cleaned using the kit according to the manufacturer's instructions. PCR amplicons of the ITS2 region were produced using the forward primer gITS2 (Ihrmark et al., 2012) and a 3:1 mixture of the reverse primers ITS4 (White et al., 1990) and ITS4arch (Kyaschenko et al., 2017a) with unique identification tags attached to both primers. 12.5 ng of DNA template was used in the 50 μl reaction (Clemmensen et al., 2016), which was run for 27–31 cycles with Dreamtaq polymerase (Thermo Fischer Scientific) and an annealing temperature of 56°C. Amplicons were cleaned with AMPure (Beckman Coulter Life Sciences) according to the manufacturer's instructions before DNA concentrations were measured on a Qubit fluorimeter (Life Technologies). Amplicons were then pooled into an equimolar mix and cleaned with the E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek). Library preparation and sequencing was conducted by SciLifeLab (NGI) on the PacBio Sequel platform (Pacific Biosciences). The PacBio platform was chosen to minimize biases due to ITS2 length variation (Castaño et al., 2020). Raw sequences were filtered in the bioinformatics pipeline SCATA (https://scata.mykopat.slu.se/, Ihrmark et al., 2012), accepting sequences with length >100 bp, mean quality >20, single base quality >3 and primer sequence similarity >90%. After removal of global singletons, sequences were compared pairwise with USEARCH (Edgar, 2010), and clustered into species hypotheses (Kõljalg et al., 2013; hereafter 'species') using single-linkage clustering with a 98.5% similarity threshold. Clusters of plant sequences were removed from further analyses.

The 209 species that accounted for >1% of the total number of sequences in any sample (together representing 85% of the total fungal sequences) were identified by comparisons to the UNITE database (Nilsson et al., 2019) and assigned to ecological guilds according to Clemmensen et al. (2015): root-associated ascomycetes (the genera *Archaeorhizomyces*, *Chaetothyriales*, *Chloridium*, *Geomyces*, *Hyaloscypha*, *Mollisia*, *Oidiodendron* and *Phialocephala;* including ericoid mycorrhizal but not ectomycorrhizal fungi), saprotrophic agaricomycetes and ectomycorrhizal fungi (Appendix S1).

2.5 | Statistical analyses

Treatment effects on the 209 identified fungal species were evaluated with canonical correspondence analysis (CCA) in CANOCO 5.12 with the four treatments applied at all sites as factorial explanatory variables. The 'No thinning + N' treatment (which was implemented at nine sites only), as well as one site with incomplete data, was removed from the dataset before the analysis. Statistical significance of treatments was evaluated by a Monte Carlo test with 999 permutations restricted to within sites only. Fungal community composition was represented graphically using detrended correspondence analysis (DCA).

For the remaining statistical analyses, thinning, N fertilization, P fertilization and latitude were included as explanatory factors in linear mixed-effect models, with site as a random factor using the lmerTest (Kuznetsova et al., 2017) and lme4 (Bates et al., 2015) packages in R version 4.0.3 (R Core Team, 2018). The interaction between N fertilization and thinning, as well as N and latitude, was included initially but removed from the models if not significant ($p < 0.05$). As the amount of N and P fertilization varied slightly between sites, loads were represented as continuous variables and normalized by the average loads, giving ranges between 0–1.4 for N and 0–1.3 for P. The latitude of the most southern plot was used as a zero reference.

Treatment effects on relative abundance of guilds and were assessed using linear mixed-effects models as described above. Square-root transformation of relative abundances of fungal guilds was necessary to achieve normal distribution of residuals. Within the ectomycorrhizal guild, collective abundances of the three most species-rich genera: *Cortinarius*, *Piloderma* and *Russula* were evaluated relative to the total fungal community and to the ectomycorrhizal community.

A core fungal community was defined as the 40 species that occurred at >1% relative abundance on at least 10 sites (representing 65% of the total number of fungal sequences; Table S3). Treatment effects on the square-root transformed relative abundance of each species in the core fungal community were evaluated as described above, and p-values were adjusted for multiple testing using the correction of Benjamini and Hochberg (1995; Appendix S2).

Treatment effects on ergosterol (mg/m^2 and μg/g OM) and enzymatic activities were evaluated in the same way. Manganese peroxidase data were square-root transformed to meet assumptions of normal distribution of residuals. To evaluate correlations with organic topsoil C stocks across treatments, we fitted linear models with either standing tree biomass, ergosterol, relative abundances of different fungal guilds or the ectomycorrhizal genus *Cortinarius* as explanatory factors and site as a random factor. Data on soil C stocks and tree biomass were collected from Jörgensen et al. (2021a).

3 | RESULTS

Mycelial biomass, estimated by ergosterol concentration in the organic topsoil, increased towards the north. Fertilization increased fungal biomass, with a stronger effect at high latitudes. We detected an interaction between N fertilization and thinning with a negative effect of thinning on fungal biomass, but only in N fertilized plots (Figure 1a; Table S4).

Out of 856,192 sequences, a total number of 575,734 sequences passed quality filtering, and after removal of unique genotypes, 293,068 sequences were clustered into 2,362 global species, whereof the 209 most abundant were identified. N-fertilization changed the composition of the fungal community (conditional effect: $p = 0.001$), and application of P together with N had an additional effect (conditional effect: $p = 0.022$; Figure 2). Thinning had no significant effect on fungal community composition. The relative abundance of root-associated ascomycetes was higher in fertilized plots (Figure 1b; Table S4), whereas ectomycorrhizal fungi decreased after fertilization (Figure 1d; Table S4). The relative abundance of the ectomycorrhizal genera *Cortinarius* and *Russula* decreased in response to fertilization, both in relation to the total fungal community (Figure 3a,c; Table S5) and in relation to other ectomycorrhizal fungi (Figure 3d,f; Table S5). The genus *Piloderma* was also negatively affected by N fertilization (Figure 3b) relative to the total fungal community but increased its share of the ectomycorrhizal community (Figure 3f; Table S5). Abundance of saprotrophic agaricomycetes did not differ among treatments, but were generally higher towards the north (Figure 1c; Table S4).

Of the core fungal community, 12 species declined in relative abundance after N fertilization, including the ectomycorrhizal *Cortinarius acutus*, *Russula decolorans* and *Suillus variegatus*, one ericoid mycorrhizal species in the order *Chaetothyriales* and the ericoid mycorrhizal fungus *Hyaloscypha hepaticicola*. The latter also increased its abundance in response to thinning. Phosphorus fertilization in combination with N had a negative impact on an unidentified *Leotiomycete*. Two *Archaeorhizomyces* species were both positively affected by N (Figure 4; Table S6).

We did not detect any significant treatment effect on manganese peroxidase activity (Figure 5a; Table S7). β-1,4-glucosidase activity declined towards the north in non-fertilized stands, but a positive interaction between N fertilization and latitude counteracted the latitudinal decline in fertilized plots (Figure 5b; Table S7). β-1,4-N-acetylglucosaminidase activity was unaffected by fertilization but positively affected by thinning (Figure 5c; Table S7). Acid phosphatase activity was higher in thinned stands and in N-fertilized stands but lower in the combined N and P fertilization treatment (Figure 5d; Table S7).

We tested the links between organic topsoil C stocks and potential fungal regulatory factors to elucidate the strongest correlations across plots and treatments. Ergosterol concentration (Figure 6a) and β-1,4-glucosidase activity (Figure S2) were positively correlated with organic topsoil C stocks, whereas *Cortinarius* relative abundance was negatively correlated with C stocks (Figure 6f).

4 | DISCUSSION

We found that fertilization changed the composition of root-associated fungal communities, both at the guild and species level (Figures 1 and 4). Interestingly, community change was particularly accentuated when P was added together with N (Figure 2). Changes in root-associated fungal communities after fertilization are thought to depend largely on reduced allocation of tree C below-ground (Högberg et al., 2003). In boreal forests, plant N and P are primarily derived from soil organic stocks via decomposition driven by ectomycorrhizal fungi. This mycorrhizal mining for nutrients is driven by allocation of plant photosynthates to fungal symbionts in response to nutrient shortage (Lindahl & Tunlid, 2015). Phosphorus limitation and a sustained need to mobilize P from organic sources may maintain plant C allocation to fungi also in N fertilized forests (Nylund & Wallander, 1992; Wallander et al., 2011), whereas combined supply of both resources would be expected to lead to more dramatic changes in the symbiosis (Suz et al., 2021).

