Science of the Total Environment 918 (2024) 170741

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Shifts in microbial community composition and metabolism correspond with rapid soil carbon accumulation in response to 20 years of simulated nitrogen deposition

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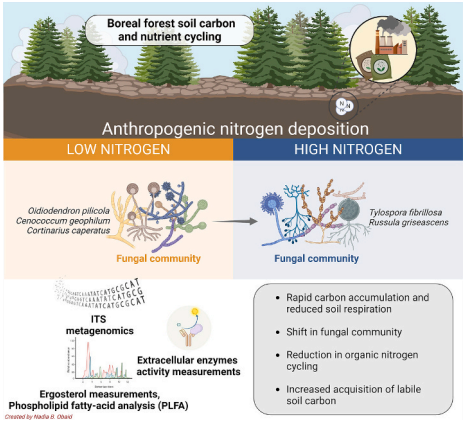
HIGHLIGHTS

- Nitrogen (N) addition shifted the dominance of fungal decomposers.
- Several taxa known to grow on organic N became less abundant.
- Organic N uptake and oxidative enzyme activity were suppressed.
- Higher activity of carbohydrate acquisition indicates decomposer energy limitation.
- Shifts in decomposer activity driven by N addition increase soil carbon stocks.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Jose Julio Ortega-Calvo

Keywords:
Boreal forest
Carbon sequestration
Ectomycorrhizal fungi
Extracellular enzymes
Microbial community
Nitrogen deposition

ABSTRACT

Anthropogenic nitrogen (N) deposition and fertilization in boreal forests frequently reduces decomposition and soil respiration and enhances C storage in the topsoil. This enhancement of the C sink can be as strong as the aboveground biomass response to N additions and has implications for the global C cycle, but the mechanisms remain elusive. We hypothesized that this effect would be associated with a shift in the microbial community and its activity, and particularly by fungal taxa reported to be capable of lignin degradation and organic N acquisition. We sampled the organic layer below the intact litter of a Norway spruce (*Picea abies* (L.) Karst) forest in northern Sweden after 20 years of annual N additions at low (12.5 kg N ha^{-1} yr^{-1}) and high (50 kg N ha^{-1} yr^{-1}) rates. We measured microbial biomass using phospholipid fatty-acid analysis (PLFA) and ergosterol

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https://doi.org/10.1016/j.scitotenv.2024.170741
Received 27 October 2023; Received in revised form 21 December 2023; Accepted 4 February 2024
Available online 6 February 2024

measurements and used ITS metagenomics to profile the fungal community of soil and fine-roots. We probed the metabolic activity of the soil community by measuring the activity of extracellular enzymes and evaluated its relationships with the most N responsive soil fungal species. Nitrogen addition decreased the abundance of fungal PLFA markers and changed the fungal community in humus and fine-roots. Specifically, the humus community changed in part due to a shift from *Oidiodendron pilicola*, *Cenococcum geophilum*, and *Cortinarius caperatus* to *Tylospora fibrillosa* and *Russula griseascens*. These microbial community changes were associated with decreased activity of Mn-peroxidase and peptidase, and an increase in the activity of C acquiring enzymes. Our results show that the rapid accumulation of C in the humus layer frequently observed in areas with high N deposition is consistent with a shift in microbial metabolism, where decomposition associated with organic N acquisition is downregulated when inorganic N forms are readily available.

1. Introduction

Soil microbes play a central role in the global carbon (C) cycle (Liang et al., 2017). By releasing the nutrients plants need to grow, they contribute to the uptake of atmospheric CO_2; however, their respiration also contributes a significant portion of soil CO_2 emissions (Crowther et al., 2019; Paul, 2015; Smith and Read, 2008). Soils in boreal forests store a substantial fraction of global soil C (Tarnocai et al., 2009), and are affected by major changes in nutrient availability such as via atmospheric nitrogen (N) deposition (Maaroufi et al., 2015; Reay et al., 2008; Thomas et al., 2015; Tipping et al., 2017). Owing to huge C stores in boreal forests (Clemmensen et al., 2021; Deluca and Boisvenue, 2012), even small changes can have an impact on the global atmospheric C balance (Lal, 2005), yet we lack a complete mechanistic understanding of how microbial processes drive such changes in response to external N inputs (Luo et al., 2016; Schmidt et al., 2011; Stocker et al., 2016; Todd-Brown et al., 2012).

While it is clear that external N inputs enhance aboveground growth and the input of C to soils by above and below ground litter in boreal forests (Blaško et al., 2022; Forsmark et al., 2021; Leppalammi-Kujansuu et al., 2014), further understanding is needed regarding how soil microbial community composition and enzymatic activities respond. Globally, plant tissue C to N ratio is a good predictor of organic matter decomposition rates, thus lower tissue C to N ratios caused by N deposition can be expected to stimulate decomposition (Averill and Waring, 2017; Stocker et al., 2016); however, studies frequently report reduced soil respiration and decomposition rates in response to N inputs (Berg, 2014; Fog, 1988; Janssens et al., 2010; Nohrstedt et al., 1989). Recent studies of the effects of N addition on C pools and fluxes across the Swedish boreal forests have shown highly consistent responses, with as much as 1000 kg C ha^{-1} yr^{-1} accumulating in the organic soil horizon (Forsmark et al., 2020a), which is driven partly by a reduction in soil respiration by 10–50 % and increased above and belowground litter inputs (Blaško et al., 2022; Forsmark et al., 2021; Maaroufi et al., 2015). Importantly, the organic soil horizon is also a major sink for added N, frequently sequestering half of the added N or more (Gundale et al., 2014; Templer et al., 2012). Therefore, insights into how the boreal microbiome responds to changes in N availability and soil stoichiometry, and in turn influences the release of C and nutrients during decomposition are key to understand the C sinks in both plant biomass and soils in boreal forests.

Litter decomposition is largely mediated by extracellular enzymes exuded into the soil environment to catalyze the release of specific resources (Sinsabaugh et al., 2008). During early stages of decomposition abundant labile resources such as cellulose, peptides, and phosphates are released by hydrolytic enzymes (Baldrian and Stursova, 2011). During later stages of decomposition, an increasing fraction of the remaining organic matter is composed of lignin and other recalcitrant compounds that require oxidative enzymes such as laccases and peroxidases or Fenton chemistry for further degradation (Lindahl and Tunlid, 2015; Sinsabaugh, 2010). Although the fungi with the strongest oxidative potential are saprotrophic, evidence is accumulating that some ectomycorrhizal fungi (EMF) have retained these genes during evolution (Argiroff et al., 2022; Bödeker et al., 2016; Floudas et al., 2012; Kohler et al., 2015; Morgenstern et al., 2008; Nicolas et al., 2019), and that they are adapted to release N from complex organic matter (Bödeker et al., 2014; Kuyper, 2017; Talbot et al., 2008). This EMF adaptation is believed to give the fungi and the associated trees access to otherwise inaccessible organic N pools (Näsholm et al., 1998; Orwin et al., 2011; Schimel and Bennett, 2004). Indeed, EMF are more abundant during late stages of decomposition, in the lower part of the humus layer (Bending and Read, 1995; Lindahl et al., 2007; Rosling et al., 2003), which is also where the soil C stock is most responsive to N enrichment (Blaško et al., 2022; Forsmark et al., 2020a; Maaroufi et al., 2015), but the role of EMF in decomposition remains elusive. Firstly, EMF have been suggested to be major drivers of decomposition (Kyaschenko et al., 2017b; Lindahl et al., 2021; Stendahl et al., 2017), and the reduced allocation of C to EMF for N acquisition may therefore lead to decreased decomposition (Chen et al., 2014; Craine et al., 2007; Moorhead and Sinsabaugh, 2006; Talbot et al., 2008). On the contrary, organic N acquisition via EMF may inhibit decomposition by reducing soil N concentrations below the demand threshold for saprotrophic microbes, i.e. the so-called Gadgil effect (Fernandez and Kennedy, 2016; Gadgil and Gadgil, 1975; Gadgil and Gadgil, 1971; Orwin et al., 2011), and a weakening of that interaction due to N enrichment would thereby lead to increased decomposition. To shed light on these mechanisms, further data are needed on how changes in microbial community composition correspond with changes in extracellular enzyme activity.

To address this knowledge gap, we utilized a long-term experimental set up in a boreal Norway spruce forest (Table S1-S2), where N has been added annually for 20 years at a low (12.5 kg N ha^{-1} yr^{-1}) and high (50 kg N ha^{-1} yr^{-1}) rate to simulate upper level N deposition rates in the boreal region and in Europe, respectively (Gundale et al., 2011). Using this experiment, we previously reported that N addition caused soil C stocks in the humus layer to increase, while soil respiration and soil microbial biomass decreased (Maaroufi et al., 2015). Furthermore, our previous work has suggested that enhanced C accumulation in response to N has to be at least partly driven by changes in microbial activity, as litter decomposition has been shown to be reduced in N treated plots (Forsmark et al., 2020b; Maaroufi et al., 2017). Therefore, to better understand why N has this impact on the soil C cycle, we focused on describing the microbial community composition, using a combination of broad community profiling via phospholipid fatty acids (PLFA) analysis, ergosterol measurements, as well as fungal DNA sequencing for in-depth analysis of fungal taxa down to the level of species. Additionally, the activities of C, N, and P acquiring enzymes and oxidative enzymes were measured to establish the relationships between fungal community composition and soil enzyme activities. Because trees allocate more C to their root systems at the end of the growing season (Högberg et al., 2010; Kaiser et al., 2010), we sampled during summer and early autumn to capture potential seasonal variation in community composition and enzyme functioning. First, we hypothesized that the addition of N would change the microbial community through a decrease in the abundance of ectomycorrhizal decomposers previously reported to be involved in organic N uptake from lignified soil organic matter, including *Cortinarius* (Bödeker et al., 2014) and *Piloderma* (Lilleskov et al., 2011), whereas *Tylospora* and *Russula* species would increase (Kyaschenko et al., 2017a; Marupakula et al., 2021; Wallander

et al., 2010). Secondly, we hypothesized that the shifts in microbial community composition would correspond with a shift in enzyme activities, and specifically by reduced activity of N acquiring and oxidative enzymes that are known to decompose organic matter, while causing an increase in P acquiring enzymes.

2. Materials and methods

2.1. Experimental design

We used an N addition experiment in an area in northern Sweden (64°14 N, 19°46E) with low ambient N deposition (<2 kg N ha^{-1} yr^{-1}). The experiment was established as a randomized complete block design in 1996 in a Norway spruce (*Picea abies* (L.) Karst) dominated forest naturally regenerated at the beginning of the 20th century (From et al., 2016). The experiment consists of control, low and high N addition rate plots. The low N addition rate at 12.5 kg N ha^{-1} yr^{-1} (hereafter referred to as the 12.5 N treatment) corresponds to upper N deposition rates in the boreal region (Gundale et al., 2011; SMHI, 2019), whereas the high N addition rate at 50 kg N ha^{-1} yr^{-1} (hereafter referred to as the 50 N treatment) is representative of high level N deposition rates in central Europe, as well as regions of north America and China (Liu et al., 2013) and also serves as a useful comparison to other N addition experiments (Hyvönen et al., 2008). The experiment consists of plots ranging in size from 1 to 2500 m^2, arranged in blocks, with each N addition rate and plot size replicated 6 times. Here, we utilized plots at 1000 m^2 and 2500 m^2 size to reach a total replication of 12 plots per N addition rate. Nitrogen has been added annually since 1996 as solid ammonium-nitrate granules directly after snowmelt, which usually occurs by the end of May. This makes it the longest running large plot experiment with N addition treatments simulating the entire range of N deposition rates observed in the boreal zone.

