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Research

Ectomycorrhizal fungi are more sensitive to high soil nitrogen levels in forests exposed to nitrogen deposition

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Summary

- Ectomycorrhizal fungi are essential for nitrogen (N) cycling in many temperate forests and responsive to anthropogenic N addition, which generally decreases host carbon (C) allocation to the fungi. In the boreal region, however, ectomycorrhizal fungal biomass has been found to correlate positively with soil N availability. Still, responses to anthropogenic N input, for instance through atmospheric deposition, are commonly negative.
- To elucidate whether variation in N supply affects ectomycorrhizal fungi differently depending on geographical context, we investigated ectomycorrhizal fungal communities along fertility gradients located in two nemo-boreal forest regions with similar ranges in soil N : C ratios and inorganic N availability but contrasting rates of N deposition.
- Ectomycorrhizal biomass and community composition remained relatively stable across the N gradient with low atmospheric N deposition, but biomass decreased and the community changed more drastically with increasing N availability in the gradient subjected to higher rates of N deposition. Moreover, potential activities of enzymes involved in ectomycorrhizal mobilisation of organic N decreased as N availability increased.
- In forests with low external input, we propose that stabilising feedbacks in tree-fungal interactions maintain ectomycorrhizal fungal biomass and communities even in N-rich soils. By contrast, anthropogenic N input seems to impair ectomycorrhizal functions.

Introduction

Ectomycorrhizal fungi play a pivotal role in nutrient cycling in many temperate forests, especially for the turnover of nitrogen (N), which is considered a central growth-limiting resource (Tamm, 1991; Högberg *et al.*, 2017). By investing photosynthetic carbon (C) in symbiotic ectomycorrhizal fungi, trees gain access to both inorganic and organic N that the fungi absorb from the soil solution or mine from solid organic matter. Because of this tight link to N cycling, and consequently to forest productivity, it is essential to obtain a better understanding of how ectomycorrhizal fungal biomass, activity and community composition respond to variation in N availability. Increased N availability can have a direct, positive effect on mycelial growth if ectomycorrhizal fungi are N-limited (Högberg *et al.*, 2021), while a direct, negative growth response is possible if C-limited fungi have to allocate more of host-supplied C towards excessive N assimilation rather than growth (Wallander, 1995). Nitrogen availability can also affect ectomycorrhizal fungi indirectly via its influence on host C supply, since trees usually allocate proportionally less C belowground as N availability increases (Högberg *et al.*, 2010; Gill & Finzi, 2016; Marshall *et al.*, 2023). Furthermore, model predictions indicate that trees should allocate less C to ectomycorrhizal fungi at larger external input of N to the ecosystem (Baskaran *et al.*, 2017). It is also likely that trees can reduce C flow to less beneficial symbionts (Hortal *et al.*, 2017), thus changing ectomycorrhizal fungal community composition in response to variation in N availability.

Input of atmospheric N has been identified as a major driver of compositional shifts within ectomycorrhizal fungal communities (Lilleskov *et al.*, 2002, 2011, 2019; van der Linde *et al.*, 2018). Different taxa have distinct tolerance thresholds for N (van der Linde *et al.*, 2018) and can be classified as nitrophobic, nitrotolerant or nitrophilic (Lilleskov *et al.*, 2011). Suz *et al.* (2021) discussed the possibility of 'ectomycorrhizal tipping points' in forests subjected to atmospheric N deposition, at which the ectomycorrhizal function would be drastically impaired by reduced belowground investment of host C. A tipping point is defined as a critical stage at which an ecosystem rapidly transitions into a new state, commonly preceded by a phase of 'hysteresis', during which the original ecosystem state is preserved by functional adaptations of biota (Dakos *et al.*, 2019). Thus, in the 'hysteresis phase', while tree productivity is maintained, turnover within the ectomycorrhizal fungal community may favour species adapted to inorganic nutrient uptake (e.g. members of the genera *Tylospora* and *Tomentella*, as well as some *Lactarius* species) over species specialised in N acquisition from organic material (e.g. members of the genera *Suillus*, *Cortinarius* and *Piloderma*; Lilleskov *et al.*, 2011, 2019; Sterkenburg *et al.*, 2015; Pellitier & Zak, 2021; Pellitier *et al.*, 2021).

Jörgensen *et al.* (2023a) investigated differences among ectomycorrhizal fungal genera in their production of extraradical mycelium relative to root colonisation. Accumulation of extraradical biomass was relatively slow for *Cortinarius*, *Hyaloscypha*, *Hygrophorus* and *Piloderma*. Of these 'slow-growing' genera, particularly *Cortinarius* and *Piloderma* have often been described as nitrophobic and C-demanding (Lilleskov *et al.*, 2011; Pellitier & Zak, 2021). By contrast, the 'fast-growing' genera *Amphinema*, *Tomentella* (incl. *Thelephora*) and *Tylospora*, commonly described as nitrophilic (Lilleskov *et al.*, 2011), proliferated with large amounts of mycelium from few root tips even in N-rich soils (Jörgensen *et al.*, 2023a), suggesting efficient C usage and potential tolerance to N-induced reductions in host C supply (Saikkonen *et al.*, 1999).

In boreal ecosystems, ectomycorrhizal fungi have been found to increase their abundance (Sterkenburg *et al.*, 2015), mycelial production (Högberg *et al.*, 2021) and species richness (Kranabetter *et al.*, 2009a,b) along natural fertility gradients from strongly limiting up to moderate N levels. Under such nutrient-limited conditions, experimental addition of N (urea) stimulated mycelial proliferation (Högberg *et al.*, 2021). N limitation of ectomycorrhizal fungi, thus, seems to be alleviated as soil fertility increases from sub-optimal levels. In more N-rich soils, however, ectomycorrhizal fungi seem to decline in favour of free-living saprotrophs in both boreal (Högberg *et al.*, 2003, 2021; Kyaschenko *et al.*, 2017) and temperate (Mayer *et al.*, 2021; Pellitier & Zak, 2021) forests.

In contrast to this seemingly unimodal response to internal ecosystem N cycling, ectomycorrhizal fungi generally respond negatively to amendments with inorganic N from external sources. The relative abundance of ectomycorrhizal species in fungal communities decreased after fertilisation of pine forests across the entire latitudinal range of Scandinavia (Jörgensen *et al.*, 2022). Standing biomass and production of mycelium decreased in response to N fertilisation (Nilsson & Wallander, 2003; Högberg *et al.*, 2014) and along gradients in atmospheric N deposition (Kjøller *et al.*, 2012; Bahr *et al.*, 2013). Furthermore, N deposition commonly has a negative effect on ectomycorrhizal species richness (Lilleskov *et al.*, 2002).

It, thus, seems that ectomycorrhizal fungi relate to variation in N availability in different ways depending on the context; in particular, whether N is released via internal recycling of organic pools or supplied from external sources. In this study, we tested the hypothesis that responses of ectomycorrhizal fungal biomass, community composition and associated enzyme activities to increased N availability would differ between two regions in the nemo-boreal parts of Sweden; one in the southernmost boreal zone with little N deposition (on average 5.8 kg N ha^{-1} yr^{-1} during the last 20 yr) and one in the northern nemoral zone with more significant N deposition (11.1 kg N ha^{-1} yr^{-1}). Samples were collected along two gradients consisting of 14 *Picea abies* forests in the boreal region and 15 in the nemoral region, which spanned equal ranges in concentrations of total and inorganic N pools in the topsoil.

We expected that (1) abundance of ectomycorrhizal fungi would be positively related to N availability in the boreal region, where increasing N would alleviate growth limitation. By contrast, there would be a negative relationship to N availability in the nemoral region, where deposition of external N would exceed optimal levels.

Furthermore, we hypothesised (2) that slow-growing and nitrophobic genera would decrease with increasing N availability in the nemoral gradient subjected to higher N deposition along with decreasing activities of enzymes involved in mobilisation of organic N. Fast-growing and nitrophilic genera were expected to increase their relative share of the community with increasing N availability, particularly in the nemoral gradient. In the boreal gradient, community changes should be absent or less pronounced.