In this study, we particularly investigated potential fungal mechanisms that may explain previously reported changes in organic topsoil C stocks following 40 years of fertilization and thinning in Swedish *Pinus sylvestris* forests (Jörgensen et al., 2021a). Taken together, we found that increased soil C storage after N fertilization correlated with both increased fungal biomass and losses of mycorrhizal fungal taxa that have previously been identified as important decomposers in boreal forest (Lindahl et al., 2021). However, we found no support for the hypothesis that this fertilization-induced effect was associated with a direct decrease in manganese peroxidase activity after fertilization.

Increased N availability following fertilization may ameliorate N-limitation of trees and associated fungi, increasing their C use efficiency and growth. Indeed, concordant with hypothesis (1a), ergosterol concentration in the organic topsoil was elevated in fertilized plots, with a stronger effect towards high latitudes (Figure 1a; Table S4). The stronger response to fertilization in the north suggests intensified nutrient limitation of the fungal community in harsher climates, potentially in combination with higher rates of N deposition towards the south (Karlsson et al., 2019) that render forests less responsive to additional input of N. Our observation stands in contrast to previous studies in Scandinavian coniferous forests,

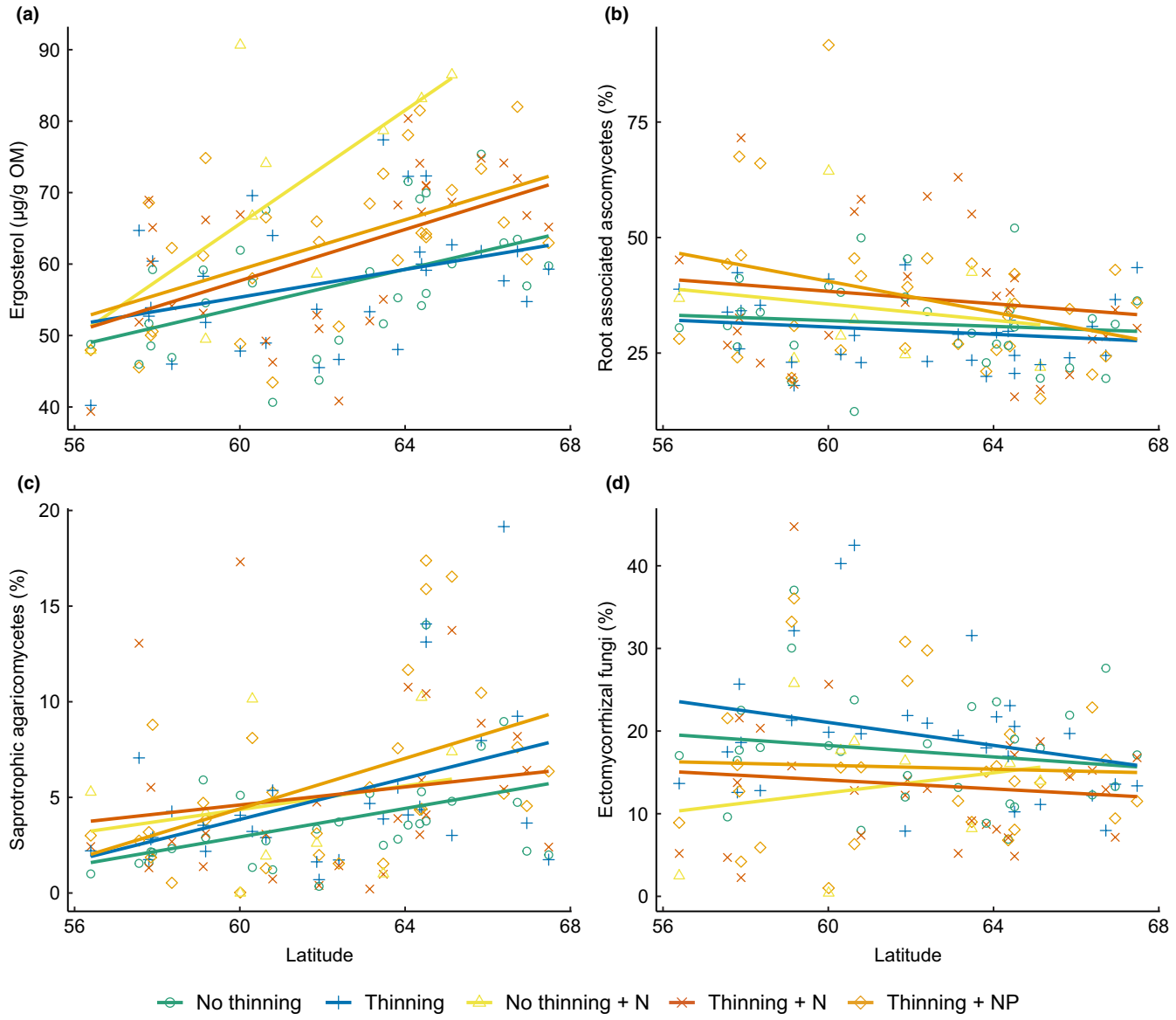

FIGURE 1 Ergosterol concentrations and relative abundances of fungal guilds in the organic topsoil of pine forest plots subjected to thinning and/or fertilization across a latitudinal transect in Sweden. (a) Ergosterol concentration (µg/g OM) in the organic topsoil, relative abundance (% of fungal sequences based on PacBio Sequel sequencing of ITS2) of (b) root-associated ascomycetes, (c) saprotrophic agaricomycetes and (d) ectomycorrhizal fungi. Lines are fitted regression lines from linear models. See Table S4 for statistics

which observed no, or negative, responses of fungal biomass to fertilization (Blasko et al., 2013; Demoling et al., 2008; Högberg, Blasko, et al., 2014; Maaroufi et al., 2019). It is possible that the *Pinus sylvestris* forests in this study were characterized by particularly low nutrient availability, and thus responded in a similar manner as the arctic tundra systems presented in Clemmensen et al. (2006). Higher mycelial biomass without increased soil respiration (even decreased respiration in non-thinned, fertilized stands; Jörgensen et al., 2021a) supports increased C use efficiency of fungi and more rapid mycelial growth after fertilization (Manzoni et al., 2018). However, the observed increases in standing fungal biomass may also be due to slower turnover rates of fungal mycelium instead of increased production (Hagenbo et al., 2017). Slow biomass turnover may also contribute to lower soil respiration by increasing apparent C use efficiency at the community level (Manzoni et al., 2018). We also found that root-associated ascomycetes proliferated in fertilized plots (Figure 1b), probably related to higher root biomass (Forsmark et al., 2021). In particular, two *Archaeorhizomyces* species responded positively to fertilization (Figure 4). One of these was highly abundant, on average accounting for 14% of the fungal sequences, and thus likely to have been largely responsible for the increase in fungal biomass after fertilization. Very little is known about the ecology of this only recently discovered genus (Rosling et al., 2011), but we postulate that *Archaeorhizomyces* species associate with roots and increase their C use efficiency and growth at high N availability. In addition, in line with hypothesis (1b), we observed a negative effect of thinning on fungal biomass, but only in fertilized stands. This supports a positive link between mycelial production and tree biomass

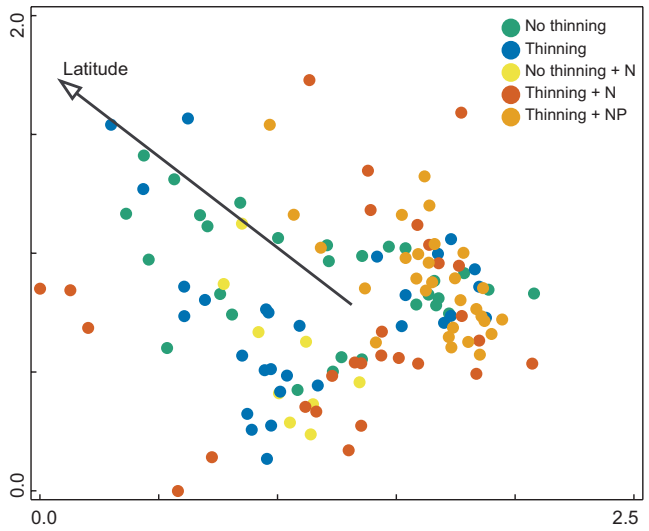

FIGURE 2 Sample plot of a detrended correspondence analysis (DCA) based on the composition of the fungal community in the organic topsoil of pine forests plots subjected to thinning and/or fertilization across a latitudinal transect in Sweden. The analysis is based on PacBio Sequel sequencing of ITS2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

in N fertilized systems and a plausible transition from N limitation to C limitation of the fungal collective after fertilization.