2.2. Soil and fine-root sampling

The organic layer under the intact litter and down to the mineral soil was sampled in summer and autumn 2016. The two time points were chosen to represent distinct states in the seasonal development of the canopy, which influences the transfer of C and N between canopy and roots (Högberg et al., 2010; Kaiser et al., 2010). The summer sampling was done one week after the solstice (June 28–July 1), which corresponds to a time of the year with maximum light availability, shoot growth, and a phase of N depletion and accumulation of carbohydrates in photosynthesizing tissues (Linder, 1995). The autumn sampling was done around the equinox (20–27 September) at a time when both N and carbohydrates are depleted in the canopy (Linder, 1995) and when the activity in the root-zone peaks (Hasselquist et al., 2012; Högberg et al., 2001) and soil respiration is high (Maaroufi et al., 2015). While the soil temperature was similar between the summer and autumn sampling (9.7 versus 9.4 °C), the daytime air temperature was 15.4 °C in the summer sampling, and the autumn sampling was done after the first frost, with daytime temperatures of 12.8 °C (ICOS, 2023).

At both sampling occasions, the entire organic layer between the intact litter layer down to the mineral soil was collected with a sharp 22 mm soil corer at 30 locations within each plot, each spaced at least 2 m apart to ensure an appropriate coverage of the spatial variation in the fungal community (Dahlberg et al., 1997). All 30 cores from each plot were pooled to create one composite sample per plot and kept cool with ice during collection, and then were weighed and sieved (2 mm) to separate roots and humus within 1 h of collection. The sieved humus was homogenized, and then separated into two sub-samples per plot. The first subsample (approximately 10 g) was freeze-dried and used to determine gravimetric moisture content, and then was ground to a fine powder on a roller mill (Stuiver et al., 2015) for measurements of total C, N, and P, phospholipid fatty acids (PLFA), and DNA extraction as described below. The second sub-sample was kept frozen and used to measure the activity of a selection of enzymes, also described below. Roots that were isolated during sieving were further hand sorted to exclude ericaceous rhizomes, and vital living Norway spruce fine-roots (< 2 mm diameter), including associated fungal mycelium, were selected for DNA extraction.

During collection and sorting, all equipment was cleaned thoroughly with 70 % ethanol between samples and then stored on ice at 4 °C during the sampling day, and frozen for long-term storage the same evening. The fine-roots were cleaned according to a protocol developed by Gottel et al. (2011) and modified by Gundale et al. (2016). Briefly, the roots were repeatedly (5 times) vigorously shaken and rinsed in distilled water alone, then shaken and rinsed in a solution of 0.05 % Tween 20 detergent, rinsed again in distilled water, and then surface-sterilized for 2 min in a 0.27 % NaOCl solution. Roots were then washed 10 times in distilled water and then frozen, freeze-dried, and ground to a fine powder for DNA extractions. In total, 72 samples were collected in the field (3 N treatments, 2 seasons, 12 replicates) and split into soil and roots for DNA sequencing, yielding a total of 144 samples for DNA sequencing.

2.3. Phospholipid fatty acid and ergosterol analysis

We used a combination of analyses of phospholipid fatty acids (PLFA) and ergosterol on the freeze-dried samples to profile the microbial community broadly, and to represent microbial biomass. Lipids were extracted from approximately 1 g soil using the Bligh and Dyer method (Bligh and Dyer, 1959; McIntosh et al., 2012; White et al., 1979) and the abundance of individual PLFA's was measured on a gas chromatograph (Perkin-Elmer Clarus 500, Mundelein, Illinois, USA) coupled to a flame ionization detector (Waltham, MA, USA) at the Swedish University of Agriculture, SLU Umeå. A total of 28 PLFA markers were identified and described using standard nomenclature, and classified according to previous work in this study system (Maaroufi et al., 2015). The sum of all PLFA markers were used as a measure of total microbial biomass, and i-15:0, a-15:0, 15:0, i-16:0, 16:1ω9, 16:1ω7, 16:0, i-17:0, cy-17:0, a-17:0, 18:1ω7, and cy-19:0 represented the total bacterial biomass (Frostegård and Bååth, 1996). The branched PLFAs i-15:0, a-15:0, i-16:0, i-17:0, and a-17:0 represented gram-positive bacteria (Wardle et al., 2013), whereas 10me16:0, 10me17:0, and 10me18:0 were used to estimate actinobacteria, and cy-17:0, cy-19:0, and 18:1ω7 represented gram-negative bacteria. PLFA 18:2ω6 alone represented fungi (Frostegård et al., 2011; Maaroufi et al., 2019). Absolute abundances were expressed in moles per gram soil C.

Free ergosterol was measured in addition to PLFA as a general fungal biomarker intended to represent living biomass (Clemmensen et al., 2013) as a complementary measure of soil fungal biomass. Ergosterol was extracted from 35 mg of the dried soil by vigorously shaking the samples in 0.25 ml MeOH (99.8 %), and the extract cleaned by centrifugation and filtration (45 μm). The extract was injected in MeOH (isocratic) at a flow rate of 1.5 ml min^{-1} on a Shimadzu prominence HPLC and separated on a reverse-phase column (Ascentis® Express C18, 2.7 μm). After 3 min, ergosterol concentrations were detected with an optical-ultraviolet detector (SPD-20 A UV/VIS).

2.4. DNA extraction and sequencing

Nucleic acids were extracted from 0.25 g of dried powdered humus samples using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions. DNA was extracted from 50 mg of freeze-dried fine-roots by first extracting and purifying in 2 % CTAB/SDS, chloroform, isopropanol and ethanol, as per Gundale et al. (2016), re-suspended in 200 ml Tris and EDTA (TE) buffer, and further purified using a Nucleospin gDNA clean up kit (Machery-Nagel, Düren, Germany).

DNA from the ITS2 region of the internal transcribed spacer (ITS) was amplified with the fungi-specific primers ITS7g (Ihrmark et al.,

2012) and ITS4 (Gardes and Bruns, 1993) which included adapter sequences for Illumina sequencing, and was subjected to a second 8-cycle amplification to attach Nextera (Illumina Inc., San Diego, CA, USA) sample barcodes. Equimolar amounts of DNA from each sample were pooled and submitted for Illumina sequencing with paired-end (325 bp forward; 275 bp reverse) sequencing on a MiSeq sequenator using the MiSeq Reagent Kit v3 chemistry (Illumina Inc., San Diego, CA, USA) at the Next-generation sequencing facility at Lund University, Lund, Sweden.

2.5. Bioinformatics

DNA sequences obtained from Illumina sequencing were trimmed and filtered using Mothur v1.34 (Schloss et al., 2009), clustered using the Gaussian mixture model clustering algorithm CROP (Hao et al., 2011) at 97 % sequence similarity, thus yielding operational taxonomic units (OTUs). All non-fungal and chimeric sequences were removed and sequences were trimmed to include only the ITS2 region using ITSx extractor v1.5.0 (Bengtsson-Palme et al., 2013). The taxonomic identity was then assigned to the set of clustered sequences by searching the Full "UNITE+INSD" (Koljalg et al., 2005; Koljalg et al., 2013) dataset (673,903 seqs, release date 2016-11-20) using the Basic Local Alignment Tool (BLASTN program 2.2.25, blast.ncbi.nlm.nih.gov). Sequences that were 96 % similar to the query sequence and top hit, with at least 80 % coverage of the query sequence length, were assigned to a taxonomic identity with genus and species. Sequences with values of 94–95 % similarity between the query sequence and top hit were assigned a taxonomic identity at the genus level only. All OTU's representing <10 total reads or occurring in only one sample were excluded. Rarefaction was performed to 31,000 reads per sample.

The ecological guild (i.e. ectomycorrhizal or non-ectomycorrhizal) of all taxa for which genera could be assigned was classified with the assistance of the database tool FunGUILD (Nguyen et al., 2016). Guild classifications were accepted, when assigned a confidence ranking of Highly Probable or Probable. EMF species identity was further verified based on the most recent knowledge of the ecology of known close relatives (genera or species) and according to Tedersoo et al. (2010). Read abundances for all OTUs for each sample were summed and the abundance of each OTU expressed as the relative abundance per sample. A matrix containing the rarefied relative abundance of each OTU per sample was then used to analyze differences in community composition. Before further analyses, 1 humus sample with very low OTU abundance and dominance of two species of *Malassizia* was removed, which gave a complete humus dataset with 71 samples and 1051 species OTUs.

2.6. Enzyme activities

The potential activity of selected enzymes originating from the soil microbiome was measured on the frozen soil according to methods in Allison (2012), that is based on methods presented by Saiya-Cork et al. (2002), and modified according to Baldrian (2009). Shortly, we used 4-methylumbelliferyl (MUB) labeled β-D-glucopyranoside, β-D-cellobioside, β-D-xylopyranoside, *N*-acetyl-β-D-glucosaminide, and phosphate to measure the activity of glucosidase (β-1.4-glucosidase, EC 3.2.1.21), endoglucanase (EC 3.2.1.4), xylosidase (β-1.4-xylosidase, EC 3.2.1.37), chitinase (β-1.4-*N*-acetylglucosaminidase, EC 3.2.1.52), and phosphatase (acid-phosphatase, EC 3.1.3.2) respectively, and 7-amido-4-methylcoumarin (AMC) labeled leucine for peptidase (leucine aminopeptidase, EC 3.4.11.1). 140 ml sodium acetate buffer (50 μM, pH 5.0) was added to 0.5 g soil. The sample was shaken for 20 min and loaded on a 96 well plate with 50 μl labeled substrate. The plate was incubated in darkness at room temperature for 10 min ($t = 0$) and fluorescence was measured with MUB after 60 min and for AMC after 20 h.

The activity of the peroxidase (Manganese(II)-peroxidase, EC 1.11.1.13) and laccase (EC 1.10.3.2) was measured as the buildup of the product of oxidized 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) and 3-dimethylaminobenzoic acid (DMAB) for peroxidase and oxidation product of 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) for laccase. Namely, 1 g soil was added to 12 ml Milli-Q water and shaken for 30 min. The samples were centrifuged at 9000 ×*g* for 10 min and the supernatant was frozen at −20 °C for subsequent analyses. The reaction buffer for peroxidase consisted of 1 ml Manganese (II) sulphate solution (1 mM), 0.5 ml DMAB solution in EtOH (50 mM) and 0.5 ml MBTH solution (1 mM) in 5 ml Succinate-lactate buffer (pH 4.5, 100 mM), whereas 1 ml EDTA (2 mM) substituted the Manganese (II) sulphate in the reaction buffer without Mn(II)sulphate (to account for background substrate oxidation). To each well on the 96 well plate 10 μl 1 mM H_2O_2, 140 μl reaction buffer and 50 μl of the extract were added and absorbance was measured every 5 min at 590 nm for 60 min. For laccase, 160 μl 100 mM sodium-acetate buffer (pH 5), 20 μl 50 mM ABTS and 20 μl sample were added to each well and the absorbance was measured at 420 nm every 10 min for 2 h.