Since our study was constrained to a single tree species, we assumed that the different primary N sources in the two regions, internal N recycling in the boreal region vs deposition of exogenous N in the nemoral region, would be a major factor behind potential context dependent N-responses of ectomycorrhizal communities between the two regions (van der Linde *et al.*, 2018).

Materials and Methods

Site selection and sampling

The study was conducted in two regions in Sweden; on the southern margin of the boreal zone at 60°N and with N deposition of *c.* 5.8 kg N ha^{-1} yr^{-1}, and on the northern margin of the nemoral zone at 56–57°N and with elevated deposition rates of 11.1 kg N ha^{-1} yr^{-1} (averages between 1998 and 2008; Swedish Meteorological and Hydrological Institute). This difference brings the boreal region just to the level where sensitive ectomycorrhizal fungi have been observed to respond, and the nemoral region close to the level where larger effects on community composition are often observed (van der Linde *et al.*, 2018). In the boreal region, the growing season is 170–190 d with a mean annual temperature of 4–6°C, and in the nemoral region, the growing season is 200–220 d with a mean annual temperature of 6–8°C (Swedish Meteorological and Hydrological Institute). We selected 29 mature (> 70 yr) *Picea abies* (L.) H. Karst-dominated stands (14 in the boreal region and 15 in the nemoral region) in a mosaic across large areas of *c.* 3000 and 8000 km^2 in the boreal and nemoral region, respectively. In both regions, sites were selected to span a wide range in ecosystem fertility based on characteristics of vegetation and measures of productivity. Less fertile sites had an understory vegetation of feather mosses (mainly *Pleurozium schreberi* (Willd. ex Brid.) and *Hylocomium splendens* (Hedw.) Schimp.), *Vaccinium myrtillus* L. and *Vaccinium vitis-idaea* L., while more fertile sites had an understory with additional contributions of ferns and grasses, or in some sites in the nemoral region, no understory. Variation within regions is likely to stem from differences in mineralogy and hydrology as well as local variation in N deposition. Biogeochemical descriptions of the sites are presented in Table 1.

In September 2018, we collected 25 soil cores (3 cm diameter) in a grid pattern across a 20 × 20 m area in each stand.

Table 1 Site description of *Picea abies* forests in the boreal and nemoral regions, ordered by N : C ratio within regions.

Region	Latitude (WGS84)	Longitude (WGS84)	Organic matter (%)	Inorganic N (μg gOM^{-1})	N : C	pH	N deposition (kg N ha^{-1} yr^{-1})
Boreal	60.24	16.99	74	0.04 (0.004)	0.028	4.0	5.4
Boreal	60.12	17.79	52	0.06 (0.005)	0.029	4.4	6.3
Boreal	60.29	17.74	66	0.04 (0.004)	0.030	4.3	5.8
Boreal	60.08	18.25	22	0.09 (0.007)	0.030	4.6	6.2
Boreal	60.09	18.28	35	0.06 (0.004)	0.031	4.5	6.2
Boreal	60.13	18.30	46	0.07 (0.007)	0.031	4.5	5.7
Boreal	60.55	17.98	53	0.06 (0.007)	0.031	4.6	4.4
Boreal	60.18	17.86	22	0.06 (0.007)	0.031	5.0	5.9
Boreal	60.29	17.05	24	0.05 (0.004)	0.033	4.3	5.4
Boreal	60.43	17.63	26	0.04 (0.011)	0.034	4.5	5.6
Boreal	59.88	18.27	31	0.05 (0.003)	0.037	4.5	6.4
Boreal	60.07	17.80	34	0.09 (0.006)	0.045	4.8	6.1
Boreal	59.96	18.19	14	0.11 (0.009)	0.046	5.2	6.2
Boreal	59.79	18.60	26	0.09 (0.004)	0.051	6.1	6.2
Nemoral	56.45	13.97	35	0.07 (0.006)	0.033	4.1	10.6
Nemoral	57.01	13.37	20	0.05 (0.003)	0.033	4.3	10.0
Nemoral	56.30	14.28	26	0.07 (0.004)	0.033	4.5	10.1
Nemoral	56.28	14.24	34	0.06 (0.004)	0.034	4.0	10.1
Nemoral	56.70	13.45	30	0.05 (0.011)	0.034	4.2	11.0
Nemoral	56.18	13.52	31	0.06 (0.007)	0.035	4.1	11.6
Nemoral	56.77	13.16	34	0.08 (0.013)	0.035	4.3	11.7
Nemoral	56.70	13.10	25	0.06 (0.005)	0.036	4.2	11.7
Nemoral	56.56	13.22	20	0.1 (0.006)	0.036	4.3	11.7
Nemoral	55.62	14.09	39	0.07 (0.004)	0.037	4.0	9.9
Nemoral	56.70	13.08	41	0.09 (0.011)	0.038	4.3	11.7
Nemoral	56.28	13.85	27	0.05 (0.006)	0.038	4.5	10.9
Nemoral	55.54	13.57	35	0.11 (0.006)	0.042	4.1	11.5
Nemoral	56.00	13.71	28	0.07 (0.004)	0.044	4.3	11.6
Nemoral	56.37	12.93	31	0.08 (0.005)	0.048	4.2	11.1

Organic matter content, concentrations of inorganic N (average of three measurements, SE in parentheses) N : C ratio and pH were measured from composite soil samples. N-deposition rates are total annual deposition rates between 1998 and 2018 (Swedish Meteorological and Hydrological Institute).

To sample comparable soil depths across sites with or without a distinct organic horizon (mor layer), the entire organic layer (when present) and the upper 7 cm of the mineral soil (or until we hit rock) were sampled. Fresh litter, coarse roots (> 2 mm) and living mosses were removed, and cores were mixed into a single composite sample per site, which was frozen (−20°C) within 8 h of collection. Five additional cores, collected as described above, were sampled at three time points between September and November (5–6 wk apart) and used for extractions of inorganic N.

Sample preparation and chemical analysis

Frozen samples were homogenised in a custom build mill, and 50 ml subsamples were freeze-dried (48 h) and finely ground in a ball mill for extraction of DNA and ergosterol as well as for analysis of C and N contents. N : C ratio was determined from 0.4 g of soil in a combustion elemental analyser (TruMac CN; LECO, St. Joseph, MI, USA). Additional subsamples of homogenised, frozen material were used to analyse enzyme activities, pH, water content and organic matter content. Organic matter content was determined based on loss on ignition of 3 g of soil at 550°C for 6 h after drying at 105°C for 24 h. pH was determined in a 1 : 5 volume ratio of soil and deionised water, using an 855 Robotic Titrosampler with an Aquatrode Plus combined pH electrode (Metrohm, Herisau, Switzerland). Nitrate and ammonium were extracted with a 1 : 2.5 weight ratio of soil and 2 M KCl, by shaking on a rotation shaker overnight and filtering through 5 and 0.45 μm syringe filters (Acrodisc, Supor Membrane filters; PALL Corp., NY, USA), and measured on an Autoanalyzer (BRAN-LUEBBE XY-2 Sampler; SEAL Analytical Inc., Emu Plains, NSW, Australia). The average concentration of inorganic N across the three sampling time points was used in subsequent analyses.

Ergosterol and enzyme assays

As a proxy for fungal biomass, we measured the amount of ergosterol (Nylund & Wallander, 1992) in 500 mg freeze-dried, ball-milled soil. Ergosterol was extracted in 10% KOH dissolved in methanol and further extracted in cyclohexane. The cyclohexane was evaporated in a flow of gaseous N_2, after which the ergosterol was resuspended in methanol. Extracts were filtered through a 0.45 μm Teflon syringe filter (Millex LCR-4; Millipore) and analysed for ergosterol using high-performance liquid chromatography following the protocol of Hagenbo *et al.* (2017).

