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Title: Peatland fungal community responses to nutrient enrichment: a story beyond nitrogen

Running title: N and PK additions changed fungal community

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Abstract

Anthropogenically elevated inputs of nitrogen (N), phosphorus (P) and potassium (K) can affect the carbon (C) budget of nutrient-poor peatlands. Fungi are intimately tied to peatland C budgets due to their roles in organic matter decomposition and symbioses with primary producers; however the influence of fertilization on peatland fungal composition and diversity remains unclear. Here we examined the effect of fertilization over 10-yrs on fungal diversity, composition, and functional guilds along an acrotelm (10-20 cm), mesotelm (30-40 cm) and catotelm (60-70 cm) depth gradient at the Mer Bleue bog, Canada. Simultaneous N and PK addition decreased the relative abundance of ericoid mycorrhizal fungi (ErMF) and increased ectomycorrhizal fungi (EcMF) and lignocellulose-degrading fungi. Fertilization effects were not more pronounced in the acrotelm relative to the catotelm, nor was there a shift towards nitrophilic taxa after N addition. The direct effect of fertilization significantly decreased the abundance of *Sphagnum*-associated fungi, primarily owing to the overarching role of limiting nutrients rather than a decline in *Sphagnum* cover. Increased nutrient loading may threaten peatland C stocks if lignocellulose-degrading fungi become abundant and accelerate decomposition of recalcitrant organic matter. Additionally, future changes in plant communities, strong water table fluctuations, and peat subsidence after long-term nutrient loading may also influence fungal functional guilds and depth-dependencies of fungal community structure.

KEYWORDS: co-limitation; fertilization; fungal guilds; stratification; vegetation; peatland

1 | INTRODUCTION

Anthropogenic activities have increased the availability, and altered the stoichiometry, of nutrients such as nitrogen (N) and phosphorus (P) in nutrient-limited ecosystems (Peñuelas et al., 2013; Wang et al., 2017). The imbalance in global atmospheric inorganic N and P deposition has resulted in a lower N:P ratio in deposition and alteration to ecosystem processes in N and P deficient ecosystems (Ackerman et al., 2019; Du et al., 2020; Peñuelas et al., 2013; Wang et al., 2017). N enrichment may also be increasing potassium (K) limitation (Hoosbeek et al., 2002). However, there is a dearth of knowledge regarding the direct and indirect long-term effects of N and P enrichment on peatland soil microorganisms (Li et al., 2021).

Peatlands are one of the largest global soil carbon (C) reservoirs (Loisel et al., 2014). Acidic *Sphagnum* peatlands (bogs and poor fens) are widespread in northern latitudes and have low nutrient availabilities owing to slow rates of organic matter decomposition, hydrologic isolation, and limited atmospheric nutrient input under non-anthropogenically altered conditions (Charman, 2002; Rydin & Jeglum, 2013). Research has largely focused on the detrimental effects of elevated N deposition on peatlands (Bragazza et al., 2012; Sheppard et al., 2014), with less focus on P, despite its integral role in peatland biogeochemistry (Bubier et al., 2007; Fritz et al., 2012; Schillereff et al., 2021). In ombrotrophic (precipitation-fed) peatlands (i.e., bogs), plant growth and microbial activities are often limited by P or co-limited by N and P as they receive nutrients exclusively from atmospheric deposition (Bridgham et al., 1996; Hill et al., 2014; Lin et al., 2014a; Wang et al., 2016) or N fixation (Yin et al., 2022; Živković et al., 2022). For example, at Mer Bleue bog in southeastern Canada, the combined addition of N, P and K showed a more profound effect on shrub biomass production and litter decomposition than N-only fertilization after 7 to 12-yrs of treatment (Larmola et al., 2013; Moore et al., 2019).

Changes in plant community composition arising from nutrient addition can also have long-term consequences for peatland C cycling. Although *Sphagnum* mosses have long been hypothesized to constrain peat decomposition (Bengtsson et al., 2018; Pipes & Yavitt, 2022; van Breemen, 1995), recent studies have demonstrated that the large quantity of polyphenolics in vascular litters could also be an important constraint on peat decomposition (Fenner & Freeman, 2020). Shifts from dominance by *Sphagnum* mosses to vascular plants (especially evergreen shrubs), may change litter quality, slowing decomposition rates and leading to C accumulation in peatlands (Fenner & Freeman, 2020; Li et al., 2021; Wang et al., 2015). Additionally, ectomycorrhizal trees and ericaceous shrubs differ in mycorrhizal symbionts, with possible consequences for alteration of decomposition dynamics that could affect a peatland's ability to sequester C (Defrenne et al., 2023; Hupperts et al., 2022).