All enzyme activities were estimated by regressing fluorescence or absorbance against time and were standardized to the amount of C in each sample to reduce the random variation caused by intermixing heavy mineral particles. Thus, the standardized measure is the potential enzyme activity in a completely organic sample. The activities of glucosidase, endoglucanase, and xylosidase were considered indicative of C acquisition, phosphatase of phosphorous acquisition, chitinase and peptidase of organic N acquisition, whereas peroxidase and laccase indicated oxidative decomposition potential. All measurements were made in humus samples derived after removing roots by sieving, hence all enzyme activity are related to the microbiome and not to processes occurring within plant biomass.

2.7. Statistical analyses

Linear mixed effects models were used to assess the effects of N (df = 2) and season (df = 1) and their interaction (df = 2) on the element ratios and pH, and measurements on the microbial community, including abundance of aggregated PLFA functional groups, and their ratios, free ergosterol, and the relative abundance of aggregated taxonomic or functional groups of the fungal community, and on the potential enzyme activities. Block ($n = 12$) was included as a random factor in the model and Tukey's honestly significant difference (HSD) test was used for post hoc.

Variation in the fungal community composition was explored with a combination of unconstrained and constrained ordinations (Oksanen, 2015), and multivariate analysis of variances on Bray-Curtis dissimilarity matrices. Detrended correspondence analyses (DCA) was applied to identify major gradients in the community and to visualize the relative contribution of sample type (humus vs. root), N addition rate, and season to variations in the fungal community composition. Canonical analysis of principal coordinates (CAP) was used to specifically target variations in community composition due to N and season. The direct and interactive effects of N and season on the location of fungal communities in multivariate space was tested separately for humus and roots using permutational multivariate analysis of variance (PERMANOVA), with block ($n = 12$) included as a random factor. An additional permutational test for homogeneity of multivariate dispersion was used to verify that differences were due to location, rather than differences in dispersion. Finally, for all significant effects identified with PERMANOVA, we followed up with additional 'contribution of variables to similarity' (SIMPER) analysis to identify which fungal OTUs contributed most to the similarity within groups and differences between groups. The correlation between selected responsive OTU's in humus samples from SIMPER analysis and enzyme activities as well as between responsive OTU's and microbial traits were calculated using Spearman's correlation. Only humus samples were used in this analysis as it is assumed that extracellular enzyme activities are responding to the soil environment and not root conditions. All statistics and graphs connected to sequencing were produced in R using phyloseq (McMurdie and

Holmes, 2013) and vegan packages (Dixon, 2003).

3. Results

3.1. Phospholipid fatty acid and ergosterol analysis

Based on PERMANOVA on all PLFA markers, microbial community composition in the humus differed significantly between N addition treatments (Table 1). The 50 N treatment markedly decreased the total PLFA by 25 % (Fig. 1A, Table 2), which was heavily influenced by a 37.5 % decrease in fungal (Fig. 1G) and a 27.1 % decrease in gram-negative bacterial (Fig. 1E) PLFA's. Gram-positive bacteria PLFA's (Fig. 1D) decreased less, and actinobacterial PLFA's (Fig. 1C) remained relatively unchanged, which led to an increase in the ratio between gram-positive and gram-negative bacteria (Fig. 1F). However, the total bacterial PLFA's were still lower in 50 N treatment overall (Fig. 1B) and the decrease in fungal PLFA's was bigger compared to bacterial PLFA's (Fig. 1I). The 12.5 N treatment did not differ significantly compared to the control in any of the PLFA markers measured (Fig. 1). In contrast to the fungal PLFA marker 18:2ω6, ergosterol increased by 30.2 % in the 50 N treatment (Fig. 1H) but was not significantly higher than the control in 12.5 N treatment. Season had a relatively minor effect on PLFA's and ergosterol (Table 2), although fungal PLFA tended to be higher ($p = 0.059$) in the autumn. No significant interactions between N and season were detected in the PLFA or ergosterol measurements (Table 2).

3.2. Fungal community

After all filtering steps, clustering, and rarefaction, sequencing generated 4,397,997 sequences belonging to 1051 operational taxonomic units (OTU's). Of these, 610 were present in both humus and root samples, 433 exclusively in the humus samples, and 8 OTU's exclusively in the root samples (Fig. S1A). Additionally, 1011 OTU's were shared between the seasons, 29 were unique for summer and 11 to autumn sampling (Fig. S1B). Regarding N treatment, 875 OTU's were shared between the three treatments, 12 were only found in 0 N, 6 in 12.5 N and 15 in 50 N treatment (Fig. S1C). Of the 1051 OTU's, 816 (representing 90 % of all sequence reads) were assignable to ecological guild; 295 OTU's, comprising 55 % of all sequence reads were classified as ectomycorrhizal (54 % of humus sequences, 57 % of root sequences), and 51 OTU's, comprising 33 % of all sequence reads were classified as ericoid fungi (22 % of humus sequences, 46 % of root sequences). The fungal community differed significantly between N treatments for both the humus and root community (PERMANOVA $p < 0.001$, Table 2). Differences in community composition between humus and roots contributed most of the variation in the combined fungal community, explaining 52.4 % of the variation (Fig. S2). CAP analysis constrained with N treatment and the PERMANOVA show that the fungal community composition in both humus and roots was significantly different in the 50 N treatment from the 0 N and the 12.5 N treatment (Fig. 2).

The humus community had a clear dominance of ascomycetes over basidiomycetes in the summer (57 % versus 40 %), shifting to become more evenly distributed in the autumn (46 % versus 51 %). The humus community was significantly affected by the N treatment based on PERMANOVA (Table 1). Among Agaricomycetes which alone constituted 33 % of the fungal community, and 73 % of the basidiomycetes, mixed responses to N at species level were observed. Specifically, EMF forming genera *Russula* and *Tylospora* increased with N addition (nitrophilic) based on linear mixed effects model (Table 2). Different OTU's within *Russula* responded in different directions (data not shown), notably, *Russula vinosa* was one of the most nitrophobic, whereas *Russula griseascens* was one of the most nitrophilic of all OTU's. The EMF genus *Cortinarius* sp. accounted for a large fraction of the OTUs (101 out of the total 1051 OTU's), and accounted for 12.4 % of all reads in the control treatment. At the genera level, *Cortinarius* were consistently nitrophobic as their relative abundance decreased by 45 % with N addition, a decline that was primarily driven by *Cortinarius caperatus* even though several OTU's at lower abundance were more strongly negatively correlated to the N treatments, most notably *Cortinarius alpinus*, *Cortinarius brunneifolius*, and *Cortinarius acutus*. The relative abundance of *Tylospora*, belonging to Atheliaceae, doubled in the 50 N treatment compared to the control. However, different *Piloderma* species, also belonging to Atheliaceae, usually declined with N treatment and *Piloderma bicolor* was among the most nitrophobic OTUs.

Using SIMPER analysis, the responsive fungal species were identified that contributed the most to dissimilarity between 0 N and 50 N samples (Table S3). Among the responsive species that cumulatively contributed to 30 % difference between N treatments, N increased the abundance of *Tylospora fibrillosa*, *Piloderma byssinum* and *Russula griseascens*, while *Oidiodendron pilicola*, *Cenococcum geophilum*, *Piloderma sphaerosporum* and *Cortinarius caperatus* decreased (Fig. 3A). Based on PERMANOVA analysis, the humus community also changed significantly over the season (Table 1), with the basidiomycetes *Tylospora fibrillosa*, *Cortinarius caperatus*, and *Piloderma sphaerosporum* increasing, and the ascomycetes *Oidiodendron pilicola*, *Cenococcum geophilum*, and *Pezoloma ericae* decreasing in the autumn (Table S4).

The root community was significantly different due to N treatment according to PERMANOVA (Table 2). SIMPER analysis identified taxa that contributed most to the differences between the 0 N and 50 N treatments (Table S5). For roots, the most responsive species that contributed to 40 % cumulative contribution of differences, N addition led to higher abundance of *Phialocephala fortinii*, *Piloderma byssinum*, *Piloderma ericae*, and *Meliniomyces variabilis*, and lower of *Meliniomyces bicolor*, *Piloderma sphaerosporum* and *Cenococcum geophilum* (Fig. 3B). Using PERMANOVA, season had no significant effect on the root community (Table 2), however based on SIMPER analysis the abundance of *Meliniomyces variabilis* and *Piloderma byssinum* increased in autumn, while abundance of *Leullia* sp., *Cenococcum geophilum* and *Piloderma sphaerosporum* decreased (Table S6).

Table 1
The effect of nitrogen treatment (0, 12.5, and 50 kg N ha^{-1} yr^{-1} × 20 years), season (summer vs. autumn), and their interaction on PLFA and sequenced microbial community composition assessed with permutational (9999) analysis of variances (PERMANOVA) testing the null-hypothesis that the community composition has the same centroid in multivariate space. The variation within groups did not differ significantly according to permutational test for homogeneity of multivariate dispersion (data not shown).

	Nitrogen treatment		Season		Nitrogen × season	
	Pseudo F-value	p-Value	Pseudo F-value	p-Value	Pseudo F-value	p-Value
PLFA						
Humus	21.428	**0.001**	1.642	0.188	0.138	0.976
Fungal community						
Total	7.075	**0.001**	0.956	0.364	0.462	0.923
Humus	7.373	**0.001**	1.799	**0.020**	0.612	0.879
Root	7.791	**0.001**	1.106	0.203	0.821	0.511

Significant effects ($p<0.05$) are highlighted in bold font.