We assayed hydrolytic activities of β-1,4-*N*-acetylglucosaminidase and acid phosphatase using fluorogenic methylumbelliferyl substrates (Saiya-Cork *et al.*, 2002) in soil suspensions adjusted to contain 5 g organic matter (dry weight) in 500 ml 50 mM sodium acetate buffer and diluted to a final concentration of 0.001 gOM ml^{-1}. Assays were done in 96-well microplates in a mixture of 200 µl of the soil suspension together with 50 µl of 200 µM 4-methylumbelliferyl N-acetyl-β-D-glucosaminid (for *N*-acetylglucosaminidase) or 4-methylumbelliferyl phosphate (for acid phosphatase) and incubated for 1 h. To stop the reaction, 10 µl of 0.5 M NaOH was added and fluorescence was measured on a fluorescence/luminescence spectrometer (LS 50B; PerkinElmer Inc., Hopkinton, MA, USA). Background fluorescence was accounted for by measuring fluorescence without incubation. To account for quenching, an umbelliferon standard was added to soil suspensions.

Manganese peroxidase activity was assayed in soil extracts of 0.001 gOM ml^{-1} using the colorimetric MBTH-DMAB method (Daniel *et al.*, 1994) as described in Kyaschenko *et al.* (2017). In short, the method relies on coupled oxidation of MBTH and DMAB, which results in an indamine dye. To mediate the reaction, EDTA or $MnSO_4$ was added together with H_2O_2. Absorbance was measured on a SpectraMax Plus 384 microplate reader (Molecular Devices, Sunnyvale, CA, USA) every 3 min for a total incubation time of 45 min. Absorbance was converted to peroxidase activity using a standard curve based on commercial manganese peroxidase from *Phanerochaete chrysosporium* (Sigma-Aldrich). Activities of manganese peroxidase were calculated as the difference in activity between the reactions with $MnSO_2$ and those with EDTA. Enzyme activities were assessed with four technical replicates per sample.

DNA extraction and fungal community characterisation

DNA was extracted from 100 µg freeze-dried and ball-milled soil using the NucleoSpin soil kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instruction. Extracts were diluted to 0.5 ng DNA $µl^{-1}$, and amplicons of the ITS2 region were produced by polymerase chain reactions (PCR) with the forward primer gITS7 and a 3 : 1 mix of the reverse primers ITS4 and ITS4arch, both with unique sample identification tags (Ihrmark *et al.*, 2012; Clemmensen *et al.*, 2023). PCR amplification was conducted in 50 µl reactions containing 12.5 ng of DNA, 0.75 mM $MgCl_2$, 0.2 mM dNTP, 0.5 µM of forward and 0.3 µM reverse primers and 0.025 U $µl^{-1}$ polymerase (DreamTaq green; Thermo Fischer Scientific, Waltham, MA, USA). PCR conditions were as follows: 5 min at 95°C; 25–31 cycles of 30 s at 94°C, 30 s at 56°C and 30 s at 72°C; 7 min at 72°C (cycle numbers were optimised for each sample individually; Castaño *et al.*, 2020). Each sample was run in technical triplicates that were pooled and cleaned using SeraMag (Cytiva, Marlborough, MA, USA) according to the manufacturer's instructions. Amplicon concentrations were measured on a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA), and equal amounts were pooled and cleaned with the E.Z.N.A Omega cycle pure kit (Omega BioTek, Nocross, GA, USA) before checking the amplicon size distribution with the 2100 Bioanalyzer system (Agilent Technologies, Santa Clara, CA, USA). Library preparation and sequencing in one SMRT cell on the PacBio Sequel Platform (Pacific Biosciences, Menlo Park, CA, USA) were conducted by SciLifeLab (NGI, Uppsala, Sweden). The PacBio platform was chosen to minimise biases due to ITS2 length variation (Castaño *et al.*, 2020).

Bioinformatics

Sequence quality filtering and clustering were performed using the bioinformatics pipeline SCATA (scata.mykopat.slu.se; Ihrmark *et al.*, 2012). Sequences with < 90% match with primers, missing 3′ or 5′ tag, being shorter than 100 bp, having mean quality scores below 20 or having individual bases with a quality score below 3, as well as unique genotypes were removed before clustering. Sequences passing quality filtering were clustered into species hypotheses (Kõljalg *et al.*, 2013) by single linkage clustering with 98.5% similarity to the closest neighbour required for sequences to enter clusters. Species hypotheses (hereafter 'species') were identified by matching the most abundant genotype from each species to the UNITE database (Nilsson *et al.*, 2019), and nonfungal species were removed. Ectomycorrhizal status was inferred from the FungalTraits database (Põlme *et al.*, 2020).

Ectomycorrhizal species were grouped within genera. For genera containing species with different symbiotic strategies, for example *Hyaloscypha*, only species with known ectomycorrhizal status were included. The relative abundance of the ectomycorrhizal guild was calculated as the proportion of summed sequence reads from all ectomycorrhizal species relative to all fungal reads. An estimate of the ergosterol attributable to ectomycorrhizal fungi was obtained by multiplying the relative abundance of ectomycorrhizal fungi (out of all fungi) by total ergosterol concentration. To assess shifts within the ectomycorrhizal community, genera present at 10 or more sites were selected for further analyses, and their relative abundances were then expressed in relation to the total number of ectomycorrhizal reads. *Cortinarius*, *Hyaloscypha*, *Hygrophorus* and *Piloderma* were classified as slow-growing, whereas *Amphinema*, *Tomentella* (incl. *Thelephora*) and *Tylospora* were classified as fast-growing (Jörgensen *et al.*, 2023a). Ectomycorrhizal genera were classified as nitrophilic or nitrophobic according to literature references (Supporting Information Table S1).

Statistical analyses

All statistical testing was done in R v.4.1.3 (R Development Core Team, 2022). Data cleaning and manipulation were done using the TIDYVERSE package (Wickham *et al.*, 2019), figures were produced with GGPLOT2 (Wickham *et al.*, 2020) and COWPLOT (Wilke, 2020), and tables were produced with SJPLOT (Lüdecke, 2018). Linear models were constructed with either total ergosterol, ergosterol attributed to ectomycorrhizal fungi, relative abundance of ectomycorrhizal fungi in the total fungal community or enzyme activities as response variables, and soil N : C ratio and region as predictors. The interaction between region and N : C ratio was included in the models if $P \leq 0.1$.

If a significant N : C × region interaction ($P \leq 0.05$) was detected, individual models were fitted to investigate the relationships between variables in each region.

Mixed linear effect models were fitted, using the packages LME4 (Bates *et al.*, 2015) and LMERTEST (Kuznetsova *et al.*, 2017), to test effects on the relative abundances of fast- and slow-growing genera (Jörgensen *et al.*, 2023a), or nitrophobic and nitrophilic genera (Table S1). Initial models included the relative abundance of genera as response variable, functional type as factors with two levels (fast/slow or nitrophobic/nitrophilic), N : C and region, and their interactions as fixed explanatory variables, and genus as a random factor. If a significant ($P \leq 0.05$) three-way interaction effect was detected, the data were split into functional types and the model run again (excluding functional type from the model). Similarly, if an N : C × region interaction was detected ($P \leq 0.05$), the data were split and a model was fitted to evaluate the correlations in each region separately.

Residuals were visually checked for homogeneity and normality, and response data were log or square-root transformed when necessary. Inorganic N concentrations were positively correlated with soil N : C ($P \leq 0.001$, Fig. 1a; Table S2), but the N : C ratio was consistently a better predictor of fungal variables and was therefore chosen to represent soil N availability in the models.

As an alternative approach, 'region' in the linear models was replaced by pH and local N-deposition rates, to test their influence as covariates together with N : C ratio on ergosterol attributed to ectomycorrhizal fungi and the relative abundances of fast/slow or nitrophobic/nitrophilic genera. In these models, all two-way interactions were included, and explanatory variables were normalised.

Relations between the relative abundance of genera and N : C ratio, region and their interaction were tested by fitting individual generalised mixed linear models (GLMs) using the anova.manyglm function in mvabund (Wang *et al.*, 2012). Models were fitted using a negative binomial linking function and assessed by ANOVA with 999 bootstraps, both at the community level and for each genus individually after adjustment for multiple testing by accounting for false discovery rates.