To evaluate tentative mechanisms explaining the effect on C stocks, we studied overarching correlations between C stocks and potential regulators across treatments. Fungal biomass correlated positively with organic topsoil C stocks (Figure 6b), and it seems plausible to attribute part of the treatment-induced gain in C stock to higher input of fungal biomass, according to hypotheses (1a and b). In particular, *Archaeorhizomycetes* may have contributed to C accumulation below-ground, in line with the idea that root-associated ascomycetes are an important source of persistent organic material (Clemmensen et al., 2013, 2015, 2021). Together with associated mycelium, roots may contribute to formation of stable organic matter due to their high content of tannins (Adamczyk et al., 2019), and a recent study in boreal forest suggested that roots contribute to increased C stocks after fertilization by increased C use efficiency and production (Forsmark et al., 2021). We do not have any data on fine-root productivity in our sites, but it is possible that increased root production also contributed the increased C stocks in our study.

Decline of saprotrophic agaricomycetes with ligninolytic potentials has been highlighted as a mechanistic link between fertilization and hampered decomposition after fertilization in North American temperate forests (Entwistle et al., 2017). However, in our forests, saprotrophic agaricomycetes were not affected by fertilization or thinning (Figure 1c), nor did they correlate with C stocks (Figure 6d).

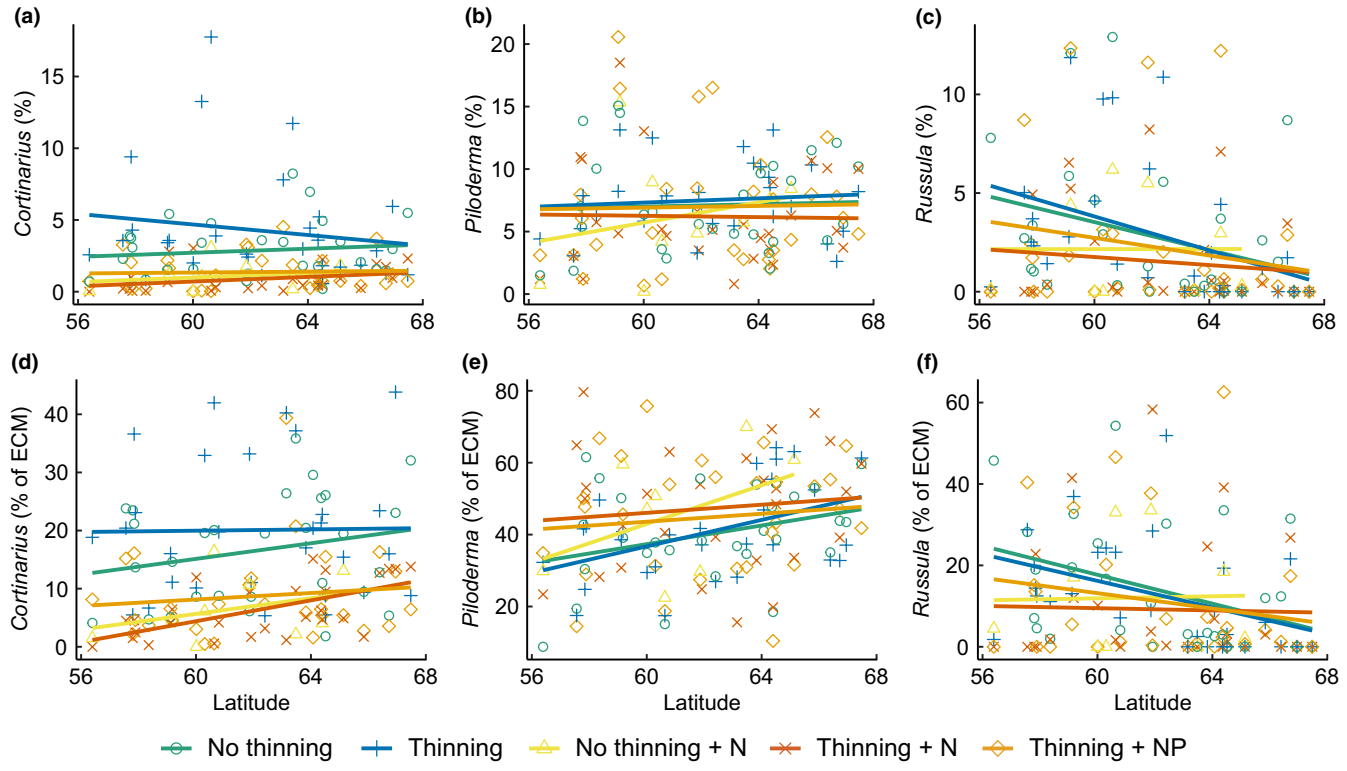

FIGURE 3 Relative abundances of ectomycorrhizal fungal genera in the organic topsoil of pine forest plots subjected to thinning and/or fertilization across a latitudinal transect in Sweden. Relative abundance (% or ITS2 sequences) of (a) *Cortinarius*, (b) *Piloderma* and (c) *Russula* in the total fungal community and (d), (e) and (f) among all ectomycorrhizal fungi (% of ectomycorrhizal (ECM) ITS2 sequences). Lines are fitted regression lines from linear models. See Table S5 for statistics

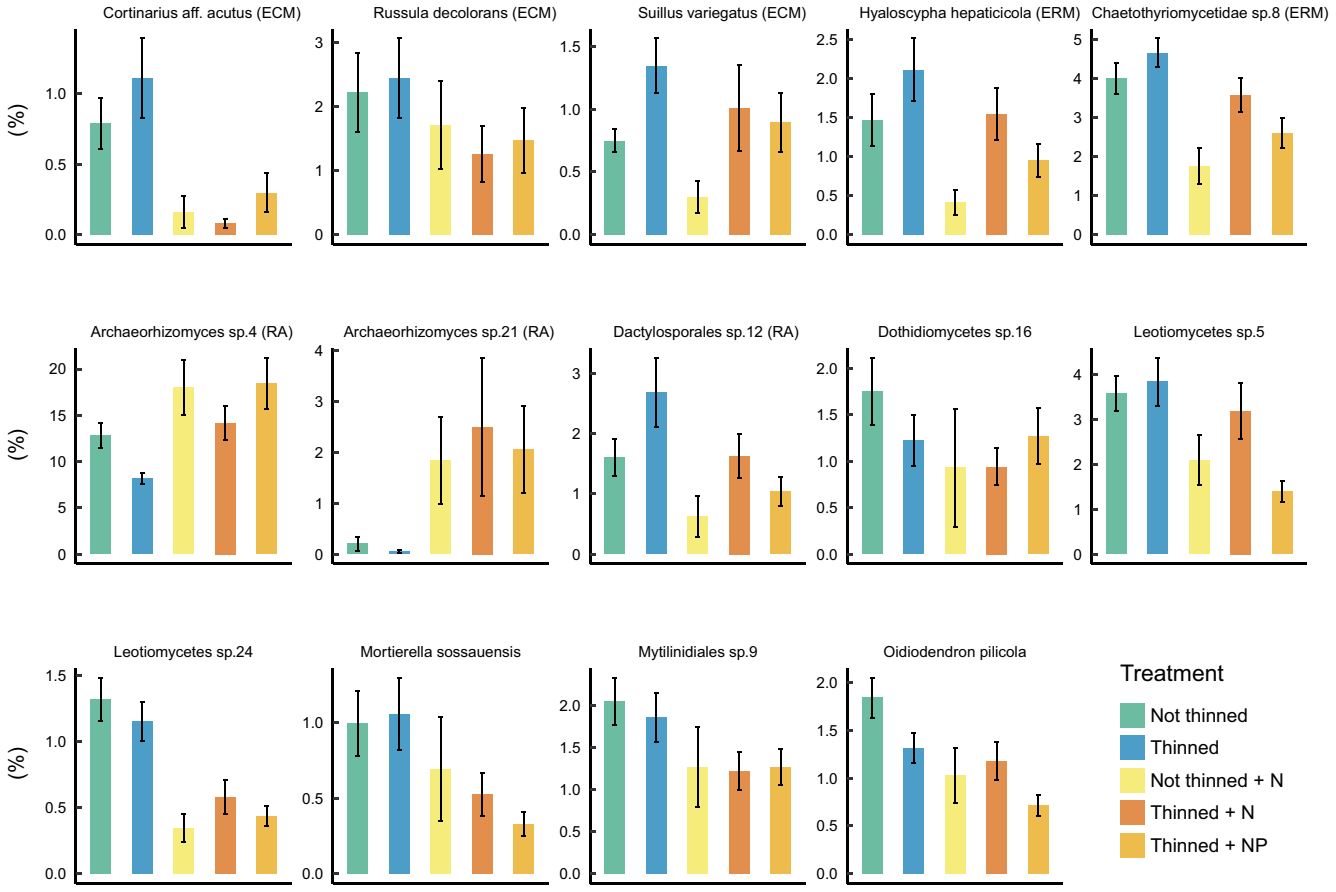

FIGURE 4 Relative abundance of species in the fungal community of organic horizon of pine plots subjected to different combinations of thinning and fertilization. Relative abundance (% of fungal reads from PacBio Sequel sequencing of ITS2 amplicons) of 14 species with a statistically significant response ($p \leq 0.05$) to any of the treatments. Abbreviations in parentheses denote functional guild: ECM = ectomycorrhiza, ERM = ericoid mycorrhiza, RA = root-associated ascomycete, no letters = unknown guild. Bars are mean abundance and error bars denote SE. See Table S6 for statistics [Correction added on 7 January 2022, after first online publication: Figure 4 has been corrected to remove duplicate labelling of Russula decolorans.]