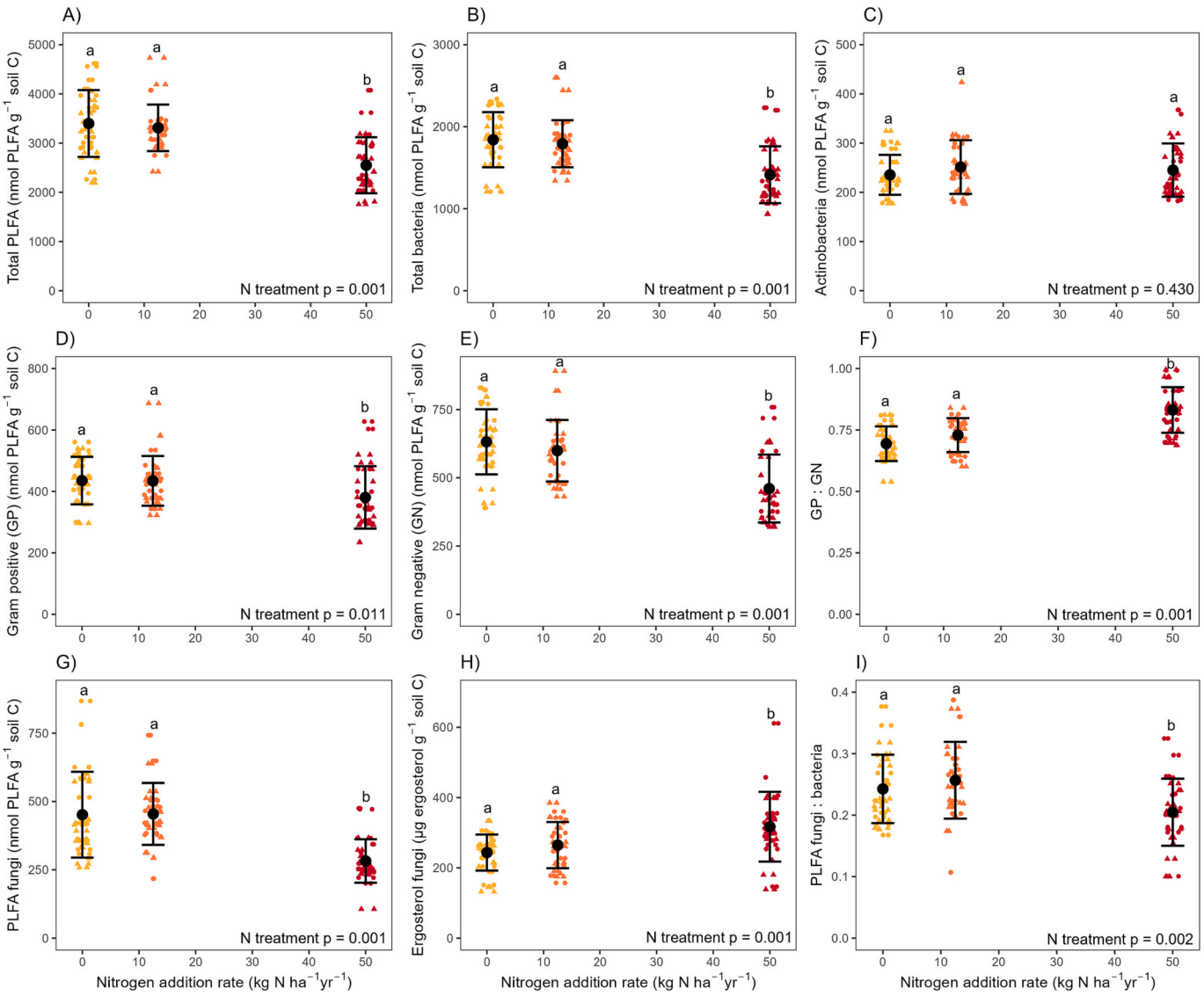

Fig. 1. Composition of the microbial community in the organic layer of a boreal forest soil sampled during summer (triangle shape) and autumn (circle shape) after 20 years of annual nitrogen addition at three levels (0, 12.5, and 50 kg N ha^{-1} yr^{-1}) based on phospholipid fatty acid (PLFA) markers and free ergosterol analysis. Black circles represent the means and the error bars represent standard deviation, the N treatment *p*-values are linear mixed effects models values (Table 1) and different letters represent a statistically significant difference based on Tukey's HSD.

3.3. Enzyme activity

The highest N addition rate (50 N) increased the activity of endoglucanase and xylosidase (β-1.4-xylosidase), decreased the activity of peptidase (Leucine aminopeptidase) and peroxidase (Manganese(II)-peroxidase), and nearly significantly ($p < 0.06$) decreased chitinase (β-1.4-*N*-acetylglucosaminidase), whereas glucosidase (β-1.4-glucosidase), phosphatase (acid-phosphatase), and laccase were unaffected (Fig. 4, Table 3). No significant differences in enzyme activities were detected between the control and the 12.5 N treatment (Fig. 4). The enzyme activity was higher in the autumn for glucosidase and peptidase, whereas the activity of phosphatase, xylosidase, chitinase, endoglucanase, peroxidase, and laccase remained constant (Fig. 4, Table 3). No interactions between N and season were detected (Table 3).

3.4. Associations between enzyme activities and humus fungal communities

Looking at association between enzyme activities and fungal species most responsive to N addition based on SIMPER analysis (Fig. 5), laccase was positively correlated with *Tylospora fibrillosa*, *Apiotrichum xylopini* and *Russula griseascens* and negatively with *Helotiales* sp. and *Piloderma sphaerosporum*. Peroxidase was positively correlated with *Oidiodendron pilicola* and *Helotiales* sp. and negatively with *Pezoloma ericae* (Fig. 5). Both chitinase and peptidase were positively correlated with *Piloderma bicolor* and peptidase was negatively correlated with *Pezoloma ericae* (Fig. 5). Phosphatase was negatively correlated with *Pezoloma ericae* (Fig. 5). *Cortinarius caperatus* was not significantly correlated with oxidative enzymes in our study (Fig. 5). In general, fungal species more abundant after N addition were positively correlated with C and P acquiring enzymes and laccase, while negatively with N acquiring enzymes (Fig. 5). Contrasting, fungal species declining with added N were positively correlated with N acquiring enzymes and peroxidase and mostly negatively with laccase, phosphatase and C acquiring enzymes (Fig. 5).

4. Discussion

The main aim of this study was to investigate a key mechanism by which N deposition enhances soil C accumulation in boreal forest, which

Table 2
The effect of nitrogen treatment (0, 12.5, and 50 kg N ha^{-1} yr^{-1} × 20 years), season (summer vs. autumn), and their interaction on aggregated microbial markers in the organic soil layer based on either Bligh & Dyer PLFA or ergosterol analysis, or the relative abundance of selected groups in the sequenced fungal community, assessed by linear mixed effects models with block (n = 12) as random factor.

	Nitrogen treatment		Season		Nitrogen × season	
	F-value	p-Value	F-value	p-Value	F-value	p-Value
Free ergosterol	8.116	**0.001**	1.703	0.197	0.533	0.590
PLFA						
Total	19.288	**0.001**	1.684	0.200	0.395	0.676
Fungi	18.884	**0.001**	3.719	0.059	0.215	0.807
Fungi:bacteria	6.731	**0.002**	2.412	0.126	0.132	0.877
Total bacteria	16.324	**0.001**	1.293	0.260	0.520	0.597
Gram positive (GP)	4.954	**0.011**	1.455	0.233	0.936	0.398
Gram negative (GN)	16.248	**0.001**	0.584	0.448	0.176	0.839
GP:GN	24.415	**0.001**	0.251	0.618	1.020	0.367
Actinobacteria	0.857	0.430	0.194	0.661	0.407	0.668
Fungal community						
Basidiomycetes: Ascomycetes	0.609	0.547	10.469	**0.002**	1.954	0.151
Ascomycetes	0.137	0.872	29.733	**0.001**	2.577	0.082
Basidiomycetes	0.154	0.858	28.895	**0.001**	2.602	0.083
Agaricomycetes	0.169	0.845	14.850	**0.001**	1.153	0.323
Russula	4.612	**0.014**	3.555	0.065	0.454	0.638
Atheliaceae	3.446	**0.039**	10.321	**0.002**	0.279	0.758
Cortinarius	9.914	**0.001**	8.414	**0.005**	1.281	0.286
Ectomycorrhiza	0.925	0.402	12.117	**0.001**	0.806	0.452

Significant effects ($p<0.05$) are highlighted in bold font.

we described in a previous study at this experimental site (Maaroufi et al., 2015). Specifically, we focused on the effect of N enrichment on microbial community composition and their associated enzyme activities using a long-term (20 years) N addition experiment. Such data are needed to explain the reduction in soil respiration and decomposition, and increase in soil C stocks that are frequently reported in boreal forests in response to external N enrichment (Blaško et al., 2022; de Vries et al., 2014; Forsmark et al., 2020a; Hyvönen et al., 2008; Janssens et al., 2010; Knorr et al., 2005).

Consistent with our first hypothesis, we found a clear shift in the microbial community composition based on PLFA and ergosterol data (Fig. 1, Tables 1 and 2). Similar to previous studies in the same experimental plots (Maaroufi et al., 2015), and a similar experiment in a Scots pine forest (Maaroufi et al., 2019), we found a clear decrease of 25 % in total PLFA between the control and 50 N treatment, but not between control and 12.5 N treatment. This effect was mainly driven by a decrease in fungal and gram-negative bacterial PLFA's, whereas the abundance of gram-positive bacterial and actinobacterial PLFA's were generally tolerant to changes in N, and in some cases even increased. Nitrogen addition significantly decreased fungal PLFA marker 18:2ω6 in the 50 N treatment but had no significant effect in the 12.5 N treatment (Fig. 1, Table 2). A reduction in fungal abundance is consistent with many studies (Fog, 1988; Janssens et al., 2010; Treseder, 2008) and it has been linked to reductions in EMF mycelial growth (Nilsson and Wallander, 2003). The abundance of this marker also showed a nearly significant ($p < 0.06$) increase by 14 %, from summer to autumn sampling which corresponds to a time of the year when the allocation of C below-ground is high (Högberg et al., 2010). Our second biomarker for fungal abundance, ergosterol, indicated a contrasting response, as this marker increased with N addition in the 50 N treatment, and was equally abundant in summer and autumn. Similar discrepancies between the two markers have been previously reported in boreal forests (Blaško et al., 2022; Kyaschenko et al., 2017b). Fungal biomass is a major pool of organic N in boreal forests, and as ergosterol is a relatively more persistent biomarker than PLFA, it may accumulate to a greater degree in soils when decomposition is disrupted, or other ecosystem perturbations occur (Clemmensen et al., 2013; Högberg, 2006; Zhao et al., 2005). For example, reductions in use of N from dead fungal necromass in response to N addition could be contributing to differences in the abundance of these two markers. Thus, the reduction in PLFA marker 18:2ω6 and accumulation of ergosterol due to N addition is likely caused by PLFA responding more strongly to a reduction in standing fungal biomass, whereas the relatively more stable ergosterol is likely responding more to the reduction in decomposition of dead fungal biomass.

DNA sequence data enabled a detailed analysis of the shifts in the fungal community composition of both humus and roots (Table 2, Fig. 2). Consistent with our hypothesis, predicting a shift from a dominance of *Cortinarius* and *Piloderma* to *Tylospora* and *Russula*, we found that the relative abundance of *Cortinarius* and *Piloderma* was 35 % and 45 % lower, respectively, in the humus samples in the 50 N treatment compared to the control. Considering that these genera together comprised one quarter of the fungal reads in the autumn, the decrease in these genera is likely to have contributed substantially to the reduction in the fungal PLFA marker 18:2ω6 we observed in response to N. Additionally, as hypothesized, the EMF genera *Russula* and *Tylospora* increased with N addition. These responses are not unique to our study system but seems to be a common response to increased availability of inorganic N (Haas et al., 2018; Jörgensen et al., 2021; Marupakula et al., 2021), and the adaptations of these taxa and the activation of specific EMF under different levels of N availability is likely to play a key role in both nutrient acquisition and C cycling. Taken together, our characterization of the fungal community revealed that the N addition not only reduced the total abundance of PLFA marker 18:2ω6 but also strongly restructured the fungal community from a dominance of EMF taxa known to be involved in organic N cycling towards taxa without the capacity to grow on organic N sources (Lilleskov et al., 2019).