In all models, concentrations of ergosterol and enzyme activities were expressed relative to organic matter content. The community composition of the most frequent ectomycorrhizal fungi was illustrated with nonmetric multidimensional scaling (NMDS) using the metaMDS function in VEGAN (Oksanen *et al.*, 2022).

Results

The gradients in the boreal and the nemoral regions overlapped in terms of N : C ratios (0.03–0.05; Table 1) and inorganic N concentrations (0.04–0.11 μg N gOM^{-1}; Fig. 1a; Table 1). The inorganic N pool was dominated by ammonium in both regions (boreal: 97.3–100%, mean = 99.6%, median = 99.8%; nemoral: 77.1–100%, mean = 96.6%, median = 99.9%). In the boreal gradient, pH correlated positively with N : C (pH range: 4.0–6.1). By contrast, pH was not correlated with N availability and had a more narrow range (4.0–4.5) in the nemoral gradient (Fig. 1b; Table S3).

PacBio sequencing generated 352 650 sequences with 136 613 sequences passing the quality filtering, which were clustered into 2488 species, of which 317 were classified as ectomycorrhizal. In total, 54 ectomycorrhizal genera were represented in the community, accounting for 2–23 (mean 10) % of the total fungal sequences (Fig. S1a). The 23 most frequent ectomycorrhizal genera that were present in 10 sites or more made up, on average, 96% of the total ectomycorrhizal community (70–100%, median 98%; Table S4). Dominant genera were *Cenococcum*, *Hyaloscypha* and *Piloderma*, accounting for 12%, 11% and 20% of the ectomycorrhizal sequence reads, respectively, in the boreal region, and 30%, 20% and 13%, respectively, in the nemoral region. Genus composition of the ectomycorrhizal fungal community differed both between the regions and along the fertility gradients. However, genera related differently to N availability in the two regions, as indicated by significant N : C × region interactions (Table 2), and the separation of the boreal and nemoral communities in the NMDS (Fig. 2).

Total ergosterol concentrations were similar across regions and declined with increasing N : C ratio (Fig. S1b; Table S5).

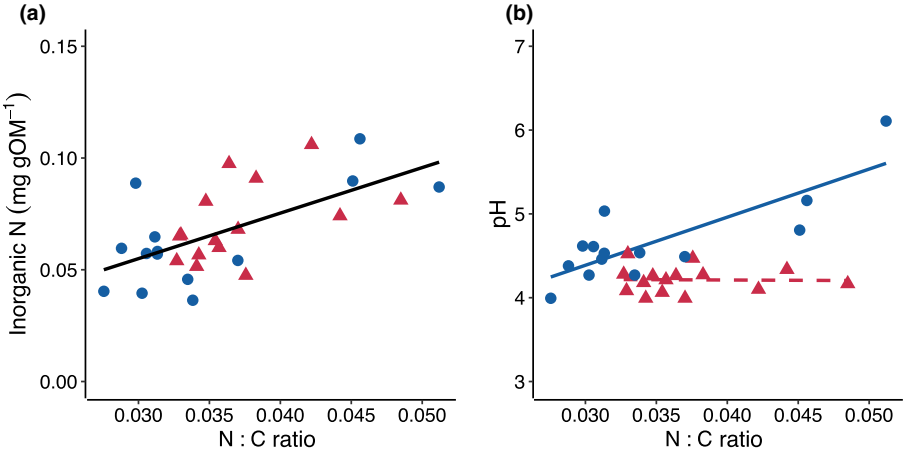

Fig. 1 Relationships of (a) inorganic N and (b) pH with N : C ratio in soil from mature *Picea abies* forests in two regions in Sweden. Shapes and colours denote sampled regions: boreal (low N deposition – blue circles) and nemoral (high N deposition – red triangles). Lines represent fitted linear regressions; solid = significant ($P \leq 0.05$), dashed = nonsignificant ($P \geq 0.05$), single black = significant main effect of N : C but no difference between regions, two coloured lines = region effect. See Supporting Information Tables S2 and S3 for statistics.

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Table 2 Results from generalised linear models, fitted using mvabund.

	Total EMF community	Hyaloscypha	Otidea	Piloderma	Tomentella	Tylospora
N : C ratio						
N : C	84 (**0.005**)	0.2 (1.000)	14.4 (**0.015**)	9.5 (0.113)	3.9 (0.530)	9.0 (0.129)
Region	156 (**0.001**)	10.5 (0.103)	4.7 (0.513)	2.2 (0.739)	3.5 (0.638)	5.3 (0.458)
N : C × Region	70 (**0.001**)	10.9 (**0.046**)	0.6 (0.970)	14.7 (**0.016**)	12.6 (**0.028**)	9.9 (**0.067**)

LR and *P*-values (in parentheses) of the response of the total ectomycorrhizal fungal (EMF) genus composition and specific genera (only genera with $P \leq 0.1$ are shown) to soil N : C ratios and region. Significant *P*-values ($P \leq 0.05$) in bold.

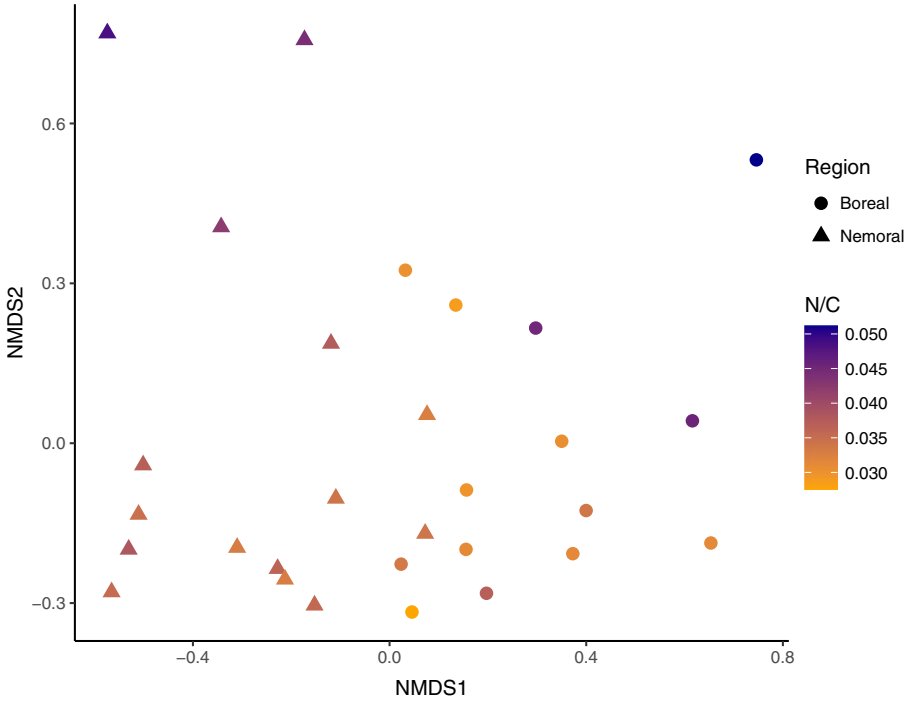

Fig. 2 Ectomycorrhizal fungal genus composition in the soil of mature *Picea abies* forests in fertility gradients in the boreal (low N deposition) and nemoral (high N deposition) regions in Sweden illustrated by nonmetric multidimensional scaling (NMDS). Symbols represent the sampled regions and colours correspond to soil N : C ratio. Fungal communities were analysed with PacBio metabarcoding of amplified ITS2 markers.

Ergosterol attributed to ectomycorrhizal fungi (total ergosterol concentrations multiplied by the relative abundance of sequences assigned to ectomycorrhizal taxa, hereafter 'ectomycorrhizal biomass') declined with increasing N : C ratio in the nemoral region, but was more stable across the N availability gradient in the boreal region, as reflected in a significant region × N : C interaction but no significant main effect of N availability (Fig. 3a; Tables 3, S6). The *a priori*-defined functional groups of fast and slow growth (Jörgensen *et al.*, 2023a) responded differently to N availability (Table S7). The relative abundance of slow-growing genera in the ectomycorrhizal fungal community did not respond to increasing N : C in either region, while the fast-growing genera increased at higher N : C in the nemoral region (highly influenced by one site) but were unresponsive to soil N : C ratio in the boreal region (Fig. 3b,c; Tables 3, S8). Nitrophobic genera declined along the N : C gradients in both regions, but with a significantly steeper decline in the nemoral region, while nitrophilic genera clearly increased in the nemoral region but were unresponsive to increased N : C in the boreal region (Fig. 3d,e; Tables 3, S9, S10). The nitrophobic/nitrophilic classification yielded stronger relations to N than the slow/fast classification.