Our current understanding of the effect of nutrient deposition on peatland fungal communities remains fragmentary (Andersen et al., 2013). Cao et al. (2022) found that long-term N and P additions had stronger effects on fungal community compared to short-term additions. Short-term increased N deposition has been observed to favor bacterial growth owing to the changes in peat chemistry (Bragazza et al., 2012), whereas the simultaneous addition of N, P and K increased fungal biomass (Basiliko et al., 2006). Long-term nutrient deposition may exert cascading effects on microbial community composition and diversity via the changes in vegetation composition and cover. For example, the encroachment of shrubs at the expense of *Sphagnum* moss after long-term N deposition (Larmola et al., 2013; Li et al., 2019) was detrimental to fungi associated with *Sphagna* via endophytic or symbiotic relationships (i.e., *Sphagnum*-associated fungi) but advantageous to fungi associated with ericaceous shrubs and trees, especially mycorrhizal fungi (Andersen et al., 2013). Within functional guilds, communities may shift as a response to nutrient addition. For example, ectomycorrhizal fungal (EcMF) species have been found to differ greatly in their response to N fertilization in uplands, with a decline in so-called nitrophobic fungi and an increase in nitrophilic fungi (Lilleskov et al., 2011, 2024). However, there have not been studies exploring whether this pattern holds in peatlands.

N fertilization has been hypothesized to shift fungal communities away from ericoid mycorrhizal fungi (ErMF) and EcMF, and toward saprotrophic basidiomycetes. ErMF primarily function to access limiting nutrients locked in organic matter and can reduce saprotroph activity via nutrient competition (Wiederman et al., 2017). In addition, saprotrophic basidiomycetes are reported to have a higher nutrient requirement than ascomycetes that dominate the ericoid mycorrhizal symbiosis (Treseder et al., 2018). N addition has been shown to increase ecosystem C loss by reducing mycorrhizal fungal activity, and probably enhancing saprotrophic activity (Vesala et al., 2021). However, ErMF colonization in ericaceous shrub roots was found to increase with fertilization at the same study site (Kiheri et al., 2020), potentially due to the capacity of ErMF to switch from mutualistic to saprotrophic lifestyle (Martino et al., 2018) or because colonization frequency may not reflect the functional status of mycorrhizal interaction. Determining the shifts in the composition of key fungal functional guilds in response to long-term changes in N and P deposition becomes important considering their essential roles in nutrient cycling and organic matter decomposition in peatlands.

Vertical stratification of fungal communities and metabolic activities is often observed in peatlands in response to increasing anoxia, root distribution, and changing substrate quality with depth (Andersen et al., 2013; Lamit et al., 2017, 2021; Lin et al., 2014b; Wang et al., 2019). It remains unclear whether the vertical stratification of fungal communities is affected by nutrient enrichment. The aforementioned nutrient-mediated shift in plant community structure and root morphology may exert cascading effects on fungi, especially mycorrhizal fungi. For example, the deeper-rooted sedges which lack mycorrhizal associations use aerenchyma to moderate the distinctive resource gradient with depth by providing oxygen and root exudates to a deeper layer than ericaceous shrubs, which lack aerenchyma (Lamit et al., 2017, 2021). In contrast, ericaceous shrubs may drive fungal community differences between surface and subsurface layers owing to their C subsidy of ErMF in the surface horizon where their roots reside (Lamit et al., 2017, 2021).

We used a pair of long-term nutrient addition experiments in an ombrotrophic peatland in southeastern Canada to examine the influence of nutrient addition on fungal communities. We specifically hypothesized that the long-term addition of N, P and K will alter fungal community structure by (H1) decreasing the relative abundance of ErMF and increasing the relative abundance of saprotrophic species in general, and white-rot basidiomycetes in particular; (H2) changing fungal relative abundance primarily in the acrotelm while minimally in the catotelm; (H3) shifting the ectomycorrhizal community from dominance by nitrophobic to nitrophilic taxa; and lastly, (H4) reducing the relative abundance of *Sphagnum*-associated fungi driven by the decline in *Sphagnum* cover.