For our second hypothesis, we predicted that changes in microbial community composition would correspond with shifts in soil enzyme activities. Particularly by decreasing activity of enzymes involved in organic N uptake and lignin decomposition after N addition, and increasing activity of P acquisition. Consistent with our hypothesis, the highest N addition rate markedly decreased the activity of peptidases and peroxidase, and nearly significantly decreased the activity of chitinase (Fig. 4). This decreased activity between control and 50 N for the N acquiring enzymes, peptidase and chitinase, indicates a reduction in the breakdown of microbial necromass. This decrease can indicate some combination of down-regulation of resorption of N in microbial necromass when the supply of mineral N is high, and that microbial biomass or necromass is less abundant. Based on the correlation analysis (Fig. 5), chitinase and peptidase were positively correlated with fungal species whose abundance decreased with N addition. This indicates that a reduction in abundance of species like *Cortinarius* and *Piloderma*, which are known for organic N acquisition (Lilleskov et al., 2011), is probably connected to the observed decrease in activity of N acquiring enzymes. The phosphatase activity, which we hypothesized to increase due to induced relative demand for P (Almeida et al., 2019; Vitousek et al., 2010), did not change after N addition. This observation corroborates the view that anthropogenic N deposition is unlikely to induce P limitations in boreal forests on minerogenic parent material (Forsmark et al., 2020b).

Mn-peroxidase plays a major role in mineralization of complex compounds such as lignin and other phenolics (Baldrian and Stursova, 2011; Sinsabaugh, 2010), and has been proposed as a mechanism to mineralize N when N is predominantly bound in recalcitrant organic matter (Bödeker et al., 2014). The observed decrease in its activity due to higher N availability observed in this and other studies (Moore et al., 2021), is likely to contribute to the decline in soil CO_2 efflux reported in this experiment (Maaroufi et al., 2015), and many others (Blaško et al., 2022; Forsmark et al., 2020a; Janssens et al., 2010), by decreasing extracellular oxidation of C, in addition to potential decreases in cellular

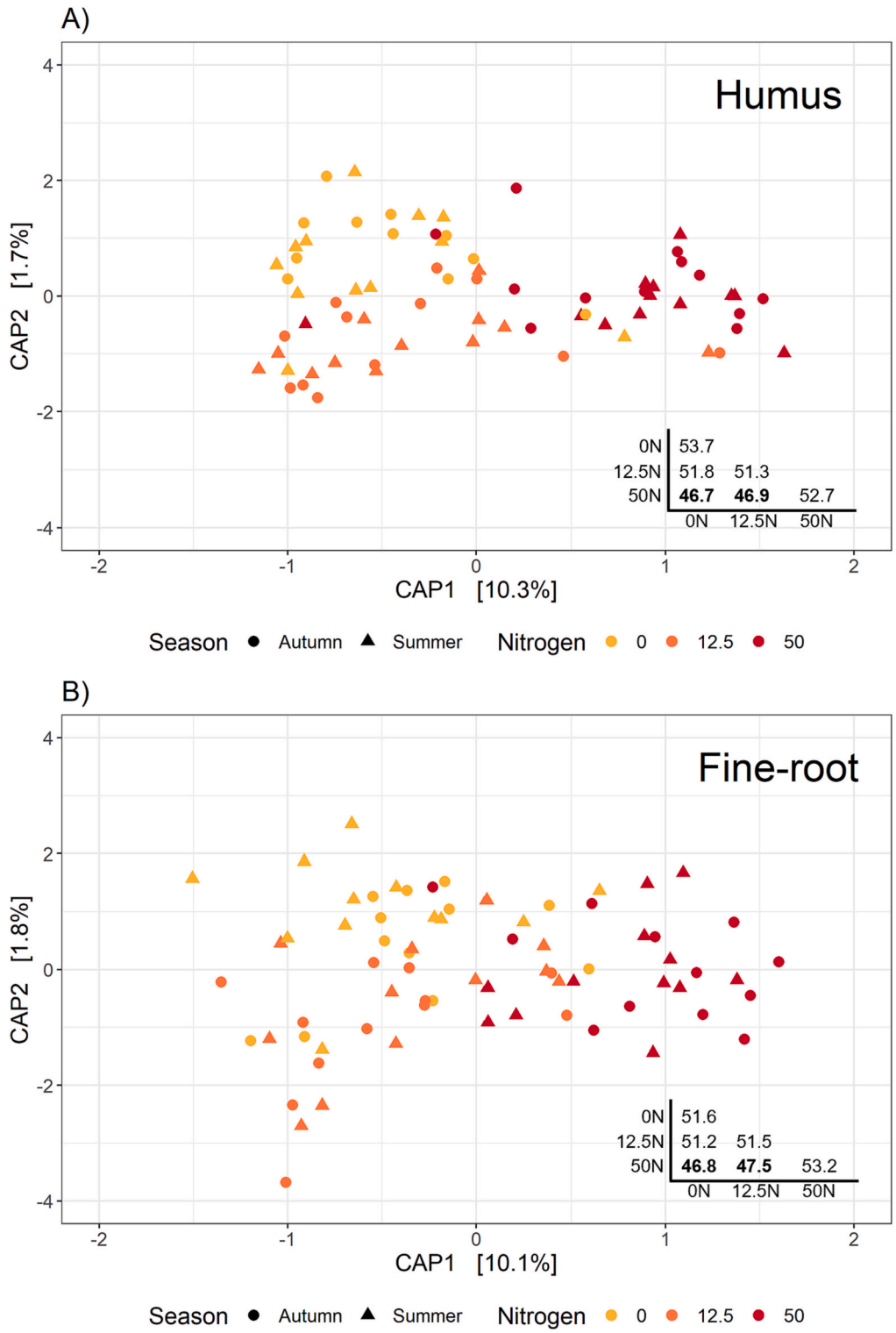

Fig. 2. Canonical analysis of principal coordinates (CAP) of fungal communities in (A) humus and (B) fine-root constrained with nitrogen addition rate (0, 12.5, and 50 kg N ha^{-1} yr^{-1} × 20 years) and season (summer (triangle shape) and autumn (circle shape)). Insert tables show percentage similarity in community composition within and between nitrogen treatments, with significant differences according to PERMANOVA in bold.

respiration due to lower microbial biomass and higher C use efficiencies. Peroxidase, like other N acquiring enzymes, was positively correlated with fungal species that are less abundant in high N environments. *Cortinarius caperatus*, which has previously been reported to be correlated with peroxidases (Bödeker et al., 2014; Lindahl et al., 2021), was not significantly correlated with peroxidase in our study, which could be due to site-specific differences. Laccase, which is an oxidative enzyme also responsible for lignin mineralization (Thurston, 1994), was unresponsive to N addition and in contrast to peroxidase was positively correlated with fungal species that increased after N addition. However, laccases are known to have a wide range of substrates in addition to lignin (Thurston, 1994), which could potentially be the reason for the difference between the observed laccase and peroxidase activity. The fungal community shift with a decrease in fungal species known for

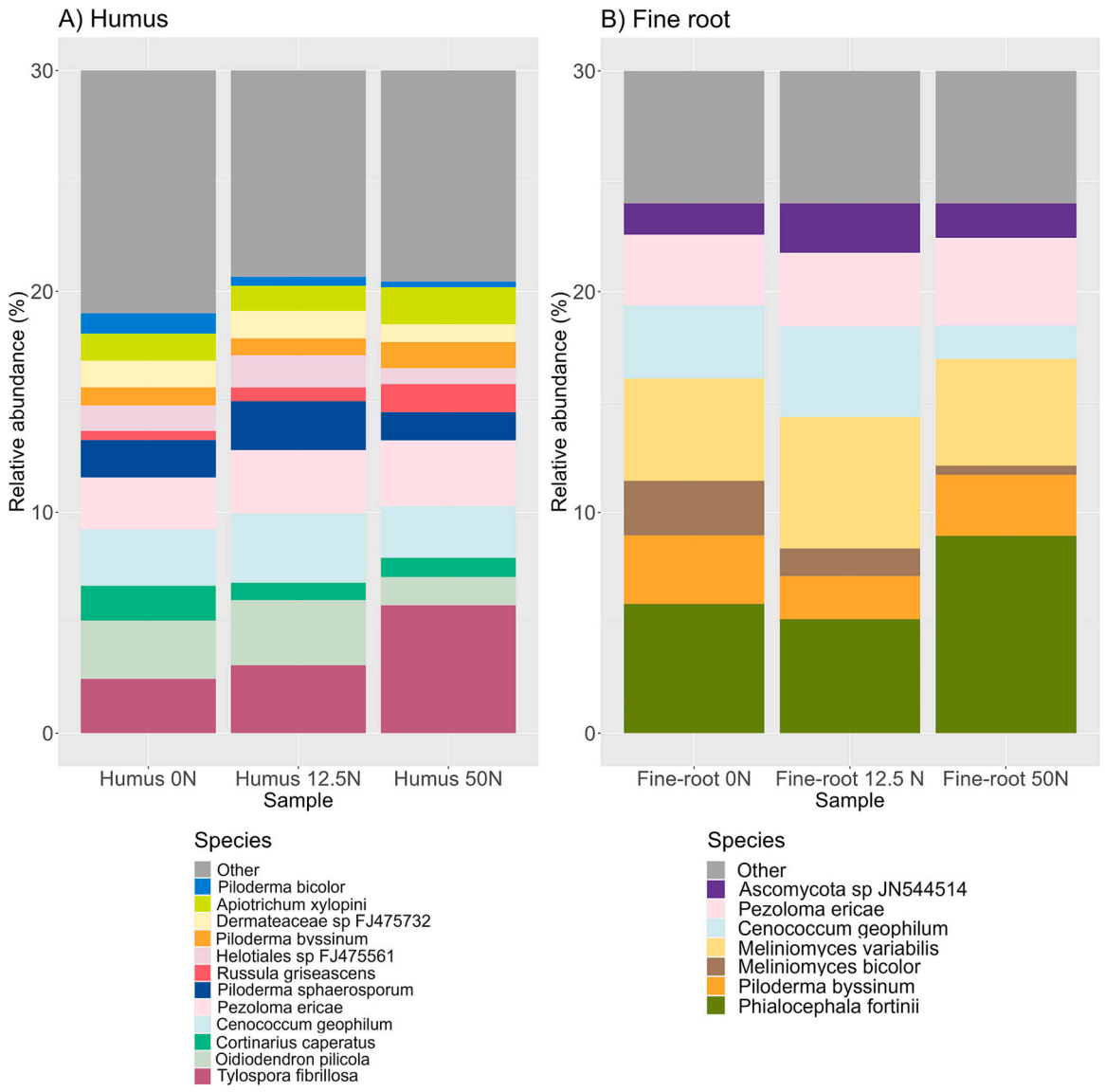

Fig. 3. The relative abundance (%) of responsive fungal species based on SIMPER analysis in (A) humus (up to 30 % cumulative contribution to differences) and (B) fine-root (up to 40 % cumulative contribution to differences) samples collected during summer and autumn on plots with annual N addition at three rates (0, 12.5, and 50 kg N ha^{-1} yr^{-1}). Species are listed by their contribution to differences based on SIMPER in increasing order, each value is the average of two sampling occasions and note that the y-axis is broken at 30 % cumulative read abundance.

organic N acquisition corresponds with a general decrease in extracellular catabolic activity associated with organic N uptake and this decrease likely reflects an increase in labile inorganic N supplied in the experimental treatments leading to reduced incentives to mine recalcitrant organic matter for organic N.