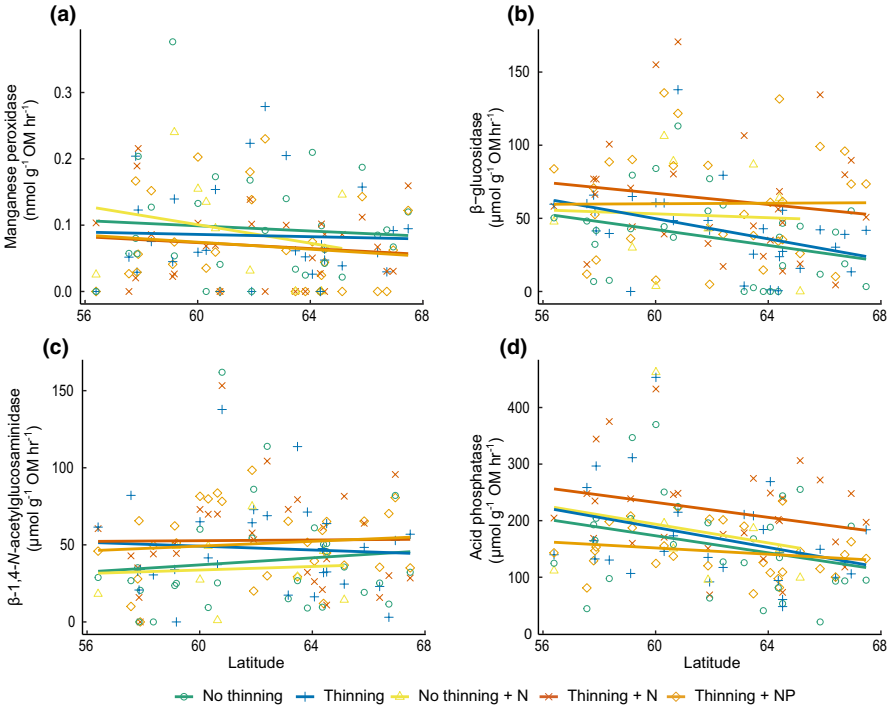

FIGURE 5 Enzyme activities in the organic topsoil of pine forest plots subjected to different combinations of thinning and fertilization along a latitudinal transect in Sweden. Enzyme activities of (a) manganese peroxidase, (b) β-1,4-glucosidase, (c) β-1,4-N-acetylglucosaminidase and (d) acid phosphatase. Lines are fitted regression lines from linear models. See Table S7 for statistics

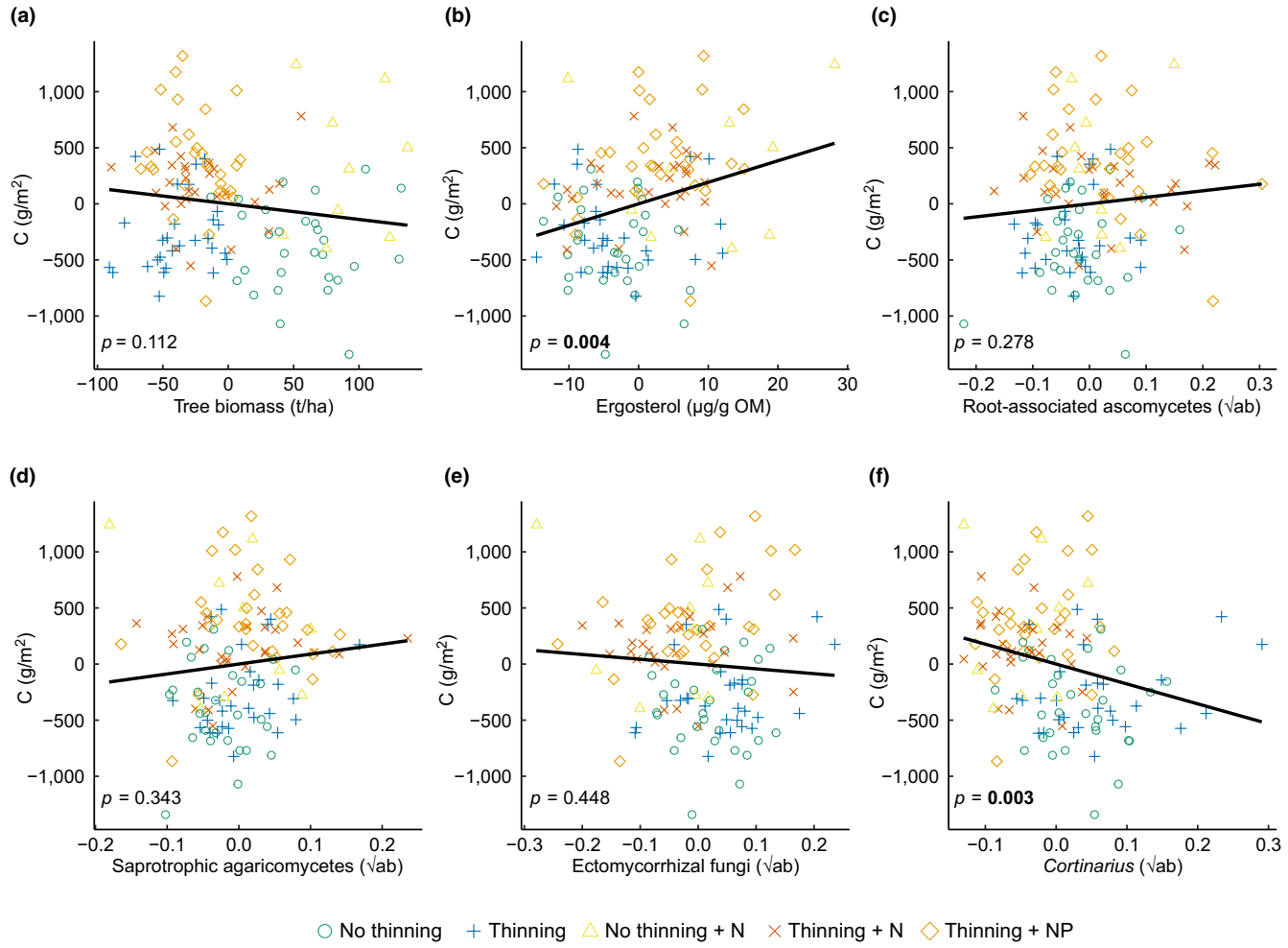

FIGURE 6 Correlations between organic topsoil C stocks and potential regulatory factors across pine forests plots subjected to different combinations of thinning and fertilization along a latitudinal transect in Sweden. Partial residuals, after accounting for site-specific random effects, of C in the organic topsoil (g/m^2) plotted against (a) standing biomass of trees (tonnes), (b) ergosterol (µg/g OM), or square root transformed relative abundances ($\sqrt{ab}$) of: (c) root-associated ascomycetes, (d) saprotrophic agaricomycetes, (e) ectomycorrhizal fungi and (f) *Cortinarius* species. Significant effects ($p \leq 0.05$), evaluated by linear models, are highlighted in bold text in the figure. $N = 124$

Instead, we found that the relative abundance of ectomycorrhizal fungi responded negatively to fertilization, with *Cortinarius* species being particularly sensitive (Figure 3a,d), in agreement with hypothesis (2a) and previous studies (Hasselquist & Högberg, 2014; Högberg et al., 2017; Lilleskov et al., 2019; van der Linde et al., 2018; Wallander et al., 2011). The genus *Piloderma*, which lack ligninolytic capacity (Kohler et al., 2015), was also negatively affected by fertilization, but less so than *Cortinarius*, leading to increased relative dominance of *Piloderma* in the ectomycorrhizal community after fertilization. The abundance of ectomycorrhizal *Cortinarius* species correlated significantly and negatively with C stocks (Figure 6f). One species, *Cortinarius acutus*, responded particularly strongly (Figure 4), and was specifically found to correlate negatively with soil C storage in a field survey of Swedish forests (Lindahl et al., 2021). *Suillus variegatus*, which is likely to use the Fenton reaction to oxidize organic matter (Shah et al., 2016), also responded negatively to fertilization. Supposedly, these ectomycorrhizal decomposers take over the role of saprotrophic agaricomycetes under the strongly N-limited conditions that prevail in boreal forests (Lindahl et al., 2021), but their N-mining activity becomes obsolete when inorganic N is provided through fertilization (Bödeker et al., 2014). The importance of N-mining by ectomycorrhizal decomposers was recently supported by a global meta-analysis, which found that stimulated tree growth at elevated CO_2 occurred at the expense of below-ground organic stocks, and primarily in forests with ectomycorrhizal hosts (Terrer et al., 2021). Similar to our study, the effect was nullified by fertilization.