2 | MATERIALS AND METHODS

2.1 | Study site

This study was conducted at Mer Bleue, an ombrotrophic peatland in southeastern Ontario (45.41° N, 75.52° W). The mean annual air temperature and precipitation are 6 °C and 943 mm, respectively (Canadian Climate Normals, 1981-2010). The bog plant community is dominated by Ericaceae (*Rhododendron groenlandicum* (Oeder) Kron & Judd, *Chamaedaphne calyculata* (L.) Moench and *Kalmia angustifolia* L.) underlain by *Sphagnum* mosses (mainly *S. capillifolium* (Ehrh.) Hedw. and *S. magellanicum* Brid.) and

Polytrichum strictum (Menzies ex Brid.). Deciduous shrubs (mainly *Vaccinium myrtilloides* Michx.), sedges (*Eriophorum vaginatum* L.), and ectomycorrhizal trees (*Larix laricina* (Du Roi) K.Koch, *Picea mariana* (Mill.) Britton, Sterns & Poggenb. and *Betula populifolia* Marshall), are distributed sparsely. Of particular relevance to fungal communities, a few scattered larger trees (mostly *B. populifolia* and *L. laricina*) grew at the experimental site only after the initiation of the experiment, likely a fertilization effect as this did not occur away from the site. The effect of this patchy tree distribution was expected to lead to patchy root colonization belowground, and hence add noise to our analysis of EcMF communities. For more details on the Mer Bleue site see Bubier et al*.* (2007) and Moore et al*.* (2019).

2.2 | Fertilization experiments

Two sets of fertilization experiments were established in 2000-2001 and 2005, respectively (Table 1). Experiment 1 included six treatments with different rates of N additions with or without P and K. To better understand the effect of different rates of N addition alone, Experiment 2 was established in 2005. In both experiments, triplicate plots (3 m x 3 m) were fertilized every three weeks from early May to late August (7 times per year) by applying ammonium nitrate (for N treatments) and/or mono potassium phosphate (for PK treatments). The two experiments combined represented a total of 27 plots (9 treatments x 3 replicates) and were established on large hummocks which covered 70% of the terrain. There have been no changes to the distribution of hummocks versus hollows with the fertilization treatments.

The rates of N addition were chosen to complement the estimated annual atmospheric deposition of 0.6 – 0.8 g N m^{-2} yr^{-1} in the Mer Bleue region and to raise them to levels encountered in Europe and elsewhere, affected by elevated N deposition, by adding 1.6, 3.2 and 6.4 g N m^{-2} yr^{-1} (Bubier et al., 2007). Rates of N_2 fixation at Mer Bleue bog are small, ~0.3 g m^{-2} yr^{-1}, compared to other peatlands (Yin et al., 2022) and are decreased by N addition and increased by P addition (Živković et al., 2022).

2.3 | Vegetation survey

Plant communities were characterized by the point intercept method of Larmola et al. (2013) in July 2014. The number of times ('hits') a specific plant species and organ (leaf/woody stem/flower/moss shoot) contacted a metal rod (4 mm in radius) over 61 grid points in a 60 cm x 60 cm frame were recorded. *Sphagnum* cover (%) was estimated by the number of hits divided by 61 and multiplying by 100. Vascular species abundance (not identical to cover) was estimated by the total number of hits of all organs per m^2 for each species.

2.4 | Peat sampling

In mid-July 2014, a single peat core was taken from a location selected at random within each plot, with 3 depth increments saved per core (27 cores x 3 depth increments = 81 samples total). The upper two depth increments of peat (10-20 and 30-40 cm below the peat surface, 10 cm length x 10 cm width x 10 cm height) were collected using a clean bread knife, and an Eijkelkamp auger (Eijkelkamp Soil & Water, Giesbeek, The Netherlands) was used for deeper peat (60-70 cm, 5.2 cm diameter x 10 cm height). At Mer Bleue, the uppermost layer (10-20 cm) represents the typical acrotelm peat which is rarely saturated (i.e., oxic) while the lowermost layer (60-70 cm) is from the catotelm where peat is rarely above the water table (i.e., anoxic). The middle layer (30-40 cm) is from the mesotelm through which the water table has seasonal oscillations and thus it is a biogeochemical 'hotspot' where roots never dry out and are rarely exposed to anoxic conditions in the growing season. At the time of collection, water table depth and the location of the water table relative to the mid-point of the depth of the peat sample were determined in each core hole and peat temperature was measured from each depth increment. Samples were subdivided into two subsamples (for DNA analyses and physicochemical analyses), transported to the laboratory on dry ice, stored at -20°C, and shipped to the USDA Forest Service, Northern Research station (Houghton, MI, USA) where they were stored at -20°C until further processing.