In addition to the general decrease in enzymes involved in degradation and uptake of N in recalcitrant organic matter, N also enhanced the activity of hydrolytic enzymes involved in labile C uptake (Fig. 4). The abundance of labile C in soil organic matter is minor compared to the large pool of recalcitrant fractions, and the upregulation of these enzymes is not likely to lead to any significant soil C loss, compared to the oxidative enzymes that can destabilize lignin complexes. The response may, however, shed some light on the underlying mechanism driving C accumulation in response to N enrichment (de Vries et al., 2014; Forsmark et al., 2020a; Maaroufi et al., 2015). Carbon acquiring enzymes, including glucosidase, endoglucanase and xylosidase were positively correlated with fungal species whose abundance increased after N addition, and negatively with species whose abundance decreased. Previous work in our study system has shown that N addition increases the production of plant biomass above and below ground (Forsmark et al., 2020a; From et al., 2016; Maaroufi et al., 2016), while reduces soil respiration (Forsmark et al., 2020a; Maaroufi et al., 2015) and decomposition (Forsmark et al., 2021; Maaroufi et al., 2016). In light of these previous studies, the upregulation of enzymes involved in the acquisition of labile C from soil organic matter appears to be linked to a major shift in C use in response to N enrichment, where less C is supplied to EMF and other microbes to stimulate depolymerization of soil organic matter for N acquisition (Allison et al., 2010; Janssens et al., 2010; Soong et al., 2020), and more C is used to build biomass (Campioli et al., 2015; Forsmark et al., 2021; Moorhead and Sinsabaugh, 2006; Spohn et al., 2016), eventually entering the soil organic matter pool as root and microbial litter.

5. Conclusions

Our study has several implications for understanding the impact of N deposition on soil C stocks. Firstly, our study clearly shows that N deposition can drive large shifts in the soil fungal community and their

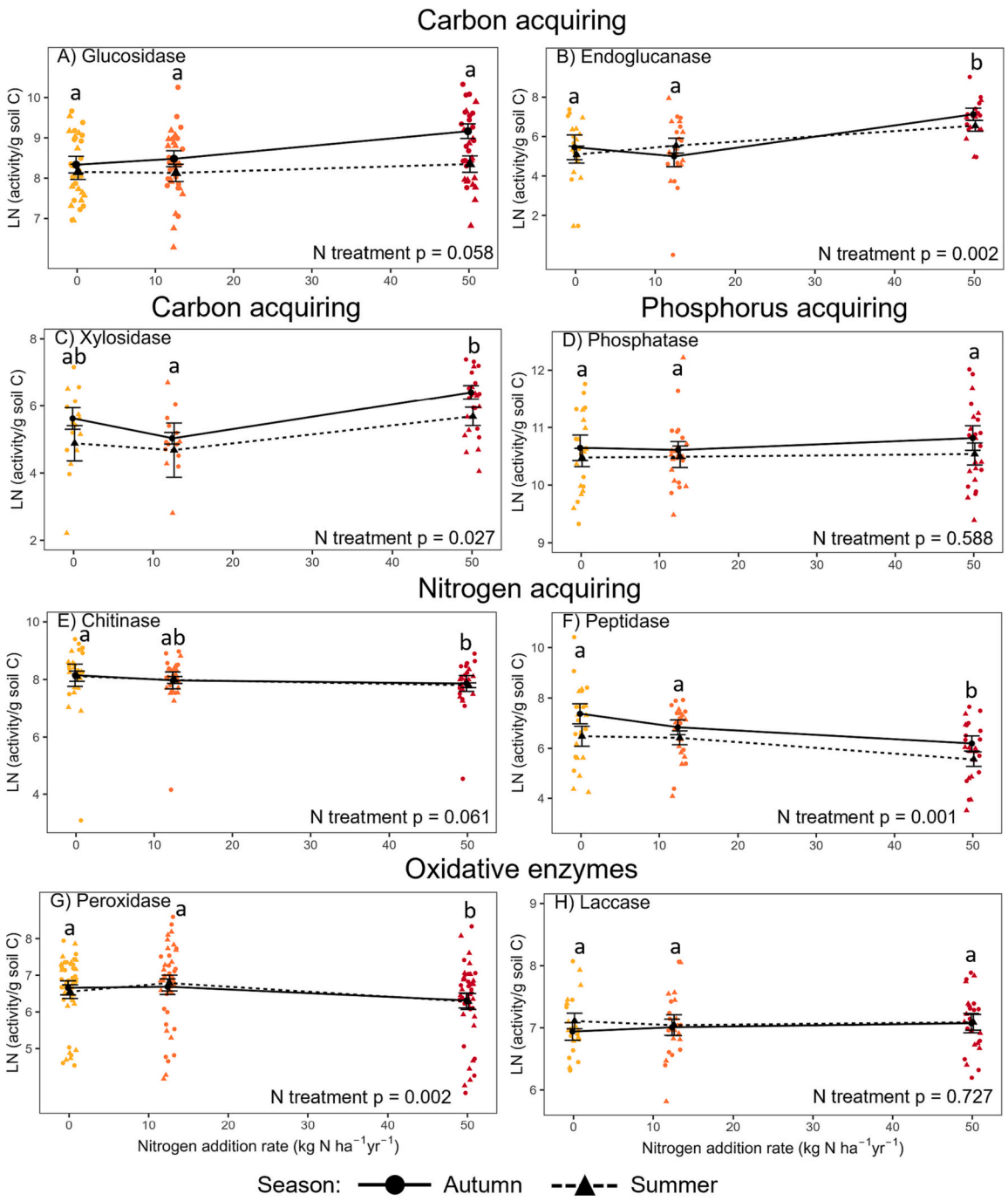

Fig. 4. Interaction plot of potential enzyme activity across three levels of nitrogen addition (0, 12.5, and 50 kg N ha^{-1} yr^{-1} × 20 years) at summer (triangle shape) and autumn (circle shape). Values are the natural logarithm of molar cleavage of enzyme specific substrates standardized to the amount of carbon in each sample (molar substrate cleavage h^{-1} g C^{-1}). Black dots represent the mean and the error bars represents standard errors of the mean (n = 12), while the N treatment p-values are values from linear mixed effects models (Table 3) and different letters represent statistical difference based on Tukey's HSD.

associated enzyme activities. In our 20 year-long experimental simulation of N deposition, these effects were most clearly pronounced for the high N addition treatment. For the low N addition treatment, corresponding with upper N deposition rates in the boreal region, no effects were detected, suggesting that very high rates of N deposition are required to drive significant changes in soil functioning. Secondly, the response of the microbial community was predictable, as the turnover of the fungal community was associated with a shift from EMF taxa capable of growing on pure organic N sources to taxa without this capability in N enriched treatments. This shift was associated with down-regulation of enzymes involved in degradation of complex organic structures and the uptake of peptides, and up-regulation of enzymes involved in uptake of simple carbohydrates. Thirdly, our results are consistent with theoretical modeling frameworks focusing on the energy demand of organic N uptake (Chen et al., 2014; Craine et al., 2007; Orwin et al., 2011), and on the role of element stoichiometry in regulating the loss of C through respiration (Chen et al., 2014; Liang et al., 2017; Luo et al., 2016). Understanding the mechanisms and functional aspects of anthropogenic N deposition effects on the changes in soil C stocks, which can have a strong effect on the global C cycle (Lal, 2005), is extremely important for a better understanding the effect of N supply on the global C cycle, especially considering a changing climate (Gruber and Galloway, 2008).

Table 3
The effect of nitrogen treatment, season, and their interaction on the natural logarithm of the potential activity (molar substrate cleavage h^{-1} g C^{-1}) of soil enzymes evaluated by linear mixed models with block (n = 12) defined as random factor.

Enzyme	Nitrogen treatment			Season			Nitrogen × season	
	Direction	F-value	p-Value	Sign	F-value	p-Value	F-value	p-Value
Glucosidase		3.000	0.058	+	11.026	**0.002**	1.632	0.205
Endoglucanase	+	7.124	**0.002**		0.251	0.619	1.405	0.256
Xylosidase	+	4.096	**0.027**		3.538	0.070	0.197	0.822
Phosphatase		0.536	0.588		2.875	0.096	0.177	0.838
Chitinase		2.951	0.061		1.965	0.167	0.003	0.997
Peptidase	−	17.966	**0.001**	+	18.859	**0.001**	0.659	0.521
Peroxidase	−	6.945	**0.002**		0.012	0.912	0.371	0.692
Laccase		0.321	0.727		1.196	0.279	0.533	0.590

Significant effects (p<0.05) are highlighted in bold font.

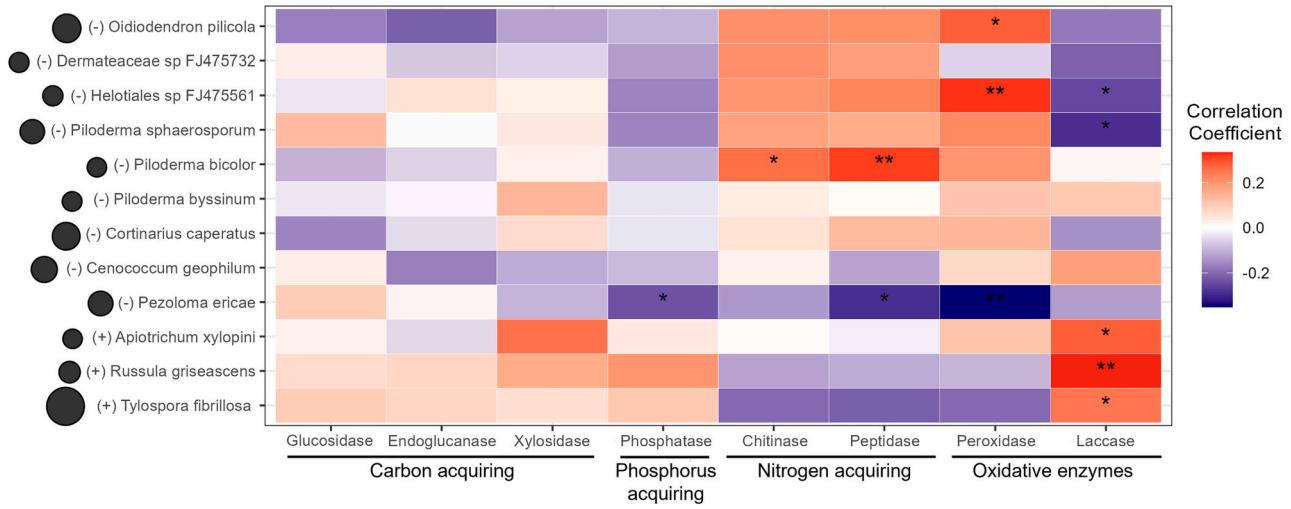

Fig. 5. Heatmap portraying Spearman's correlation coefficient between enzyme activity for carbon, phosphorus and nitrogen acquiring enzymes and oxidative enzymes and fungal species identified by SIMPER analysis to contribute to 30 % cumulative contribution. The circles by fungal species represent the individual species cumulative contribution to differences between 0 and 50 N samples according to SIMPER analysis and their sign indicates if their abundance decreased (−) or increased (+) with nitrogen addition. The statistically significant p-values are portrayed by stars (* represents $p < 0.05$, and ** $p < 0.01$).