We did, however, not find a significant decline in manganese peroxidase activity after fertilization, and therefore little direct support for hypothesis (2b), although there was a trend towards decreased activity when thinning and fertilization were combined (Figure 5a). Previous studies have found correlations between fungal community composition and manganese peroxidase activity (Bödeker et al., 2014; Entwistle et al., 2017; Kyaschenko et al., 2017a), and linked this to changes in soil C stocks (Kyaschenko et al., 2017b; Stendahl et al., 2017). However, causal links were not

as straightforward in this study, presumably with other mechanisms at play. For example, release of ectomycorrhizal competition could have stimulated peroxidase production by litter-associated saprotrophic basidiomycetes (Kyaschenko et al., 2017b), but with little impact on over-all organic matter turnover. Alternatively, our sampling methods or assays were not sufficiently accurate to detect plot-level effects in oxidative enzyme activity.

Fertilization stimulated cellulolytic β-1,4-glucosidase activity (Figure 5b) as in previous studies (e.g. Carreiro et al., 2000), and more so at higher latitudes, suggesting a stronger N limitation of cellulose decomposers in the north. Nevertheless, C stocks in the organic topsoil increased after fertilization (with a positive rather than negative correlation with β-1,4-glucosidase activity; Figure S2), further supporting the idea that accumulation of organic matter is independent of rates of cellulose decomposition (Kyaschenko et al., 2017b). Carbon in the upper part of the organic topsoil has a much faster turnover than in the lower part, and likely, decomposition of labile, cellulose-rich, litter components plays a subordinate role in the overall regulation of total C stocks, which rather depend on the turnover of more persistent C pools (Craine et al., 2007; Kyaschenko et al., 2019).

β-1,4-N-acetylglucosaminidase activity increased after thinning (Figure 5c). This enzyme is used by fungi to decompose chitin (Maillard et al., 2018) and has previously been observed to increase after root disruption, presumably as an effect of turnover of dead mycorrhizal mycelium (Kohout et al., 2018; Lindahl et al., 2010). These previous studies observed increased activities within days and up to one year after root death, but in our study, thinning occurred several years before sampling. Our results suggest that the effects of root death on β-1,4-N-acetylglucosaminidase activity may linger longer than previously assumed, but it remains to be investigated whether thinning has long-term effects on fungal biomass turnover.

Acid phosphatase activities increased in N fertilized stands, but decreased when P was added together with N (Figure 5d), which is in agreement with previous studies in N fertilized forests (Heuck et al., 2018; Saiya-Cork et al., 2002). This indicates that N fertilization can increase the demand for organic P by some soil organisms, possibly roots or root-associated fungi. However, even though P additions influenced rhizosphere processes and fungal community composition, we did not observe a tree growth response, although C sequestration in the organic topsoil was stimulated (Jörgensen et al., 2021a).

Taken together, we propose that, under ambient conditions and low levels of N deposition, soil C stocks are mainly regulated by rates of decomposition, in line with previous studies (Janssens et al., 2010). However, the importance of below-ground inputs, including mycelium of root-associated fungi, for C sequestration increased in the N amended systems, particularly at high latitudes. The reason may be a shift in the mechanism of N acquisition from microbial mining of organic N, which leads to losses of C, to uptake of inorganic N provided through fertilization (Craine et al., 2007) and higher C input in the form of fungal mycelium. We acknowledge that these conclusions are based on correlations, potentially subjected to confounding factors, calling for additional experiments that can confirm the regulatory importance of these mechanisms. Although N fertilization promotes C sequestration and biomass production, it also seems to disrupt mechanisms for nutrient cycling from organic matter in the boreal forest ecosystem. Hence, we do not yet know the long-term consequences. Previous studies have shown that the ectomycorrhizal guild, in terms of collective abundance, recovers within a relatively short period after the N amendment is terminated (Högberg, Yarwood, et al., 2014; Högberg et al., 2011). However, effects on community composition may be more long lasting; more persistent effects on fruit body production by certain species have been observed (Strengbom et al., 2001). Since traits and N responses differ among ectomycorrhizal taxa, long-term community shifts may have a major impact on ecosystem functioning (Lindahl et al., 2021; van der Linde et al., 2018; Zak et al., 2019).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

J.S., G.G. and B.D.L. conceived the study; K.J. collected the data and performed the analyses; K.J. wrote the first draft and all authors contributed to interpretation and writing.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.s4mw6m97j (Jörgensen et al., 2021b). Sequence data is published in NCBI-SRA under project PRJNA738321.

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REFERENCES

Adamczyk, B., Sietiö, O.-M., Straková, P., Prommer, J., Wild, B., Hagner, M., Pihlatie, M., Fritze, H., Richter, A., & Heinonsalo, J. (2019). Plant roots increase both decomposition and stable organic matter formation in boreal forest soil. *Nature Communications*, *10*(1), 1–9. https://doi.org/10.1038/s41467-019-11993-1

Almeida, J. P., Rosenstock, N. P., Forsmark, B., Bergh, J., & Wallander, H. (2019). Ectomycorrhizal community composition and function in a spruce forest transitioning between nitrogen and phosphorus limitation. *Fungal Ecology*, *40*, 20–31. https://doi.org/10.1016/j.funeco.2018.05.008

Arnolds, E. (1991). Decline of ectomycorrhizal fungi in Europe. *Agriculture, Ecosystems & Environment*, *35*(2), 209–244. https://doi.org/10.1016/0167-8809(91)90052-Y

Bahr, A., Ellström, M., Akselsson, C., Ekblad, A., Mikusinska, A., & Wallander, H. (2013). Growth of ectomycorrhizal fungal mycelium along a Norway spruce forest nitrogen deposition gradient and its effect on nitrogen leakage. *Soil Biology and Biochemistry*, *59*, 38–48. https://doi.org/10.1016/j.soilbio.2013.01.004

Baskaran, P., Hyvönen, R., Berglund, S. L., Clemmensen, K. E., Ågren, G. I., Lindahl, B. D., & Manzoni, S. (2017). Modelling the influence of ectomycorrhizal decomposition on plant nutrition and soil carbon sequestration in boreal forest ecosystems. *New Phytologist*, *213*(3), 1452–1465. https://doi.org/10.1111/nph.14213

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Blasko, R., Högberg, P., Bach, L. H., & Högberg, M. N. (2013). Relations among soil microbial community composition, nitrogen turnover, and tree growth in N-loaded and previously N-loaded boreal spruce forest. *Forest Ecology and Management*, *302*, 319–328. https://doi.org/10.1016/j.foreco.2013.02.035

Boberg, J., Finlay, R. D., Stenlid, J., Näsholm, T., & Lindahl, B. D. (2008). Glucose and ammonium additions affect needle decomposition and carbon allocation by the litter degrading fungus *Mycena epipterygia*. *Soil Biology & Biochemistry*, *40*(4), 995–999. https://doi.org/10.1016/j.soilbio.2007.11.005

Bödeker, I. T. M., Clemmensen, K. E., de Boer, W., Martin, F., Olson, Å., & Lindahl, B. D. (2014). Ectomycorrhizal Cortinarius species participate in enzymatic oxidation of humus in northern forest ecosystems. *The New Phytologist*, *203*(1), 245–256. https://doi.org/10.1111/nph.12791