2.5 | Physicochemical properties of peat

A variety of elemental concentrations, pH, humification index and organic chemical composition of peat were measured. Using moist peat from each sample, humification level was characterized using the von Post humification index (Von Post, 1922), and peat pH was measured using a 2:1 ratio (volume:volume) of distilled water to peat. A subsample of peat was oven-dried at 60 °C to a constant weight and ground using a Wiley Mini Mill (Thomas Scientific, Swedesboro, NJ, USA) with size 60 mesh. Total C, N, and S concentrations were determined by dry combustion on a VarioMacro CNS Analyzer (Elementar Gmbh, Langenselbold, Germany) in the Watmough lab at Trent University as described in Watmough et al*.* (2022). P and K were analyzed using ICP-MS (Varian 810, now part of Agilent Technologies, Santa Clara, CA, USA) in the Spiers lab at Laurentian University after combustion with a muffle furnace and a modified EPA3050A block digestion (see Birnbaum et al*.*, 2023 for details). Fourier-transform infrared spectroscopy (FTIR) analysis of dried peat and assignment of peaks to carbohydrate and aromatic classes was carried out in the Chanton lab at Florida State University, as described in Verbeke et al*.* (2022).

2.6 | DNA extraction, amplicon library preparation and sequencing

In a 50 mL centrifuge tube, 10 mL of wet peat was placed with twenty 3.2 mm chrome-steel beads. The sample was pulverized for two minutes on a modified mini-beadbeater-96 (BioSpec Products, Bartlesville, OK, USA). A subsample of 0.5 g pulverized peat was used for DNA extraction with a PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA, now Qiagen). The extraction procedure followed the manufacturer's instructions, with the addition of a 10-min vortex followed by incubation at 65 °C for 30-min, all in the C1 lysis buffer. DNA was cleaned using a MoBio PowerClean® Pro DNA Clean-Up Kit and quantified with a Qubit Fluorometer (Invitrogen, Life Technologies, Carlsbad, CA, USA).

Cleaned DNA extracts were pooled and sequenced at the U.S. Department of Energy Joint Genome Institute (JGI, Walnut Creek, CA, USA). The amplicon library preparation and sequencing were performed following Joint Genome Institute Protocols for Illumina MiSeq community amplicon sequencing (Coleman-Derr et al., 2016; Lamit et al., 2017). Briefly, the fungal ITS2 region was targeted with the forward primer fITS9 (GAACGCAGCRAANNGYGA) (Ihrmark et al., 2012) and the reverse primer ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990). The full-length primer was composed of an Illumina adapter, an 11-bp barcode unique to each sample on the reverse primer, a 10-bp primer pad, a 3-bp spacer pad and the primer sequence (Lamit et al., 2017). DNA samples were combined in equimolar aliquots and sequenced on an Illumina Miseq platform (Illumina, Inc., San Diego, CA, USA) with 2×300 bp chemistry. Eight samples were lost due to poor sequencing, which occurred independently and were not treatment or depth specific.

2.7 | Bioinformatics analyses

Paired-end reads were demultiplexed according to their unique barcodes with Qiime 1.9.1 (Caporaso et al., 2010), and then PhiX 174, Illumina adapters and human contaminants were filtered using BBduk (https://sourceforge.net/projects/bbmap/). Cutadapt was used to remove primers and paired-end reads were merged by BBmerge (Bushnell et al., 2017). The merged reads were discarded if the expected errors (calculated based on error probabilities from Phred scores) exceeded one, if there were Ns, or if the sequence length was shorter than 250-bp using USEARCH (Edgar & Flyvbjerg, 2015). The ITS2 regions were extracted by ITSx (Bengtsson-Palme et al., 2013). Reference-based chimeras were detected with UCHIME2 (Edgar, 2016) using the UNITE database (2017-06-28 release; https://unite.ut.ee/) (Nilsson et al., 2015). The subsequent reads were dereplicated and operational taxonomic units (OTUs) clustered with the default 97% similarity using USPARSE-OTU algorithm (Edgar, 2013).

The generated OTUs were assigned to taxonomy using Qiime with the BLAST algorithm and UNITE database (2020-02-20 release). OTUs classified as nonfungal, no BLAST hit, or assigned only to fungal class or higher, were subjected to manual BLASTn searches in the NCBI nucleotide database. Fungal OTUs were only retained if the BLASTn hits had a percent identity match of at least 75%, with coverage of at least 50% of the sequence length, and if there were no better matches with nonfungal organisms. Fungal OTUs were assigned to putative functional guilds using FUNGuild and FungalTraits (Põlme et al., 2020), and the assignments were refined, if necessary, based on literature searches and our expertise in fungal ecology in peatlands (Wang et al., 2024). The OTU matrix was rarefied using the number of sequences corresponding to the sample with the least reads (i.e., 100,251).