In models where the spatial structure of the data (i.e. region) was substituted by pH and local N-deposition rates, N : C had a negative main effect on ectomycorrhizal biomass and relative abundances of nitrophobic ectomycorrhizal fungal genera. These negative relationships with N : C were stronger at high rates of N deposition, as indicated by a significant negative N : C × N-deposition interaction. By contrast, the nitrophilic and fast-growing genera were positively related to N : C, with stronger responses at higher rates of N deposition (positive N : C × N-deposition interaction). Neither pH nor N deposition had any main effect on the response variables, although a negative N : C × pH interaction effect on the slow-growing genera was detected (Table 4).

Five ectomycorrhizal genera correlated significantly with N : C ratio (Fig. 4; Table 2). The slow-growing *Piloderma*, as well as *Otidea* (not shown in Fig. 4) declined with increasing N : C ratio, with a significantly stronger decline of *Piloderma* in the nemoral region. The fast-growing *Tomentella* and *Tylospora* increased with

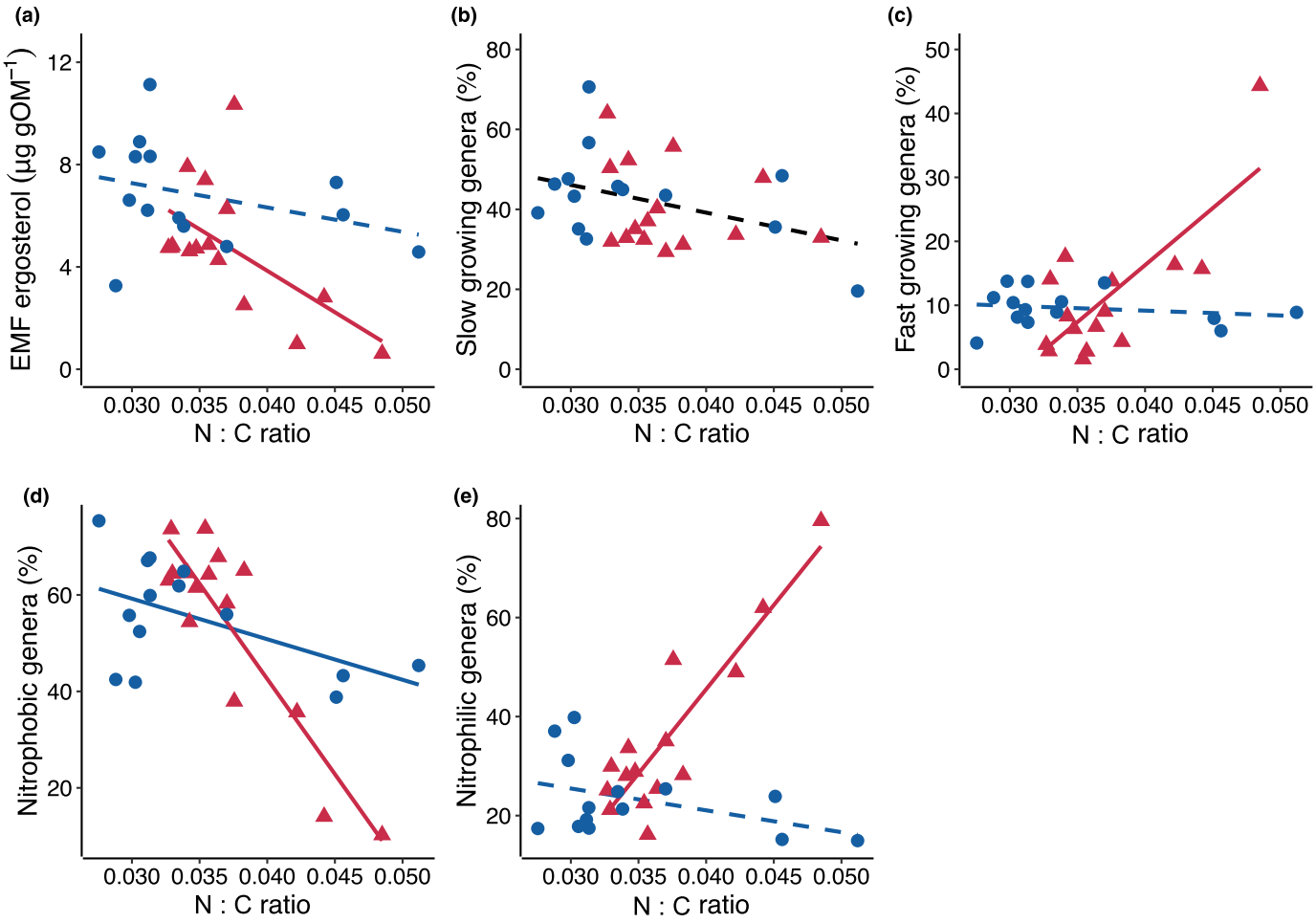

Fig. 3 Responses of the ectomycorrhizal fungal community across soil N/C gradients in mature *Picea abies* forests in two forest regions. (a) Ergosterol attributed to ectomycorrhizal fungi (EMF; µg gOM^{-1}) and relative abundance (% ectomycorrhizal out of total ITS2 sequence reads) of (b) slow and (c) fast-growing genera (defined according to Jörgensen *et al*., 2023a), and (d) nitrophobic and (e) nitrophilic genera (Supporting Information Table S1). Shapes and colours denote sampled regions: boreal (low N deposition – blue circles) and nemoral (high N deposition – red triangles). Lines represent fitted linear regressions; solid = significant ($P \leq 0.05$), dashed = nonsignificant ($P \geq 0.05$), single black = significant main effect of N : C but no difference between regions, two coloured lines = region effect. See Tables 3 and S6–S10 for statistics.

increasing N : C ratio in the nemoral region but were unresponsive in the boreal region. Meanwhile, *Hyaloscypha* responded differently than other slow-growing genera, correlating negatively with the N : C ratio in the boreal region but positively in the nemoral region (Fig. 4).

Acid phosphatase activities were generally higher in the boreal than in the nemoral region and quite stable across the N : C gradient (Fig. 5a; Table 5). There was a marginally significant ($P = 0.061$) negative correlation between *N*-acetylglucosaminidase and N : C, and activities were higher in the boreal region (Fig. 5b; Table 5). The ratios between acid phosphatase and *N*-acetylglucosaminidase increased with N : C ratio in the nemoral gradient as indicated by a marginally significant ($P = 0.058$) N : C × Region interaction (Fig. 5c; Table 5). Manganese peroxidase activity was negatively correlated with N : C ratio and generally higher in the nemoral than in the boreal region (Fig. 5d; Table 5).

Discussion

We sampled mature spruce forests along fertility gradients in two regions in Sweden to test whether ectomycorrhizal fungi respond differently to variation in N availability under conditions of low rates of N deposition, in the boreal region, or relatively high rates of N deposition, in the nemoral region. To attain equivalent spans of boreal and nemoral N levels, in spite of the boreal region generally being less nutrient-rich, we included particularly fertile boreal sites with mull type soils, rich in N. Ectomycorrhizal fungal abundance remained stable across the boreal gradient (it did not increase, as we hypothesised). By contrast, and in line with our hypothesis, responses of ectomycorrhizal fungi were much more pronounced in the nemoral region with higher N deposition. Concordantly, N-deposition × N : C interactions were highly significant in statistical models where the effect of region was represented by actual N-deposition levels in the stands.

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Table 3 Coefficients and 95% confidence intervals (in parentheses) from mixed effects linear models of ectomycorrhizal fungal biomass (ectomycorrhizal fungal (EMF) ergosterol) and the relative abundance of slow and fast, and nitrophobic and nitrophilic ectomycorrhizal fungi genera.