The agreement of the data from our detailed profiling of the microbial community composition and metabolism with these modeling frameworks can therefore enable more precise predictions of the future soil C balance (Averill and Waring, 2017; Luo et al., 2016; Stocker et al., 2016; Terrer et al., 2021).

CRediT authorship contribution statement

Benjamin Forsmark: Conceptualization, Formal analysis, Investigation, Visualization, Writing – original draft. **Tinkara Bizjak:** Formal analysis, Investigation, Visualization, Writing – original draft. **Annika Nordin:** Conceptualization, Supervision, Writing – review & editing. **Nicholas P. Rosenstock:** Conceptualization, Formal analysis, Investigation, Writing – review & editing. **Håkan Wallander:** Conceptualization, Writing – review & editing. **Michael J. Gundale:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

AN declares employment with Stora Enso, and BF with the forestry cooperative Södra Skogsägarna. TB, NPR, HW and MJG declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are openly available on the SafeDeposit at the Swedish University of Agriculture server http://www.safedeposit.se/, reference ID: 425.

Acknowledgements

The study was funded by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS) and the Knut and Alice Wallenberg Foundation. The field experiment and support for our measurements were provided by the Swedish infrastructure for terrestrial ecosystem sciences (SITES) and the Unit for Field-based Research (ESF). We are thankful to Nadia Binte Obaid for creating the graphical abstract using BioRender.com and Erik Lilleskov, Mark Bonner, and Scott Buckley for providing valuable feedback on an early version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.170741.

References

Allison, S.D., 2012. Fluorimetric and Oxidative Enzyme Assay Protocol.

Allison, S., Weintraub, M., Gartner, T., Waldrop, M., 2010. Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function. In: Shukla, G., Varma, A. (Eds.), Soil Enzymology. 22. Springer-Verlag, Berlin Heidelberg.
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 Ecol. 40, 20–31.
Argiroff, W.A., Zak, D.R., Pellitier, P.T., Upchurch, R.A., Belke, J.P., 2022. Decay by ectomycorrhizal fungi couples soil organic matter to nitrogen availability. Ecol. Lett. 25, 391–404.
Averill, C., Waring, B., 2017. Nitrogen limitation of decomposition and decay: how can it occur? Glob. Chang. Biol. 24, 1417–1427.
Baldrian, P., 2009. Microbial enzyme-catalyzed processes in soils and their analysis. Plant Soil Environ. 55, 370–378.
Baldrian, P., Stursova, M., 2011. Enzymes in forest soils. In: Shukla, G., Varma, A. (Eds.), Soil Enzymology. 22. Springer-Verlag, Berlin, Berlin, pp. 61–73.
Bending, G.D., Read, D.J., 1995. The structure and function of the vegetative mycelium of ectomycorrhizal plants. V. Foraging behaviour and translocation of nutrients from exploited litter. New Phytol. 130, 401–409.
Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., Wit, P., Sanchez-García, M., Ebersberger, I., Sousa, F., Amend, A., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for use in environmental sequencing. Methods Ecol. Evol. 4, 914–919.
Berg, B., 2014. Decomposition patterns for foliar litter - a theory for influencing factors. Soil Biol. Biochem. 78, 222–232.
Blaško, R., Forsmark, B., Gundale, M.J., Lim, H., Lundmark, T., Nordin, A., 2022. The carbon sequestration response of aboveground biomass and soils to nutrient enrichment in boreal forests depends on baseline site productivity. Sci. Total Environ. 838, 156327.
Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37.
Bödeker, I.T.M., Clemmensen, K.E., de Boer, W., Martin, F., Olson, A., Lindahl, B.D., 2014. Ectomycorrhizal Cortinarius species participate in enzymatic oxidation of humus in northern forest ecosystems. New Phytol. 203, 245–256.
Bödeker, I.T.M., Lindahl, B.D., Olson, A., Clemmensen, K.E., 2016. Mycorrhizal and saprotrophic fungal guilds compete for the same organic substrates but affect decomposition differently. Funct. Ecol. 30, 1967–1978.
Campioli, M., Vicca, S., Luyssaert, S., Bilcke, J., Ceschia, E., Chapin Iii, F.S., et al., 2015. Biomass production efficiency controlled by management in temperate and boreal ecosystems. Nat. Geosci. 8, 843–846.
Chen, R.R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X.G., et al., 2014. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. Glob. Chang. Biol. 20, 2356–2367.
Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., et al., 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science 339, 1615–1618.
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. Ecol. Lett. 24, 1193–1204.
Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105–2113.
Crowther, T.W., Jvd, Hoogen, Wan, J., Mayes, M.A., Keiser, A.D., Mo, L., et al., 2019. The global soil community and its influence on biogeochemistry. Science 365.
Dahlberg, A., Jonsson, L., Nylund, J.E., 1997. Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old-growth Norway spruce forest in south Sweden. Canadian Journal of Botany-Revue Canadienne De Botanique 75, 1323–1335.
de Vries, W., Du, E.Z., Butterbach-Bahl, K., 2014. Short and long-term impacts of nitrogen deposition on carbon sequestration by forest ecosystems. Curr. Opin. Environ. Sustain. 9-10, 90–104.
Deluca, T.H., Boisvenue, C., 2012. Boreal forest soil carbon: distribution, function and modelling. Forestry 85, 161–184.
Dixon, P., 2003. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14, 927–930.
Fernandez, C.W., Kennedy, P.G., 2016. Revisiting the 'Gadgil effect': do interguild fungal interactions control carbon cycling in forest soils? New Phytol. 209, 1382–1394.
Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R.A., Henrissat, B., et al., 2012. The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336, 1715–1719.
Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biol. Rev. Camb. Philos. Soc. 63, 433–462.
Forsmark, B., Nordin, A., Maaroufi, N.I., Lundmark, T., Gundale, M.J., 2020a. Low and high nitrogen deposition rates in northern coniferous forests have different impacts on aboveground litter production, soil respiration, and soil carbon stocks. Ecosystems 23, 1423–1436.
Forsmark, B., Wallander, H., Nordin, A., Gundale, M.J., Stevens, C., 2020b. Long-term nitrogen enrichment does not increase microbial phosphorus mobilization in a northern coniferous forest. Funct. Ecol. 35, 277–287.
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 Biol. Biochem. 155.
From, F., Lundmark, T., Mörling, T., Pommerening, A., Nordin, A., 2016. Effects of simulated long-term N deposition on Picea abies and Pinus sylvestris growth in boreal forest. Can. J. For. Res. 46, 1396–1403.
Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fertil. Soils 22, 59–65.
Frostegård, A., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biol. Biochem. 43, 1621–1625.
Gadgil, R.L., Gadgil, P.D., 1971. Mycorrhiza and litter decomposition. Nature 233, 133.
Gadgil, R., Gadgil, P., 1975. Suppression of litter decomposition by mycorrhizal roots of Pinus radiata. N. Z. J. For. Sci. 35–41.
Gardes, M., Bruns, T.D., 1993. ITS Primers with enhanced specificity for Basidiomycetes application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118.
Gottel, N.R., Castro, H.F., Kerley, M., Yang, Z., Pelletier, D.A., Podar, M., et al., 2011. Distinct microbial communities within the endosphere and rhizosphere of Populus deltoides roots across contrasting soil types. Appl. Environ. Microbiol. 77, 5934–5944.
Gruber, N., Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen cycle. Nature 451, 293–296.
Gundale, M.J., Deluca, T.H., Nordin, A., 2011. Bryophytes attenuate anthropogenic nitrogen inputs in boreal forests. Glob. Chang. Biol. 17, 2743–2753.
Gundale, M.J., From, F., Bach, L.H., Nordin, A., 2014. Anthropogenic nitrogen deposition in boreal forests has a minor impact on the global carbon cycle. Glob. Chang. Biol. 20, 276–286.
Gundale, M.J., Almeida, J.P., Wallander, H., Wardle, D.A., Kardol, P., Nilsson, M.C., et al., 2016. Differences in endophyte communities of introduced trees depend on the phylogenetic relatedness of the receiving forest. J. Ecol. 104, 1219–1232.
Haas, J.C., Street, N.R., Sjödin, A., Lee, N.M., Högberg, M.N., Näsholm, T., et al., 2018. Microbial community response to growing season and plant nutrient optimisation in a boreal Norway spruce forest. Soil Biol. Biochem. 125, 197–209.
Hao, X., Jiang, R., Chen, T., 2011. Clustering 16S rRNA for OTU prediction: a method of unsupervised Bayesian clustering. Bioinformatics 27, 611–618.
Hasselquist, N.J., Metcalfe, D.B., Högberg, P., 2012. Contrasting effects of low and high nitrogen additions on soil CO2 flux components and ectomycorrhizal fungal sporocarp production in a boreal forest. Glob. Chang. Biol. 18, 3596–3605.
Högberg MN. Discrepancies between ergosterol and the phospholipid fatty acid 18 : 2 omega 6,9 as biomarkers for fungi in boreal forest soils. Soil Biol. Biochem. 2006; 38: 3431–3435.
Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., et al., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789–792.
Högberg, M.N., Briones, M.J.I., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., et al., 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 Phytol. 187, 485–493.
Hyvönen, R., Persson, T., Andersson, S., Olsson, B., Ågren, G.I., Linder, S., 2008. Impact of long-term nitrogen addition on carbon stocks in trees and soils in northern Europe. Biogeochemistry 89, 121–137.
ICOS, 2023. https://www.icos-sweden.se/Svartberget.
Ihrmark, K., Bodeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., et al., 2012. New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82, 666–677.
Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., et al., 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nat. Geosci. 3, 315–322.
Jörgensen, K., Granath, G., Strengbom, J., Lindahl, B.D., 2021. Links between boreal forest management, soil fungal communities and below-ground carbon sequestration. Funct. Ecol. 36, 392–405.
Kaiser, C., Koranda, M., Kitzler, B., Fuchslueger, L., Schnecker, J., Schweiger, P., et al., 2010. Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. New Phytol. 187, 843–858.
Knorr, M., Frey, S.D., Curtis, P.S., 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86, 3252–3257.
Kohler, A., Kuo, A., Nagy, L.G., Morin, E., Barry, K.W., Buscot, F., et al., 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. Nat. Genet. 47, 410–415.
Koljalg, U., Larsson, K.H., Abarenkov, K., Nilsson, R.H., Alexander, I.J., Eberhardt, U., et al., 2005. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol. 166, 1063–1068.
Koljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., et al., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277.
Kuyper, T.W., 2017. Carbon and energy sources of mycorrhizal fungi: obligate symbionts or latent Saprotrophs. In: Johnson, N.C., Gehring, C., Jansa, J. (Eds.), Mycorrhizal Mediation of Soil - Fertility, Structure, and Carbon Storage. Elsevier, Amsterdam, Netherlands, pp. 357–374.
Kyaschenko, J., Clemmensen, K.E., Hagenbo, A., Karltun, E., Lindahl, B.D., 2017a. Shift in fungal communities and associated enzyme activities along an age gradient of managed Pinus sylvestris stands. ISME J. 11, 863–874.
Kyaschenko, J., Clemmensen, K.E., Karltun, E., Lindahl, B.D., 2017b. Below-ground organic matter accumulation along a boreal forest fertility gradient relates to guild interaction within fungal communities. Ecol. Lett. 20, 1546–1555.
Lal, R., 2005. Forest soils and carbon sequestration. For. Ecol. Manag. 220, 242–258.
Leppalammi-Kujansuu, J., Salemaa, M., Kleja, D.B., Linder, S., Helmisaari, H.-S., 2014. Fine root turnover and litter production of Norway spruce in a long-term temperature and nutrient manipulation experiment. Plant Soil 374, 73–88.
Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nat. Microbiol. 2, 17105.