2.8 | Statistical analyses

A variety of statistical analyses were used to address our hypotheses about fertilization effects on fungal communities and their depth-dependency. Except for the Permutational analysis of variance (PERMANOVA), statistical analyses were performed on the two experiments separately.

Differences between fertilization treatments and sampling depths in OTU richness, Shannon’s diversity index, Pielou’s evenness index, the relative abundances of different fungal functional guilds were examined using linear mixed model with *lmerTest* package (Kuznetsova et al., 2017) in R 4.1.2 (R Core Team, 2021). The linear mixed models included fertilization treatments, sampling depths and their interactions as fixed factors (both were categorical variables), and individual peat core as a random factor nested within treatment. The mixed models were fitted with Kenward-Roger approximation for *F*-test. If there was evidence for an interactive effect, *emmeans* package (Lenth, 2021) was used to obtain marginal means and conducted *post hoc* pairwise comparisons. If there was no evidence for an interactive effect, only the main effects (fertilization treatment or sampling depth) were shown.

Differences between fertilization treatments in vegetation abundance (total abundance of ericaceous shrubs, and cover of *Sphagnum* or *Polytrichum* mosses) were assessed using one-way ANOVA followed by Tukey's multiple comparisons. The canonical analysis of principal coordinates (CAP; Anderson & Willis, 2003) with Bray-Curtis dissimilarity was conducted to visualize the overall responses of fungal community using PRIMER 7.0.21 (PRIMER-e, Quest Research Limited, Auckland, New Zealand).

The PERMANOVA with Bray-Curtis dissimilarity was used to examine the effects of fertilization treatments and sampling depths on OTU composition, followed by a test of homogeneity of multivariance dispersions (PERMDISP), using PRIMER 7.0.21. The PERMANOVA models included fertilization treatments, sampling depths and their interactions as fixed factors, and individual peat core as a random factor nested within treatment. The PERMANOVAs were conducted for two experiments separately, as well as the dataset with two experiments pooled together. The OTU matrices were fourth-root transformed to downweight the influence of most abundant taxa prior to the analysis.

The *indicspecies* package (De Cáceres & Legendre, 2009) was used to identify OTUs with specific preferences to certain fertilization treatments or sampling depths. The contribution of plants (total abundance of ericaceous shrubs, and cover of *Sphagnum* or *Polytrichum* mosses) and environmental variables (C, N, P and K concentrations; pH; von Post humification index; concentrations of carbohydrates and aromatic compounds; relative water table depth) to *Sphagnum*-associated fungal composition was assessed by hierarchical partitioning using *rdacca.hp* package. The hierarchical partitioning algorithm enables the estimation of the relative importance of individual predictors by considering their unique contribution to the total model R^2, along with their average shared contributions with other predictors, which is useful when dealing with complex datasets when there are potential collinearities among predictors (or matrices of predictors) (Lai et al., 2022). It calculates the variable importance from all subset models, leading to an unordered assessment of importance. Variables with negative values of 'individual importance' (Supplementary Table S1) correspond to cases where the predictor variables explain less variation than random normal variables and therefore were removed until all remaining variables had positive values. Eventually the 'individual percent' (i.e., the individual effect of each variable divided by total adjusted R^2 from 'individual importance') were used to show the relative contribution of plants and environmental variables to the compositions of *Sphagnum*-associated fungi (Supplementary Table S1).

In this study, we adopt a language of evidence, following the approach outlined by Muff et al. (2021). Instead of describing results as significant or not, we describe our results along a spectrum of evidence, ranging from very strong evidence (equivalent to $p < 0.001$), strong evidence ($p < 0.01$), moderate evidence ($p < 0.05$), weak evidence ($p < 0.1$), to no evidence ($p \geq 0.1$).

3 | RESULTS

3.1 | Fungal composition and diversity responses to fertilization

The rarefied dataset contained 73 samples (*n* = 48 in Experiment 1, and *n* = 25 in Experiment 2; see Materials and Methods for details of missing samples) and 7,318,323 sequences in total (4,812,048 per Experiment 1, and 2,506,275 per Experiment 2), with an average of 220 OTUs per sample (SD = 110, Range = 63-537). Overall, fungal communities were dominated by Helotiales (Ascomycota), followed by Agaricales (Basidiomycota), Mortierellales (Mortierellomycota), Sebacinales (Basidiomycota) and Thelephorales (Basidiomycota) (Figure 1).