	EMF ergosterol		Slow genera		Fast genera		Nitrophobic genera		Nitrophilic genera	
Predictors	Estimates	*P*	Estimates	*P*	Estimates	*P*	Estimates	*P*	Estimates	*P*
(Intercept)	2.31 (1.16 to 3.46)	**<0.001**	0.35 (0.17 to 0.53)	**<0.001**	0.21 (0.08 to 0.34)	**0.002**	0.30 (0.13 to 0.48)	**0.001**	0.13 (0.03 to 0.24)	**0.014**
N : C	−12.51 (−44.97 to 19.94)	0.435	−1.62 (−5.26 to 2.01)	0.378	−1.23 (−4.76 to 2.30)	0.490	−1.34 (−4.87 to 2.19)	0.456	0.32 (−1.87 to 2.51)	0.774
Region	3.87 (1.68 to 6.07)	**0.001**	−0.02 (−0.06 to 0.02)	0.360	−0.45 (−0.68 to −0.21)	**<0.001**	0.30 (0.06 to 0.54)	**0.014**	−0.31 (−0.46 to −0.17)	**<0.001**
N : C × Region	−117.20 (−176.83 to −57.57)	**<0.001**			11.80 (5.31 to 18.28)	**0.001**	−8.99 (−15.48 to −2.51)	**0.007**	8.99 (4.96 to 13.02)	**<0.001**

Significant *P*-values ($P \leq 0.05$) in bold.

Table 4 Coefficients and 95% confidence intervals (in parentheses) from mixed effects linear models testing the effect of N : C ratio, pH, annual N-deposition rates and their two-way interactions on ergosterol attributed to ectomycorrhizal fungi ectomycorrhizal fungi (EMF), and relative abundances of functional groups of ectomycorrhizal fungi from mature *Picea abies* forests in two regions in Sweden.

	EMF biomass		Slow genera		Fast genera		Nitrophobic genera		Nitrophilic genera	
Predictors	Estimates	*P*	Estimates	*P*	Estimates	*P*	Estimates	*P*	Estimates	*P*
(Intercept)	1.78 (1.49 to 2.06)	**<0.001**	0.66 (0.61 to 0.72)	**<0.001**	0.32 (0.26 to 0.37)	**<0.001**	0.73 (0.68 to 0.77)	**<0.001**	0.54 (0.49 to 0.59)	**<0.001**
N : C	−0.41 (−0.66 to −0.17)	**0.002**	−0.02 (−0.07 to 0.02)	0.263	0.07 (0.02 to 0.11)	**0.008**	−0.12 (−0.16 to −0.08)	**<0.001**	0.08 (0.03 to 0.12)	**0.001**
pH	0.16 (−0.24 to 0.55)	0.421	0.05 (−0.03 to 0.12)	0.199	0.04 (−0.03 to 0.12)	0.243	−0.04 (−0.10 to 0.03)	0.267	0.02 (−0.05 to 0.08)	0.649
N deposition	−0.12 (−0.40 to 0.16)	0.373	−0.00 (−0.05 to 0.05)	0.994	0.00 (−0.05 to 0.06)	0.968	−0.00 (−0.05 to 0.04)	0.936	0.04 (−0.00 to 0.09)	0.072
N : C × pH	−0.01 (−0.16 to 0.14)	0.903	−0.03 (−0.06 to −0.00)	**0.033**	−0.02 (−0.05 to 0.01)	0.278	0.01 (−0.02 to 0.04)	0.449	−0.01 (−0.03 to 0.02)	0.652
N : C × N deposition	−0.36 (−0.63 to −0.09)	**0.011**	−0.01 (−0.06 to 0.03)	0.546	0.06 (0.00 to 0.11)	**0.038**	−0.08 (−0.12 to −0.03)	**0.001**	0.07 (0.02 to 0.12)	**0.005**
pH × N deposition	0.15 (−0.27 to 0.57)	0.462	0.01 (−0.06 to 0.09)	0.723	0.04 (−0.04 to 0.12)	0.345	−0.04 (−0.11 to 0.03)	0.288	0.06 (−0.02 to 0.13)	0.135

Significant *P*-values ($P \leq 0.05$) in bold.

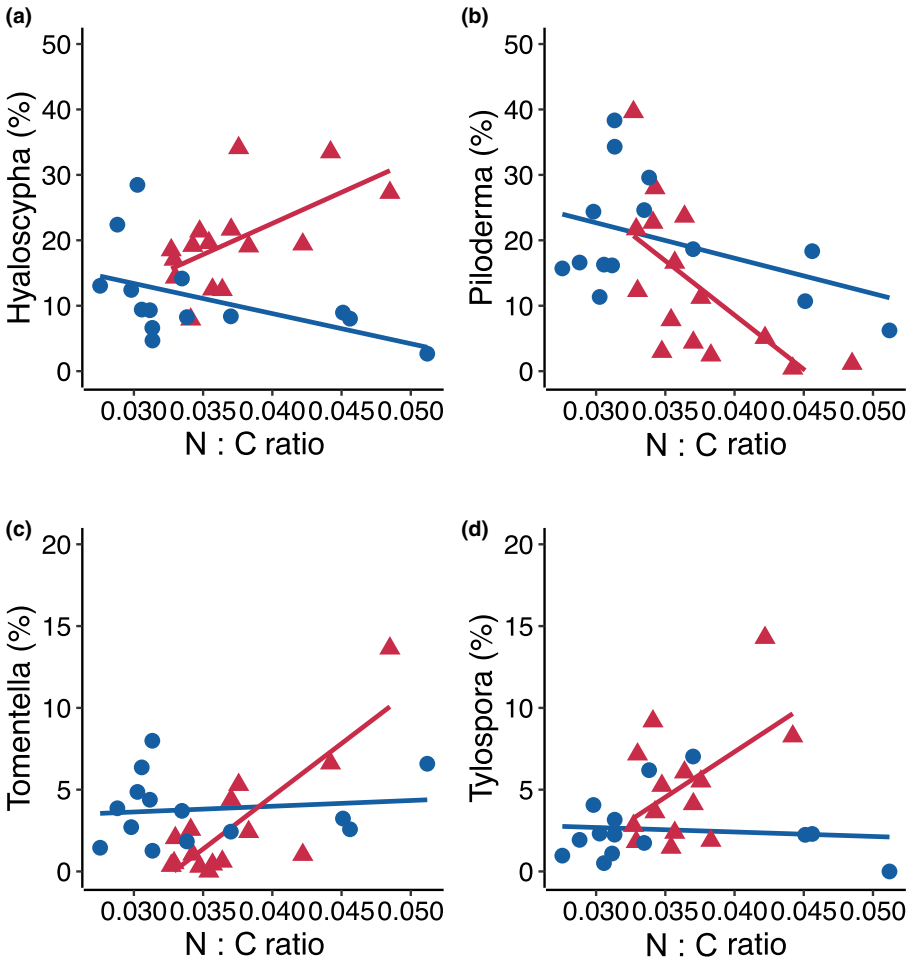

Fig. 4 Relative abundance of ectomycorrhizal genera (% of ectomycorrhizal ITS2 sequence reads) that correlated significantly ($P \leq 0.05$) with variation in N/C ratio. Samples were collected from the organic horizon and top 7 cm of mineral soil across *Picea abies* forests in two regions in Sweden. (a) *Hyaloscypha* was classified as slow-growing but nitrophilic, (b) *Piloderma* was classified as slow-growing and nitrophobic and (c) *Tomentella* and (d) *Tylospora* as fast-growing and nitrophilic based on (Jörgensen *et al*., 2023a) and references in Supporting Information Table S1. Lines represent fitted linear regressions. Shapes and colours denote sampled regions: boreal (low N deposition – blue circles) and nemoral (high N deposition – red triangles).

Nitrogen deposition and host species have been identified as principal drivers of variation in ectomycorrhizal community composition across Europe (van der Linde *et al*., 2018), and our study encompassed a single host species only. Still, other factors, for example climatic dissimilarities between the tested regions, as well as differences with respect to geology and history of land use, may also have affected the propensity of ectomycorrhizal communities to respond to variation in N availability in our comparison.