Carreiro, M. M., Sinsabaugh, R. L., Repert, D. A., & Parkhurst, D. F. (2000). Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology*, *81*(9), 2359–2365. https://doi.org/10.2307/177459

Castaño, C., Alday, J. G., Lindahl, B. D., Martínez de Aragón, J., de-Miguel, S., Colinas, C., Parladé, J., Pera, J., & Bonet, J. A. (2018). Lack of thinning effects over inter-annual changes in soil fungal community and diversity in a Mediterranean pine forest. *Forest Ecology and Management*, *424*, 420–427. https://doi.org/10.1016/j.foreco.2018.05.004

Castaño, C., Berlin, A., Durling, M. B., Ihrmark, K., Lindahl, B. D., Stenlid, J., Clemmensen, K. E., & Olson, Å. (2020). Optimized metabarcoding with Pacific biosciences enables semi-quantitative analysis of fungal communities. *New Phytologist*, *228*(3). https://doi.org/10.1111/nph.16731

Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R. D., Wardle, D. A., & Lindahl, B. D. (2013). Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*, *339*(6127), 1615–1618. https://doi.org/10.1126/science.1231923

Clemmensen, K. E., Durling, M. B., Michelsen, A., Hallin, S., Finlay, R. D., & Lindahl, B. D. (2021). A tipping point in carbon storage when forest expands into tundra is related to mycorrhizal recycling of nitrogen. *Ecology Letters*, *24*, 1193–1204. https://doi.org/10.1111/ele.13735

Clemmensen, K. E., Finlay, R. D., Dahlberg, A., Stenlid, J., Wardle, D. A., & Lindahl, B. D. (2015). Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytologist*, *205*(4), 1525–1536. https://doi.org/10.1111/nph.13208

Clemmensen, K. E., Ihrmark, K., Durling, M. B., & Lindahl, B. D. (2016). Sample preparation for fungal community analysis by high-throughput sequencing of barcode amplicons. In F. Martin & S. Uroz (Eds.), *Microbial environmental genomics (MEG)* (Vol. 1399, pp. 61–88). Humana Press Inc.

Clemmensen, K. E., Michelsen, A., Jonasson, S., & Shaver, G. R. (2006). Increased ectomycorrhizal fungal abundance after long-term fertilization and warming of two arctic tundra ecosystems. *New Phytologist*, *171*(2), 391–404. https://doi.org/10.1111/j.1469-8137.2006.01778.x

Collado, E., Castaño, C., Bonet, J. A., Hagenbo, A., Martínez de Aragón, J., & de-Miguel, S. (2020). Divergent above- and below-ground responses of fungal functional groups to forest thinning. *Soil Biology and Biochemistry*, *150*. https://doi.org/10.1016/j.soilbio.2020.108010

Craine, J. M., Morrow, C., & Fierer, N. (2007). Microbial nitrogen limitation increases decomposition. *Ecology*, *88*(8), 2105–2113. https://doi.org/10.1890/06-1847.1

Daniel, G., Volc, E., & Kubatova, E. (1994). Pyranose oxidase, a major source of H_2O_2 during wood degradation by *Phanerochaete chrysosporium*, *Trametes versicolor*, and *Oudemansiella mucida*. *Applied and Environmental Microbiology*, *60*(7), 2524–2532. https://doi.org/10.1128/aem.60.7.2524-2532.1994

DeLuca, T. H., & Boisvenue, C. (2012). Boreal forest soil carbon: Distribution, function and modelling. *Forestry: An International Journal of Forest Research*, *85*(2), 161–184. https://doi.org/10.1093/forestry/cps003

Demoling, F., Nilsson, L. O., & Bååth, E. (2008). Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. *Soil Biology and Biochemistry*, *40*(2), 370–379. https://doi.org/10.1016/j.soilbio.2007.08.019

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Entwistle, E. M., Zak, D. R., & Argiroff, W. A. (2017). Anthropogenic N deposition increases soil C storage by reducing the relative abundance of lignolytic fungi. *Ecological Monographs*, *88*(2), 225–244. https://doi.org/10.1002/ecm.1288

Forsmark, B., Nordin, A., Rosenstock, N. P., Wallander, H., & Gundale, M. J. (2021). Anthropogenic nitrogen enrichment increased the efficiency of belowground biomass production in a boreal forest. *Soil Biology and Biochemistry*. https://doi.org/10.1016/j.soilbio.2021.108154

Forstner, S. J., Wechselberger, V., Stecher, S., Müller, S., Keiblinger, K. M., Wanek, W., Schleppi, P., Gundersen, P., Tatzber, M., Gerzabek, M. H., & Zechmeister-Boltenstern, S. (2019). Resistant soil microbial communities show signs of increasing phosphorus limitation in two temperate forests after long-term nitrogen addition. *Frontiers in Forests and Global Change*, *2*(73). https://doi.org/10.3389/ffgc.2019.00073

Franklin, O., Näsholm, T., Högberg, P., & Högberg, M. N. (2014). Forests trapped in nitrogen limitation – An ecological market perspective on ectomycorrhizal symbiosis. *The New Phytologist*, *203*(2), 657–666. https://doi.org/10.1111/nph.12840

Gauthier, S., Bernier, P., Kuuluvainen, T., Shvidenko, A. Z., & Schepaschenko, D. G. (2015). Boreal forest health and global change. *Science*, *349*(6250), 819–822. https://doi.org/10.1126/science.aaa9092

Hagenbo, A., Clemmensen, K. E., Finlay, R. D., Kyaschenko, J., Lindahl, B. D., Fransson, P., & Ekblad, A. (2017). Changes in turnover rather than production regulate biomass of ectomycorrhizal fungal mycelium across a *Pinus sylvestris* chronosequence. *New Phytologist*, *214*(1), 424–431. https://doi.org/10.1111/nph.14379

Hasselquist, N. J., & Högberg, P. (2014). Dosage and duration effects of nitrogen additions on ectomycorrhizal sporocarp production and functioning: An example from two N-limited boreal forests. *Ecology and Evolution*, *4*(15), 3015–3026. https://doi.org/10.1002/ece3.1145

Heuck, C., Smolka, G., Whalen, E. D., Frey, S., Gundersen, P., Moldan, F., Fernandez, I. J., & Spohn, M. (2018). Effects of long-term nitrogen addition on phosphorus cycling in organic soil horizons of temperate forests. *Biogeochemistry*, *141*(2), 167–181. https://doi.org/10.1007/s10533-018-0511-5

Högberg, M. N., Bååth, E., Nordgren, A., Arnebrant, K., & Högberg, P. (2003). Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs - A hypothesis based on field observations in boreal forest. *New Phytologist*, *160*(1), 225–238. https://doi.org/10.1046/j.1469-8137.2003.00867.x

Högberg, M. N., Blasko, R., Bach, L. H., Hasselquist, N. J., Egnell, G., Näsholm, T., & Högberg, P. (2014). The return of an experimentally N-saturated boreal forest to an N-limited state: Observations on the soil microbial community structure, biotic N retention capacity and gross N mineralisation. *Plant and Soil*, *381*(1–2), 45–60. https://doi.org/10.1007/s11104-014-2091-z

Högberg, M. N., Briones, M. J. I., Keel, S. G., Metcalfe, D. B., Campbell, C., Midwood, A. J., Thornton, B., Hurry, V., Linder, S., Näsholm, T., & Högberg, P. (2010). Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. *New Phytologist*, *187*(2), 485–493. https://doi.org/10.1111/j.1469-8137.2010.03274.x

Högberg, M. N., Yarwood, S. A., & Myrold, D. D. (2014). Fungal but not bacterial soil communities recover after termination of decadal nitrogen additions to boreal forest. *Soil Biology and Biochemistry*, *72*, 35–43. https://doi.org/10.1016/j.soilbio.2014.01.014

Högberg, P., Fan, H., Quist, M., Binkley, D., & Tamm, C. O. (2006). Tree growth and soil acidification in response to 30 years of experimental nitrogen loading on boreal forest. *Global Change Biology*, *12*(3), 489–499. https://doi.org/10.1111/j.1365-2486.2006.01102.x

Högberg, P., Johannisson, C., Yarwood, S., Callesen, I., Näsholm, T., Myrold, D. D., & Högberg, M. N. (2011). Recovery of ectomycorrhiza after 'nitrogen saturation' of a conifer forest. *New Phytologist*, *189*(2), 515–525. https://doi.org/10.1111/j.1469-8137.2010.03485.x