At the genus level, there was weak evidence that the relative abundance of the dominant ErMF genus *Hyaloscypha* (formerly *Pezoloma*, Fehrer et al., 2019) decreased after N+PK additions ($p < 0.1$; Figure 3a; Table S4). Additionally, there was weak to moderate evidence that the relative abundances of *Mortierella* ($p < 0.05$), *Thelephora* ($p < 0.05$) and *Trechispora* ($p < 0.1$) increased after the additions of N and N+PK at high rates.

We found that long-term N addition shifted fungal community diversity and composition, and these effects tended to be most pronounced when P and K were also added. There was moderate evidence that N+PK additions increased Shannon's diversity index ($p < 0.05$) via the concomitant increase of OTU richness and Pielou's evenness ($p < 0.05$; Table 3). Moreover, there was moderate to very strong evidence that both treatment ($p < 0.05$) and depth ($p < 0.001$) affected fungal OTU composition, while there was no evidence for an interaction between treatment and depth ($p > 0.1$; Figure 2; Table 2). Fungal indicator OTUs of the control were mainly EcMF and endophytes, whereas fertilization (especially N+PK) increased the relative abundance of saprotrophs and pathogens (Table S2).

3.2 | Effect of fertilization on functional guilds

In support of H1, there was weak to moderate evidence that N+PK additions decreased the relative abundance of ErMF ($p < 0.05$; Figure 4b;

Table S5), and increased the relative abundances of lignocellulose-degrading fungi ($p < 0.1$; Figure 5a), saprotrophs ($p < 0.05$; Figure 5d) and dark septate root endophyte ($p < 0.1$; Figure S1a). However, except for EcMF, the results of fertilization effect on the relative abundance of mycorrhizal or saprotrophic fungi at different depths did not support H2. Unexpectedly, there was very strong evidence that the effect of N+PK additions on ErMF abundance was most profound at the depth of 60-70 cm in Experiment 1 ($p < 0.001$; Figure 4b). In contrast, there was moderate evidence for the strongest effect of N-only addition on EcMF abundance at the depth of 10-20 cm in Experiment 2 ($p < 0.05$; Figure 4f; Table S5). Additionally, there was no evidence for depth-dependent responses to nutrient additions for lignocellulose-degrading and other saprotrophic fungi, and dark septate root endophytes($p > 0.1$; Figures 5 and S1).

The results of fertilization effect on nitrophilic and nitrophobic taxa of EcMF community did not support H3. There was, to the contrary, weak to moderate evidence that the additions of N+PK ($p < 0.1$) or high rate of N individually (3.2 and 6.4N; $p < 0.05$) increased the relative abundance of EcMF (Figure 4d), including both the nitrophobic and nitrophilic taxa (Table 4).

The relative abundance of *Sphagnum*-associated fungi decreased after nutrient additions and was apparently better predicted by fertilization than *Sphagnum* cover (Table S1), which did not support H4. There was weak evidence that PK, 3.2N+PK and 6.4N treatments decreased the relative abundance of *Sphagnum*-associated fungi ($p < 0.1$; Figure 4g; Table S6). Additionally, there was weak evidence that the most abundant *Sphagnum*-associated fungal species, *Clavaria sphagnicola*, declined after PK or N+PK additions ($p < 0.1$; Figure S2a), while the second most abundant species, *Entoloma chamaemori,* remained largely unaffected. Similarly, there was weak evidence that N additions (especially at high rates) increased the relative abundance of *C. sphagnicola* but decreased that of *E. chamaemori*, *Hygrocybe miniata*, *Pseudoplectania episphagnum*, and *Galerina* spp. ($p <$ 0.1; Figure S2a).

3.3 | Vertical stratification of functional guilds

There was moderate evidence that OTU composition at 60-70 cm differed most strongly from the 10-20 cm depth ($p < 0.05$; Figure 2b). Similarly, there was moderate to strong evidence that OTU richness declined with depth ($p <$ 0.01), while Pielou's evenness increased with depth ($p < 0.05$; Table 3).