In the boreal region, there was a clear, positive correlation between soil N availability and pH, as previously documented along natural soil fertility gradients (Sterkenburg *et al*., 2015; Högberg *et al*., 2017). However, fungal responses to soil fertility (N and pH) were weak in this region. Meanwhile, in the nemoral region subjected to high N deposition, pH was consistently low without much variation across sites with different N availability (Fig. 1). Yet, mycorrhizal responses were stronger in the nemoral region, suggesting a subordinate role of pH in shaping fungal communities in the context of our study. Hence, while being aware of potential confounding factors, we interpret the results in relation to anthropogenic N supply.

When N availability in forests increases, either naturally or by human activities, there is a threshold level above which ectomycorrhizal fungal abundance and activities decline (Högberg *et al*., 2021; Pellitier & Zak, 2021; Suz *et al*., 2021). This response is thought to be conditional on reduced belowground allocation of tree C to roots and mycorrhizal fungi (Wallander & Nylund, 1992; Högberg *et al*., 2003; Gill & Finzi, 2016; Marshall *et al*., 2023) and a preferential use of C for root growth rather than for allocation to fungal symbionts (Forsmark *et al*., 2021). In the nemoral region, exposed to elevated N deposition, ectomycorrhizal biomass decreased with increased N availability and was low in the most fertile end of the gradient (Fig. 3a).

The ectomycorrhizal fungal community shifted along the gradient, in line with our hypothesis, with progressive loss of nitrophobic (Fig. 3d), supposedly C-demanding (Saikkonen *et al*., 1999) genera, such as *Piloderma*, which are likely to rely on N mining from organic material (Pellitier & Zak, 2021). Ectomycorrhizal N mining is a C-demanding process that is generally suppressed by high inorganic N availability (Bödeker *et al*., 2014; Hobbie *et al*., 2014; Clemmensen *et al*., 2015; Entwistle *et al*., 2017, 2018; Lindahl *et al*., 2021). Accordingly, activities of manganese peroxidase and *N*-acetylglucosaminidase, proposed to be involved in ectomycorrhizal mobilisation of N from organic matter (Sterkenburg *et al*., 2018), declined at high N availability. The ratio between acid phosphatase and *N*-acetylglucosaminidase also increased with N availability in the nemoral region, indicating increased demand for organically bound P relative to N (Heuck *et al*., 2018), although P limitation of trees rarely occurs

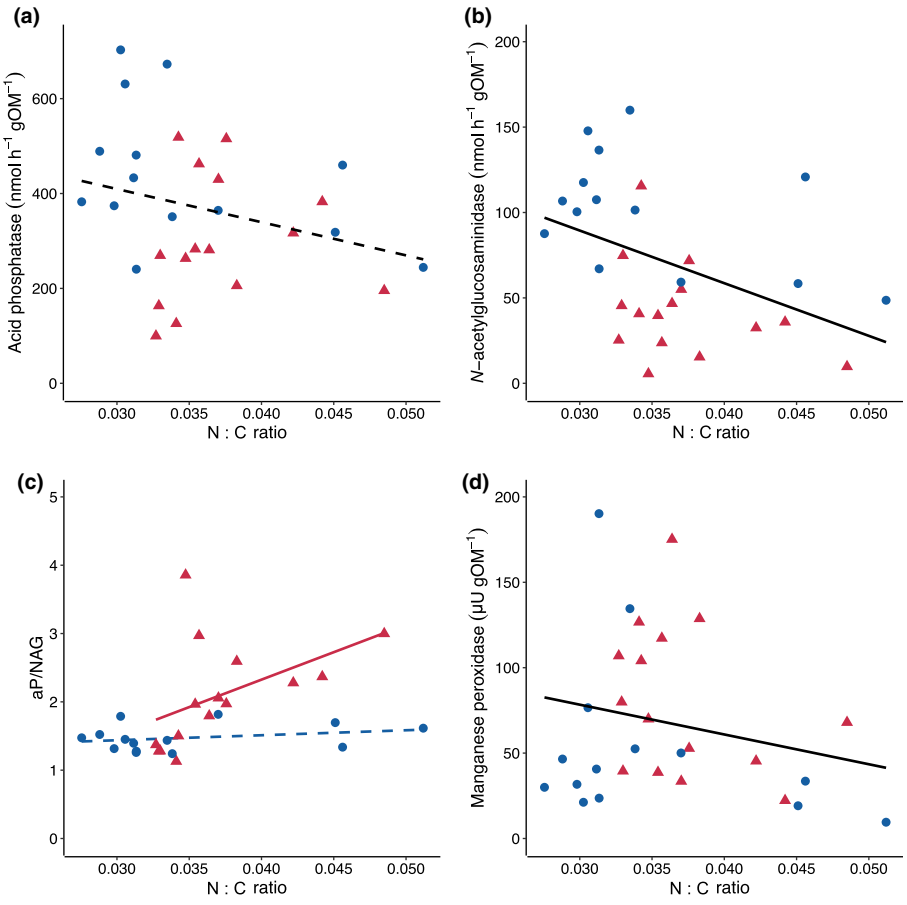

Fig. 5 Activities of extracellular enzymes estimated in the organic horizon and top 7 cm of mineral soil of *Picea abies* forests in two forest regions. (a) Acid phosphatase (b) β-1.4-*N*-acetylglucosaminidase (c) the ratio of acid phosphatase and β-1.4-*N*-acetylglucosaminidase (aP/NAG) and (d) manganese peroxidase activities plotted across gradients in soil N : C ratio. Shapes and colours denote sampled regions: boreal (low N deposition – blue circles) and nemoral (high N deposition – red triangles). Lines represent fitted linear regressions; solid = significant ($P \leq 0.05$), dashed = nonsignificant ($P \geq 0.05$), single black = significant main effect of N : C but no difference between regions, two coloured lines = region effect. See Table 5 for statistics.

Table 5 Coefficients and 95% confidence intervals (in parentheses) from linear models testing the effect of N : C ratio, region and their interaction on potential extracellular enzyme activities in the organic topsoil and top 7 cm of soil sampled from mature *Picea abies* forests in two regions in Sweden.

	Acid phosphatase		*N*-acetylglucosaminidase		Acid phosphatase/ *N*-acetylglucosaminidase		Manganese peroxidase	
Predictors	Estimates	*P*	Estimates	*P*	Estimates	*P*	Estimates	*P*
(Intercept)	6.33 (5.31 to 7.35)	**<0.001**	5.86 (4.46 to 7.26)	**<0.001**	1.22 (−0.24 to 2.68)	0.098	5.43 (3.94 to 6.92)	**<0.001**
N : C	−8.41 (−36.92 to 20.10)	0.550	−37.35 (−76.52 to 1.82)	0.061	7.31 (−33.83 to 48.45)	0.718	−50.03 (−91.71 to −8.35)	**0.021**
Region	−0.41 (−0.75 to −0.08)	**0.018**	−0.97 (−1.43 to −0.50)	**<0.001**	−2.11 (−4.89 to 0.68)	0.132	0.66 (0.16 to 1.15)	**0.011**
N : C × Region					73.03 (−2.56 to 148.62)	0.058		

Significant *P*-values ($P \leq 0.05$) in bold.

in Swedish nemoral/boreal forests even under high N addition rates (Jörgensen *et al.*, 2021).

At high N availability in the nemoral region, there was a relative increase in fast-growing (Fig. 3c) and nitrophilic (Fig. 3e) ectomycorrhizal genera, such as *Tomentella* and *Tylospora*, which may be more efficient in converting a meagre supply of host-derived C into extraradical mycelium (Jörgensen *et al.*, 2023a) and better adapted for inorganic N uptake, which potentially mitigates leaching losses (Bahr *et al.*, 2013). It is, however, important to point out that the increase in fast-growing/nitrophilic taxa was relative, not absolute and should be interpreted together with the overall decrease in ectomycorrhizal biomass. Thus, these ectomycorrhizal genera were probably not stimulated by high N availability but rather persisted better under these conditions relative to other genera. Suz *et al.* (2021) predicted an increase in ectomycorrhizal genera with contact exploration type as the ectomycorrhizal tipping-point approaches, because they are thought to produce little mycelial biomass and generally perceived as nitrotolerant or nitrophilic. In support of this, we did observe increased relative abundance of the contact type *Hyaloscypha* with increased N availability in the nemoral gradient (Fig. 4a). However, other common contact type genera such as *Russula* and *Lactarius* did not respond, and we propose that ectomycorrhizal exploration types, as currently defined, do not respond to N availability in a consistent manner (Jörgensen *et al.*, 2023a).