Högberg, P., Näsholm, T., Franklin, O., & Högberg, M. N. (2017). Tamm review: On the nature of the nitrogen limitation to plant growth in Fennoscandian boreal forests. *Forest Ecology and Management*, *403*, 161–185. https://doi.org/10.1016/j.foreco.2017.04.045

Ihrmark, K., Bödeker, I. T. M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K. E., & Lindahl, B. D. (2012). New primers to amplify the fungal ITS2 region – Evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology*, *82*(3), 666–677. https://doi.org/10.1111/j.1574-6941.2012.01437.x

Janssens, I. A., Dieleman, W., Luyssaert, S., Subke, J.-A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A. J., Grace, J., Matteucci, G., Papale, D., Piao, S. L., Schulze, E.-D., Tang, J., & Law, B. E. (2010). Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience*, *3*(5), 315–322. https://doi.org/10.1038/ngeo844

Jörgensen, K., Granath, G., Lindahl, B., & Strengbom, J. (2021). Forest management to increase carbon sequestration in boreal *Pinus sylvestris* forests. *Plant and Soil*, *466*, 165–178. https://doi.org/10.1007/s11104-021-05038-0

Jörgensen, K., Granath, G., Strengbom, J., & Lindahl, B. (2021). Data from: Links between boreal forest management, soil fungal communities and belowground carbon sequestration. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.s4mw6m97j

Karlsson, P. E., Pihl Karlsson, G., Hellsten, S., Akselsson, C., Ferm, M., & Hultberg, H. (2019). Total deposition of inorganic nitrogen to Norway spruce forests – Applying a surrogate surface method across a deposition gradient in Sweden. *Atmospheric Environment*, *217*. https://doi.org/10.1016/j.atmosenv.2019.116964

Kohler, A., Kuo, A., Nagy, L. G., Morin, E., Barry, K. W., Buscot, F., Canbäck, B., Choi, C., Cichocki, N., Clum, A., Colpaert, J., Copeland, A., Costa, M. D., Doré, J., Floudas, D., Gay, G., Girlanda, M., Henrissat, B., Herrmann, S., … Martin, F. (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature Genetics*, *47*(4), 410–415. https://doi.org/10.1038/ng.3223

Kohout, P., Charvátová, M., Štursová, M., Mašínová, T., Tomšovský, M., & Baldrian, P. (2018). Clearcutting alters decomposition processes and initiates complex restructuring of fungal communities in soil and tree roots. *The ISME Journal*, *12*(3), 692–703. https://doi.org/10.1038/s41396-017-0027-3

Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., Bahram, M., Bates, S. T., Bruns, T. D., Bengtsson-Palme, J., Callaghan, T. M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G. W., Hartmann, M., Kirk, P. M., Kohout, P., … Larsson, K.-H. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*, *22*(21), 5271–5277. https://doi.org/10.1111/mec.12481

Kuznetsova, A., Brockhoff, P. B., Christensen, R. H. B., & Jensen, S. P. (2017). lmerTest: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13), 1–26.

Kyaschenko, J., Clemmensen, K. E., Hagenbo, A., Karltun, E., & Lindahl, B. D. (2017). Shift in fungal communities and associated enzyme activities along an age gradient of managed *Pinus sylvestris* stands. *The ISME Journal*, *11*(4), 863–874. https://doi.org/10.1038/ismej.2016.184

Kyaschenko, J., Clemmensen, K. E., Karltun, E., & Lindahl, B. D. (2017). Below-ground organic matter accumulation along a boreal forest fertility gradient relates to guild interaction within fungal communities. *Ecology Letters*, *20*(12), 1546–1555. https://doi.org/10.1111/ele.12862

Kyaschenko, J., Ovaskainen, O., Ekblad, A., Hagenbo, A., Karltun, E., Clemmensen, K. E., & Lindahl, B. D. (2019). Soil fertility in boreal forest relates to root-driven nitrogen retention and carbon sequestration in the mor layer. *New Phytologist*, *221*(3), 1492–1502. https://doi.org/10.1111/nph.15454

Lilleskov, E. A., Kuyper, T. W., Bidartondo, M. I., & Hobbie, E. A. (2019). Atmospheric nitrogen deposition impacts on the structure and function of forest mycorrhizal communities: A review. *Environmental Pollution*, *246*, 148–162. https://doi.org/10.1016/j.envpol.2018.11.074

Lindahl, B. D., de Boer, W., & Finlay, R. D. (2010). Disruption of root carbon transport into forest humus stimulates fungal opportunists at the expense of mycorrhizal fungi. *The ISME Journal*, *4*(7), 872–881. https://doi.org/10.1038/ismej.2010.19

Lindahl, B. D., Ihrmark, K., Boberg, J., Trumbore, S. E., Högberg, P., Stenlid, J., & Finlay, R. D. (2007). Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *The New Phytologist*, *173*(3), 611–620. https://doi.org/10.1111/j.1469-8137.2006.01936.x

Lindahl, B. D., Kyaschenko, J., Varenius, K., Clemmensen, K. E., Dahlberg, A., Karltun, E., & Stendahl, J. (2021). A group of ectomycorrhizal fungi restricts organic matter accumulation in boreal forest. *Ecology Letters*, *47*(7), 1341–1351. https://doi.org/10.1111/ele.13746

Lindahl, B. D., & Tunlid, A. (2015). Ectomycorrhizal fungi – Potential organic matter decomposers, yet not saprotrophs. *The New Phytologist*, *205*(4), 1443–1447. https://doi.org/10.1111/nph.13201

Maaroufi, N. I., Nordin, A., Hasselquist, N. J., Bach, L. H., Palmqvist, K., & Gundale, M. J. (2015). Anthropogenic nitrogen deposition enhances carbon sequestration in boreal soils. *Global Change Biology*, *21*(8), 3169–3180. https://doi.org/10.1111/gcb.12904

Maaroufi, N. I., Nordin, A., Palmqvist, K., Hasselquist, N. J., Forsmark, B., Rosenstock, N. P., Wallander, H., & Gundale, M. J. (2019). Anthropogenic nitrogen enrichment enhances soil carbon accumulation by impacting saprotrophs rather than ectomycorrhizal fungal activity. *Global Change Biology*, *25*(9), 2900–2914. https://doi.org/10.1111/gcb.14722

Maassen, S., Fritze, H., & Wirth, S. (2006). Response of soil microbial biomass, activities, and community structure at a pine stand in northeastern Germany 5 years after thinning. *Canadian Journal of Forest Research*, *36*(6), 1427–1434. https://doi.org/10.1139/X06-039

Maillard, F., Didion, M., Fauchery, L., Bach, C., & Buée, M. (2018). N-acetylglucosaminidase activity, a functional trait of chitin degradation, is regulated differentially within two orders of ectomycorrhizal fungi: Boletales and Agaricales. *Mycorrhiza*, *28*(4), 391–397. https://doi.org/10.1007/s00572-018-0833-0

Manzoni, S., Capek, P., Mooshammer, M., Lindahl, B. D., Richter, A., & Santruckova, H. (2017). Optimal metabolic regulation along resource stoichiometry gradients. *Ecology Letters*, *20*(9), 1182–1191. https://doi.org/10.1111/ele.12815

Manzoni, S., Capek, P., Porada, P., Thurner, M., Winterdahl, M., Beer, C., Bruchert, V., Frouz, J., Herrmann, A. M., Lindahl, B. D., Lyon, S. W., Santruckova, H., Vico, G., & Way, D. (2018). Reviews and syntheses: Carbon use efficiency from organisms to ecosystems – Definitions, theories, and empirical evidence. *Biogeosciences*, *15*(19), 5929–5949. https://doi.org/10.5194/bg-15-5929-2018

Mayer, M., Prescott, C. E., Abaker, W. E. A., Augusto, L., Cécillon, L., Ferreira, G. W. D., James, J., Jandl, R., Katzensteiner, K., Laclau, J.-P., Laganière, J., Nouvellon, Y., Paré, D., Stanturf, J. A., Vanguelova, E. I., & Vesterdal, L. (2020). Tamm review: Influence of forest management activities on soil organic carbon stocks: A knowledge synthesis. *Forest Ecology and Management*, *466*. https://doi.org/10.1016/j.foreco.2020.118127

Nilsson, L. O., & Wallander, H. (2003). Production of external mycelium by ectomycorrhizal fungi in a Norway spruce forest was reduced in response to nitrogen fertilization. *New Phytologist*, *158*(2), 409–416. https://doi.org/10.1046/j.1469-8137.2003.00728.x

Nilsson, R. H., Larsson, K.-H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F. O., Tedersoo, L., Saar, I., Kõljalg, U., & Abarenkov, K. (2019). The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, *47*(D1), D259–D264. https://doi.org/10.1093/nar/gky1022

Nilsson, U., Agestam, E., Ekö, P.-M., Elfving, B., Fahlvik, N., Johansson, U., Karlsson, K., Lundmark, T., & Wallentin, C. (2010). *Thinning of Scots pine and Norway spruce monocultures in Sweden* (Rapport No. 219; Studia Forestalia Suecica).