The lower relative abundance of mycorrhizal functional guilds at the deep peat layer and the lack of vertical stratification for other functional guilds only partially supported H2. There was moderate to strong evidence that the relative abundances of ErMF was the lowest at the depth of 60-70 cm after the addition of N ($p < 0.05$) or PK ($p < 0.01$) individually in Experiment 1 (Figure 4b). Similarly, there was strong evidence that a sharp decline in the relative abundance of ErMF at the depth of 60-70 cm compared to the upper two depths was observed in Experiment 2 ($p < 0.01$; Figure 4c; Table S5). Additionally, there was strong evidence that the relative abundance of EcMF decreased with depth under the 6.4N treatment ($p < 0.01$; Figure 4f). No evidence of vertical stratification was observed for *Sphagnum*-associated fungi ($p > 0.1$; Figure 4h,i), lignocellulose-degrading fungi ($p > 0.1$; Figure 5b,c), saprotrophs ($p > 0.1$; Figure 5e,f), or dark septate root endophytes ($p >$ 0.1; Figure S1b,c).

3.4 | Effect of fertilization on vegetation abundance

Responses of plant community to fertilization were primarily exhibited by the moss layer. There was no evidence that the abundance of Ericaceae (at the family-level) responded to fertilization ($p > 0.1$), with moderate evidence that the abundance of *C. calyculata* increased at the 3.2N treatment compared to the control ($p < 0.05$; Figure 6a). In contrast, there was moderate to strong evidence that N, PK or N+PK fertilizations generally reduced the cover of *Sphagnum* (*p* < 0.01) and *Polytrichum* mosses (*p* < 0.05), with stronger effect of N+PK than N-only treatments (Figure 6b,c).

4 | DISCUSSION

4.1 | Fungal community responses to fertilization: overall effects

Long-term N addition did not strongly alter fungal diversity, except at the highest rate, which even enriched fungal diversity, contrasting with the consensus that fertilization often reduces fungal diversity (Allison et al., 2007; Liu et al., 2012). Our finding that the changes in fungal guild were more pronounced with the addition of N+PK vs. N alone could be related to a tendency of N and P co-limitation of plant and microbial communities at Mer Bleue bog (Larmola et al., 2013; Wang et al., 2016).

4.2 | Mycorrhizal versus saprotrophic fungi in response to fertilization

Our previous work using quantitative PCR of a fungal DNA marker suggested minimal change in absolute fungal biomass after N+PK additions (Guo, 2015), which supports the interpretation that the decrease in relative abundance of ErMF in our study reflects the suppression of ErMF (i.e., decline in absolute abundance) instead of the stimulation of other guilds. This decline might reflect lower allocation by hosts to ErMF partners under elevated nutrient availability. ErMF might also be suppressed because the loss of living *Sphagnum* and *Polytrichum* mosses has led to peat subsidence (Juutinen et al., 2018), which increased soil wetness, unfavorable to either partner in the ericoid mycorrhizal symbiosis.

In contrast to ErMF, we found that dark septate root endophytes were more abundant in N or N+PK additions. Dark septate root endophytic fungi may be better adapted to periodic waterlogged conditions than mycorrhizal fungi in peatlands (e.g., Kiheri et al., 2020). The role of dark septation root endophytic fungi in soil organic matter decomposition warrants additional study (Netherway et al., 2024).

The observed increase in saprotrophs concomitant with the decrease in ErMF bears further examination from a functional perspective. The increase in the abundance of saprotrophs, especially lignocellulose-degrading fungi at the expense of mycorrhizal fungi after fertilization, could weaken the ‘Gadgil effect’, where the competition between mycorrhizal and saprotrophic fungi leads to the suppression of decomposition (Fernandez & Kennedy, 2016; Gadgil & Gadgil, 1975), and might contribute to the enhanced organic matter decomposition in northern peatlands (Larmola et al., 2013; Limpens et al., 2011). However, N addition can also lead to suppression of decomposition and soil oxidative enzyme activity (e.g., Berg & Matzner, 1997; Bowden et al., 2019). Such altered community functional changes likely represent impacts on both saprotroph- and mycorrhizally-mediated decomposition (Lindahl & Tunlid, 2015; Zak et al., 2019), so changes observed in these communities in the present study justify deeper investigation into the links between fertility, fungal community composition, and decomposition in peatlands (Defrenne et al., 2023).