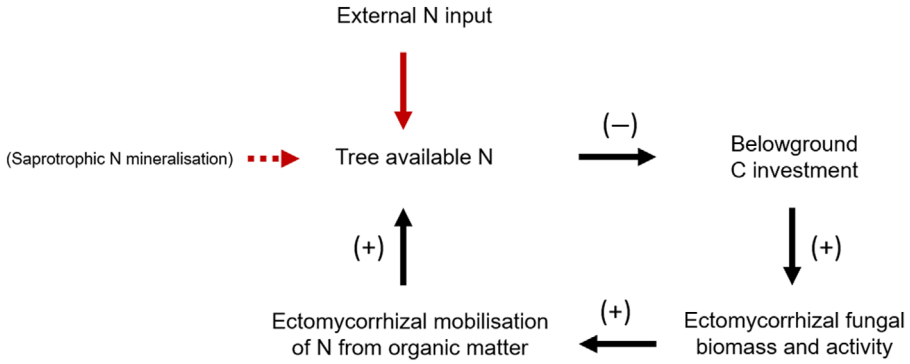

Fig. 6 Proposed stabilising feedback loop (black arrows) that maintains high ectomycorrhizal biomass in naturally fertile boreal forests with low N deposition, but which is disrupted in forests subjected to high rates of external N input. In the feedback loop, high levels of plant-available N decrease belowground C investment in ectomycorrhiza, leading to reduced mycorrhizal acquisition of N from organic matter and subsequent delivery to trees. This constraint on nutrient uptake restores tree C investment in mycorrhiza, stabilising mycorrhizal biomass and activity. However, this feedback is disrupted by externally applied inorganic N (red, solid), which make trees less dependent on ectomycorrhizal fungi, leading to overall lower belowground C investment and declining ectomycorrhizal biomass and activity. Although saprotrophic organisms can mineralise N and contribute to the plant available pool (red, hatched), N mineralisation is, generally, low in strongly nutrient-limited, ectomycorrhizal-dominated coniferous forests.

The nitrophobic/nitrophilic classification largely overlapped with our fast/slow categorisation but yielded stronger responses to N availability. All fast-growing genera were also classified as nitrophilic. However, there were some notable differences among slow-growing genera; *Hyaloscypha* and *Hygrophorus* were classified as nitrophilic in spite of slow extraradical growth (Jörgensen *et al.*, 2023a). By contrast, the nitrophobic *Cenococcum* and *Russula* were not classified as fast or slow but tended to have slow extraradical growth. The better performance of the nitrophobic/-nitrophilic classification largely depended on the inconsistent and abundant *Hyaloscypha*, which also diverge by being an ascomycete more closely related to ericoid mycorrhizal fungi.

In both regions, we observed decreasing manganese peroxidase (Fig. 5d) and *N*-acetylglucosaminidase activities (Fig. 5b) as N availability increased, supposedly indicating reduced N mining from organic matter. However, in the boreal region ectomycorrhizal fungal biomass was stable across the N availability gradient, and the relative abundances of fast-growing, and nitrophilic taxa did not increase in N-rich stands. This was despite that the forests at the rich end of the boreal gradient were situated on mull soils with high pH, which were particularly fertile in a boreal forest context. In a previous study, spanning a wider fertility range that also included more nutrient-poor *Pinus* stands in the boreal region, Sterkenburg *et al.* (2015) observed increased ectomycorrhizal abundance at higher fertility. Similarly, Högberg *et al.* (2021) found ectomycorrhizal biomass production to increase along a gradient of increasing fertility, although drastically decreasing at the richest end of the gradient (an exceptional site with high nitrate levels).

We propose that stable ectomycorrhizal biomass along the boreal fertility gradient depended on a balanced co-limitation by N and C of mycorrhizal fungal growth. Elevated N status of trees would reduce C investment in ectomycorrhiza and decrease ectomycorrhizal biomass and N acquisition from soil pools. However, in boreal forests, where N-mineralisation is low and trees largely depend on ectomycorrhizal N mobilisation from organic stocks (Phillips *et al.*, 2013), reduced ectomycorrhizal activity may have a negative impact on tree nutrition, consequently restoring tree C supply to roots and associated fungi (Fig. 6). Such a stabilising feedback may act to maintain ectomycorrhizal biomass along gradients of natural fertility in systems where trees rely strongly on N uptake via ectomycorrhizal mechanisms. We further propose that this feedback loop was disrupted by high input of external N in the nemoral gradient, where trees increasingly may take up inorganic N directly rather than spending C on promoting ectomycorrhizal nutrient acquisition from organic matter. Furthermore, reduced ectomycorrhizal fungal abundance and decomposer activity may drive observed increases in accumulation of organic matter in the topsoil after fertilisation of boreal forests (Jörgensen *et al.*, 2022). This postulation may potentially be tested, for example by comparing ^{15}N abundances in soils and foliage (Högberg *et al.*, 2020) across gradients with different modes of N supply (natural or anthropogenic).

Conclusion

As hypothesised, the responses of ectomycorrhizal fungi to variation in N supply differed between two regions with low or high N deposition, despite similar ranges in N : C and inorganic N concentration across both gradients. This suggests that N from anthropogenic input has different effects on ectomycorrhizal fungi than N stemming from endogenous processes. Thereby, N deposition alters how the ectomycorrhizal fungal community responds to overall variation in forest N availability. In the nemoral region with atmospheric N deposition around critical threshold levels (van der Linde *et al.*, 2018) ectomycorrhizal fungal biomass, particularly that of nitrophobic genera, decreased drastically at high N levels. However, despite some shifts in the community composition and enzyme activities towards more fertile forests, we did not observe a similar decline even in the most fertile forests in the boreal region.

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Competing interests

None declared.

Author contributions

BDL, HW, KEC and KJ designed the study. BDL, KEC and KJ performed field sampling. KJ performed laboratory analyses and statistical analyses and wrote the first draft of the manuscript. All authors contributed to the interpretation of results and revisions of the manuscript.

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Data availability

Data and code needed to reproduce analyses are available on Dryad Digital Repository and Zenodo (doi: 10.5061/dryad.5hqbzkhcp; Jörgensen *et al.*, 2023b). Sequence data are published in NCBI-SRA under project PRJNA796801.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Ergosterol concentrations and relative abundance of ectomycorrhizal fungi across the N : C gradients.

Table S1 Summary of ectomycorrhizal fungal responses to N fertilisation/N deposition/natural fertility in forested ecosystems from previously published studies.

Table S2 Model output of linear models testing relationships between N : C ratio and inorganic N concentrations.

Table S3 Model output of linear models testing the relationship between N : C ratio and pH ratio.

Table S4 Relative abundance of ectomycorrhizal fungal genera in the core community.

Table S5 Model output of linear models testing the relationship between N : C ratio and ergosterol concentrations.

Table S6 Model output of linear models testing relationships between N : C and relative abundance of ectomycorrhizal fungi in the different regions.

Table S7 Model output of linear models testing relationships between N : C, region on growth speed (slow/fast) on the relative abundance of ectomycorrhizal fungal genera.

Table S8 Model output of linear models testing relationships between N : C and relative abundance of fast-growing ectomycorrhizal fungi.

Table S9 Model output of linear models testing relationships between N : C, region on N-response (nitrophobic/nitrophilic) on the relative abundance of ectomycorrhizal fungal genera.

Table S10 Model output of linear models testing relationships between N : C and relative abundance of nitrophobic/nitrophilic ectomycorrhizal fungi.

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