Lilleskov, E.A., Hobbie, E.A., Horton, T.R., 2011. Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. Fungal Ecol. 4, 174–183.
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. Environ. Pollut. 246, 148–162.
Lindahl, B.D., Tunlid, A., 2015. Ectomycorrhizal fungi - potential organic matter decomposers, yet not saprotrophs. New Phytol. 205, 1443–1447.
Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Hogberg, P., Stenlid, J., et al., 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytol. 173, 611–620.
Lindahl, B.D., Kyaschenko, J., Varenius, K., Clemmensen, K.E., Dahlberg, A., Karltun, E., et al., 2021. A group of ectomycorrhizal fungi restricts organic matter accumulation in boreal forest. Ecol. Lett. 24, 1341–1351.
Linder, S., 1995. Foliar analysis for detecting and correcting nutrient imbalances in Norway spruce. In: Staaf, H., Tyler, G. (Eds.), Ecological Bulletins; Effects of Acid Deposition and Tropospheric Ozone on Forest Ecosystems in Sweden, vol. 44. Munksgaard {a}, 35 Norre Sogade, DK 1370 Copenhagen, Denmark, pp. 178–190.
Liu, X.J., Zhang, Y., Han, W.X., Tang, A.H., Shen, J.L., Cui, Z.L., et al., 2013. Enhanced nitrogen deposition over China. Nature 494, 459–462.
Luo, Y.Q., Ahlstrom, A., Allison, S.D., Batjes, N.H., Brovkin, V., Carvalhais, N., et al., 2016. Toward more realistic projections of soil carbon dynamics by Earth system models. Glob. Biogeochem. Cycles 30, 40–56.
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. Glob. Chang. Biol. 21, 3169–3180.
Maaroufi, N.I., Nordin, A., Palmqvist, K., Gundale, M.J., 2016. Chronic nitrogen deposition has a minor effect on the quantity and quality of aboveground litter in a boreal forest. PLoS One 11, e0162086.
Maaroufi, N.I., Nordin, A., Palmquist, K., Gundale, M.J., 2017. Nitrogen enrichment impacts on boreal litter decomposition are driven by changes in soil microbiota rather than litter quality. Sci. Rep. 7.
Maaroufi, N.I., Nordin, A., Palmqvist, K., Hasselquist, N.J., Forsmark, B., Rosenstock, N. P., et al., 2019. Anthropogenic nitrogen enrichment enhances soil carbon accumulation by impacting saprotrophs rather than ectomycorrhizal fungal activity. Glob. Chang. Biol. 25, 2900–2914.
Marupakula, S., Mahmood, S., Clemmensen, K.E., Jacobson, S., Hogbom, L., Finlay, R.D., 2021. Root associated fungi respond more strongly than rhizosphere soil fungi to N fertilization in a boreal forest. Sci. Total Environ. 766, 142597.
McIntosh, A.C.S., Macdonald, S.E., Gundale, M.J., 2012. Tree species versus regional controls on ecosystem properties and processes: an example using introduced Pinus contorta in Swedish boreal forests. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 42, 1228–1238.
McMurdie, P., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8 (4).
Moore, J.A.M., Anthony, M.A., Pec, G.J., Trocha, L.K., Trzebny, A., Geyer, K.M., et al., 2021. Fungal community structure and function shifts with atmospheric nitrogen deposition. Glob. Chang. Biol. 27, 1349–1364.
Moorhead, D.L., Sinsabaugh, R.L., 2006. A theoretical model of litter decay and microbial interaction. Ecol. Monogr. 76, 151–174.
Morgenstern, I., Klopman, S., Hibbett, D.S., 2008. Molecular evolution and diversity of lignin degrading heme peroxidases in the agaricomycetes. J. Mol. Evol. 66, 243–257.
Näsholm, T., Ekblad, A., Nordin, A., Giesler, R., Högberg, M., Högberg, P., 1998. Boreal forest plants take up organic nitrogen. Nature 392, 914–916.
Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., et al., 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248.
Nicolas, C., Martin-Bertelsen, T., Floudas, D., Bentzer, J., Smits, M., Johansson, T., et al., 2019. The soil organic matter decomposition mechanisms in ectomycorrhizal fungi are tuned for liberating soil organic nitrogen. ISME J. 13, 977–988.
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 Phytol. 158, 409–416.
Nohrstedt, H.Ö., Arnebrant, K., Bååth, E., Söderström, B., 1989. Changes in carbon content, respiration rate, ATP content, and microbial biomass in nitrogen-fertilized pine forest soils in Sweden. Can. J. For. Res. 19, 323–328.
Oksanen, J., 2015. Multivariate Analysis of Ecological Communities in R: vegan Tutorial.
Orwin, K.H., Kirschbaum, M.U., St John, M.G., Dickie, I.A., 2011. Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: a model-based assessment. Ecol. Lett. 14, 493–502.
Paul, E.A., 2015. Soil Microbiology, Ecology and Biochemistry. Academic Press.
Reay, D.S., Dentener, F., Smith, P., Grace, J., Feely, R.A., 2008. Global nitrogen deposition and carbon sinks. Nat. Geosci. 1, 430–437.
Rosling, A., Landeweert, R., Lindahl, B.D., Larsson, K.H., Kuyper, T.W., Taylor, A.F.S., et al., 2003. Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. New Phytol. 159, 775–783.
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 Biol. Biochem. 34, 1309–1315.
Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85, 591–602.
Schloss PD, Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W. Introducing mothur: open-source, platform-independent, community-sup- ported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 2009; 75.
Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., et al., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56.
Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem. 42, 391–404.
Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., et al., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. 11, 1252–1264.
SMHI. In: Institute SMaH, editor, 2019.
Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis. Great Britain Elsevier - Academic press.
Soong, J.L., Fuchslueger, L., Maranon-Jimenez, S., Torn, M.S., Janssens, I.A., Penuelas, J., et al., 2020. Microbial carbon limitation: the need for integrating microorganisms into our understanding of ecosystem carbon cycling. Glob. Chang. Biol. 26, 1953–1961.
Spohn, M., Pötsch, E.M., Eichorst, S.A., Woebken, D., Wanek, W., Richter, A., 2016. Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. Soil Biol. Biochem. 97, 168–175.
Stendahl, J., Berg, B., Lindahl, B.D., 2017. Manganese availability is negatively associated with carbon storage in northern coniferous forest humus layers. Sci. Rep. 7, 6.
Stocker, B.D., Prentice, I.C., Cornell, S.E., Davies-Barnard, T., Finzi, A.C., Franklin, O., et al., 2016. Terrestrial nitrogen cycling in Earth system models revisited. New Phytol. 210, 1165–1168.
Stuiver, B.M., Gundale, M.J., Wardle, D.A., Nilsson, M.C., 2015. Nitrogen fixation rates associated with the feather mosses Pleurozium schreberi and Hylocomium splendens during forest stand development following clear-cutting. For. Ecol. Manag. 347, 130–139.
Talbot, J.M., Allison, S.D., Treseder, K.K., 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Funct. Ecol. 22, 955–963.
Tarnocai, C., Canadell, J.G., Schuur, E.A.G., Kuhry, P., Mazhitova, G., Zimov, S., 2009. Soil organic carbon pools in the northern circumpolar permafrost region. Glob. Biogeochem. Cycles 23, 1–11.
Tedersoo, L., May, T.W., Smith, M.E., 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20, 217–263.
Templer, P.H., Mack, M.C., Chapin, F.S., Christenson, L.M., Compton, J.E., Crook, H.D., et al., 2012. Sinks for nitrogen inputs in terrestrial ecosystems: a meta-analysis of N-15 tracer field studies. Ecology 93, 1816–1829.
Terrer, C., Phillips, R.P., Hungate, B.A., Rosende, J., Pett-Ridge, J., Craig, M.E., et al., 2021. A trade-off between plant and soil carbon storage under elevated CO(2). Nature 591, 599–603.
Thomas, R.Q., Brookshire, E.N.J., Gerber, S., 2015. Nitrogen limitation on land: how can it occur in Earth system models? Glob. Chang. Biol. 21, 1777–1793.
Thurston, C.F., 1994. The structure and function of fungal laccases. Microbiology 140, 19–26.
Tipping, E., Davies, J.A.C., Henrys, P.A., Kirk, G.J.D., Lilly, A., Dragosits, U., et al., 2017. Long-term increases in soil carbon due to ecosystem fertilization by atmospheric nitrogen deposition demonstrated by regional-scale modelling and observations. Sci. Rep. 7, 11.
Todd-Brown, K.E.O., Hopkins, F.M., Kivlin, S.N., Talbot, J.M., Allison, S.D., 2012. A framework for representing microbial decomposition in coupled climate models. Biogeochemistry 109, 19–33.
Treseder, K.K., 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecol. Lett. 11, 1111–1120.
Vitousek, P.M., Porder, S., Houlton, B.Z., Chadwick, O.A., 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecol. Appl. 20, 5–15.
Wallander, H., Johansson, U., Sterkenburg, E., Brandström Durling, M., Lindahl, B.D., 2010. Production of ectomycorrhizal mycelium peaks during canopy closure in Norway spruce forests. New Phytol. 187, 1124–1134.
Wardle, D.A., Gundale, M.J., Jäderlund, A., Nilsson, M.-C., 2013. Decoupled long-term effects of nutrient enrichment on aboveground and belowground properties in subalpine tundra. Ecology 94, 904–919.
White, D.C., Nickels, J.S., King, J.D., Bobbie, R.J., 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40.
Zhao, X.R., Lin, Q., Brookes, P.C., 2005. Does soil ergosterol concentration provide a reliable estimate of soil fungal biomass? Soil Biol. Biochem. 37, 311–317.