Nohrstedt, H. O. (2001). Response of coniferous forest ecosystems on mineral soils to nutrient additions: A review of Swedish experiences. *Scandinavian Journal of Forest Research*, *16*(6), 555–573. https://doi.org/10.1080/02827580152699385

Nylund, J.-E., & Wallander, H. (1992). Ergosterol analysis as a means of quantifying mycorrhizal biomass. In J. R. Norris, D. J. Read, & A. K. Varma (Eds.), *Methods in microbiology* (Vol. *24*, pp. 77–88). Academic Press. https://doi.org/10.1016/S0580-9517(08)70088-6

Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., Phillips, O. L., Shvidenko, A., Lewis, S. L., Canadell, J. G., Ciais, P., Jackson, R. B., Pacala, S. W., McGuire, A. D., Piao, S., Rautiainen, A., Sitch, S., & Hayes, D. (2011). A large and persistent carbon sink in the world's forests. *Science*, *333*(6045), 988–993. https://doi.org/10.1126/science.1201609

R Core Team. (2020). *R: A language and environment for statistical computing*. (4.0.3). [Computer software]. R Foundation for Statistical Computing.

Rosling, A., Cox, F., Cruz-Martinez, K., Ihrmark, K., Grelet, G.-A., Lindahl, B. D., Menkis, A., & James, T. Y. (2011). Archaeorhizomycetes: Unearthing an ancient class of ubiquitous soil fungi. *Science*, *333*(6044), 876–879. https://doi.org/10.1126/science.1206958

Royal Swedish Academy of Agriculture and Forestry. (2015). *Forests and forestry in Sweden*. Royal Swedish Academy of Agriculture and Forestry. https://www.ksla.se/pdf-meta/forests-and-forestry-in-sweden_2015-2/

Saarsalmi, A., & Mälkönen, E. (2001). Forest fertilization research in Finland: A literature review. *Scandinavian Journal of Forest Research*, *16*(6), 514–535. https://doi.org/10.1080/02827580152699358

Saiya-Cork, K. R., Sinsabaugh, R. L., & Zak, D. R. (2002). The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biology and Biochemistry*, *34*(9), 1309–1315. https://doi.org/10.1016/S0038-0717(02)00074-3

Shah, F., Nicolás, C., Bentzer, J., Ellström, M., Smits, M., Rineau, F., Canbäck, B., Floudas, D., Carleer, R., Lackner, G., Braesel, J., Hoffmeister, D., Henrissat, B., Ahrén, D., Johansson, T., Hibbett, D. S., Martin, F., Persson, P., & Tunlid, A. (2016). Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. *New Phytologist*, *209*(4), 1705–1719. https://doi.org/10.1111/nph.13722

Sinsabaugh, R. L., Carreiro, M. M., & Repert, D. A. (2002). Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry*, *60*(1), 1–24. https://doi.org/10.1023/A:1016541114786

Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C., Contosta, A. R., Cusack, D., Frey, S., Gallo, M. E., Gartner, T. B., Hobbie, S. E., Holland, K., Keeler, B. L., Powers, J. S., Stursova, M., Takacs-Vesbach, C., Waldrop, M. P., Wallenstein, M. D., … Zeglin, L. H. (2008). Stoichiometry of soil enzyme activity at global scale. *Ecology Letters*, *11*(11), 1252–1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x

Stendahl, J., Berg, B., & Lindahl, B. D. (2017). Manganese availability is negatively associated with carbon storage in northern coniferous forest humus layers. *Scientific Reports*, *7*(1), 15487. https://doi.org/10.1038/s41598-017-15801-y

Sterkenburg, E., Clemmensen, K. E., Ekblad, A., Finlay, R. D., & Lindahl, B. D. (2018). Contrasting effects of ectomycorrhizal fungi on early and late stage decomposition in a boreal forest. *The ISME Journal*, *12*(9), 2187–2197. https://doi.org/10.1038/s41396-018-0181-2

Strengbom, J., Axelsson, E. P., Lundmark, T., & Nordin, A. (2018). Trade-offs in the multi-use potential of managed boreal forests. *Journal of Applied Ecology*, *55*(2), 958–966. https://doi.org/10.1111/1365-2664.13019

Strengbom, J., Nordin, A., Näsholm, T., & Ericson, L. (2001). Slow recovery of boreal forest ecosystem following decreased nitrogen input. *Functional Ecology*, *15*(4), 451–457. https://doi.org/10.1046/j.0269-8463.2001.00538.x

Sun, T., Hobbie, S. E., Berg, B., Zhang, H., Wang, Q., Wang, Z., & Hättenschwiler, S. (2018). Contrasting dynamics and trait controls in first-order root compared with leaf litter decomposition. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(41), 10392–10397. https://doi.org/10.1073/pnas.1716595115

Suz, L. M., Bidartondo, M. I., van der Linde, S., & Kuyper, T. W. (2021). Ectomycorrhizas and tipping points in forest ecosystems. *New Phytologist*, *231*(5), 1700–1707. https://doi.org/10.1111/nph.17547

Talbot, J. M., & Treseder, K. K. (2012). Interactions among lignin, cellulose, and nitrogen drive litter chemistry–decay relationships. *Ecology*, *93*(2), 345–354. https://doi.org/10.1890/11-0843.1

Tamm, C. O. (1991). *Nitrogen in terrestrial ecosystems: Questions of productivity, vegetational changes, and ecosystem stability*. Ecological Studies 81. Springer-Verlag.

Terrer, C., Phillips, R. P., Hungate, B. A., Rosende, J., Pett-Ridge, J., Craig, M. E., van Groenigen, K. J., Keenan, T. F., Sulman, B. N., Stocker, B. D., Reich, P. B., Pellegrini, A. F. A., Pendall, E., Zhang, H., Evans, R. D., Carrillo, Y., Fisher, J. B., Van Sundert, K., Vicca, S., & Jackson, R. B. (2021). A trade-off between plant and soil carbon storage under elevated CO_2. *Nature*, *591*(7851), 599–603. https://doi.org/10.1038/s41586-021-03306-8

Teste, F. P., Lieffers, V. J., & Strelkov, S. E. (2012). Ectomycorrhizal community responses to intensive forest management: Thinning alters impacts of fertilization. *Plant and Soil*, *360*(1–2), 333–347. https://doi.org/10.1007/s11104-012-1231-6

van der Linde, S., Suz, L. M., Orme, C. D. L., Cox, F., Andreae, H., Asi, E., Atkinson, B., Benham, S., Carroll, C., Cools, N., De Vos, B., Dietrich, H.-P., Eichhorn, J., Gehrmann, J., Grebenc, T., Gweon, H. S., Hansen, K., Jacob, F., Kristöfel, F., … Bidartondo, M. I. (2018). Environment

and host as large-scale controls of ectomycorrhizal fungi. *Nature*, *558*(7709), 243–248. https://doi.org/10.1038/s41586-018-0189-9

Wallander, H., Ekblad, A., & Bergh, J. (2011). Growth and carbon sequestration by ectomycorrhizal fungi in intensively fertilized Norway spruce forests. *Forest Ecology and Management*, *262*(6), 999–1007. https://doi.org/10.1016/j.foreco.2011.05.035

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols* (pp. 315–322). Elsevier. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zak, D. R., Holmes, W. E., Burton, A. J., Pregitzer, K. S., & Talhelm, A. F. (2008). Simulated atmospheric deposition increases soil organic matter by slowing decomposition. *Ecological Applications*, *18*(8), 2016–2027. https://doi.org/10.1890/07-1743.1

Zak, D. R., Pellitier, P. T., Argiroff, W. A., Castillo, B., James, T. Y., Nave, L. E., Averill, C., Beidler, K. V., Bhatnagar, J., Blesh, J., Classen, A. T., Craig, M., Fernandez, C. W., Gundersen, P., Johansen, R., Koide, R. T., Lilleskov, E. A., Lindahl, B. D., Nadelhoffer, K. J., … Tunlid, A. (2019). Exploring the role of ectomycorrhizal fungi in soil carbon dynamics. *New Phytologist*, *223*(1), 33–39. https://doi.org/10.1111/nph.15679

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