In contrast, the increased relative abundance of EcMF under N+PK addition and high rate of N addition runs counter to the general finding of decrease in mycorrhizal abundance after fertilization (Lilleskov et al., 2024). A key difference in the present study is the fertilization effect on tree encroachment on these peatlands. The invasion of trees has been observed in several heavily fertilized plots (T.R. Moore et al., unpublished), which may account for the increasing abundance of EcMF related to the control. We hypothesize that the different responses of ErMF and EcMF may be amplified by differential C allocation belowground between shrubs and trees in response to nutrient patches: whereas belowground allocation by shrubs would likely be suppressed by complete fertilization (i.e., N+PK), that by trees could be stimulated. This likely difference arises from the experimental design of the current study, in which the size of the plots (3 m x 3 m) encompasses entire shrub plants, but only small portions of the root systems of the trees, favoring preferential tree root proliferation in nutrient-enriched plots (e.g., Hodge, 2004). The differences in the foraging strategy of the Ericaceae *versus* tree root systems would only reinforce this. Bog specialist Ericaceae roots form adventitiously on buried shrub stems and do not extend far from those stems (Lems, 1956; MacDonald et al., 1995), constraining them to the plot in which they are growing; in contrast, ectomycorrhizal tree roots explore very long distances from the tree stem while foraging for nutrients (Putz et al., 2024; Lilleskov, unpublished). Foraging tree roots would be likely to encounter and proliferate preferentially in these nutrient-enriched hot spots, increasing overall abundance of tree roots relative to Ericaceae shrub roots. Large tree hosts have a strong demand for nutrients, increasing belowground C allocation in these patches (Prescott et al., 2020). This hypothesized driver of counterintuitive EcMF responses to fertilization requires experimental testing.

4.3 | Effects of fertilization on mycorrhizal fungi abundance are depth-dependent

The nature of the interaction effects between fertilization and depth on the relative abundance of ErMF and EcMF was surprising. We anticipated a more substantial and consistent effect of fertilization in the oxic acrotelm than in the anoxic catotelm where most fungi are not expected to survive. Although the majority of Ericaceae roots were distributed in the top 20 cm of the surface peat at Mer Bleue (Murphy, 2009), the lack of a clear N+PK effect in the shallowest layer stood in contrast with the strong evidence for fertilization effects in the deepest peat. This could be in part to do with higher variability within treatments at the surface, as mean effects were quite substantial. The substantial presence of ErMF at depth contrasts with the pattern of stratification of fungal abundance observed in the PEATcosm mesocosm study with *Sphagnum* peat monoliths (without fertilization), in which there were few ErMF below 30-40 cm (Lamit et al., 2021). The acrotelm–catotelm boundary was located at ~25 cm depth in PEATcosm (Lamit et al., 2021) vs. ~40 cm depth with strong seasonal oscillations from ~25 to 65 cm depth at Mer Bleue (Juutinen et al., 2010, 2018). This thicker acrotelm at Mer Bleue may explain the greater presence of ErMF at depth in the current study. Furthermore, the subsidence of peat surface in heavily fertilized plots (e.g., 6.4N and 6.4N+PK) led to the rise in water table to ~20 cm depth (Juutinen et al., 2018). This thinner acrotelm after subsidence may contribute to the decrease of ErMF abundance in N+PK fertilized plots, in which no vertical stratification of ErMF community was detected.

4.4 | Nitrogen addition did not shift the ectomycorrhizal community from nitrophobic to nitrophilic taxa

The lack of a clear shift in ectomycorrhizal community from nitrophobic to nitrophilic taxa after N addition, or in the relative abundances of several typical nitrophobic EcMF, e.g., *Cortinarius* and *Suillus* spp. (Lilleskov et al., 2001, 2002, 2011, 2024), were unexpected. Given the nutrient-deficient nature of northern acidic peatlands, the addition of N will likely lead to P-limitation and *vice versa* (Wang et al., 2016). Therefore, we hypothesize that the increase of these EcMF may be owing to continued high C allocation into the nutrient hot spots hypothesized earlier in the discussion, which are expected to be especially enhanced under continued N or P limitation (Lilleskov et al., 2024). The continued persistence of nitrophobes, especially *Cortinarius*, under these N enriched conditions indicates that high N availability is not always sufficient to decrease the abundance of so-called nitrophobic fungi, consistent with the alternative hypothesis that C allocation, rather than nutrient supply *per se*, can regulate nitrophobe and nitrophile relative abundance (Poznanovic et al., 2015; Lilleskov et al., 2024). Poznanovic et al. (2015) found that so-called nitrophilic mycorrhizal fungi dominated on seedling roots in very high C:N woody debris under very dense shade, consistent with adaptation to C limitation rather than excess N. Therefore, it would be particularly enlightening to test the effect of spatial scale of fertilizer additions, and co-limitation by N & P, on C allocation to roots and associated ectomycorrhizal fungal community response.

4.5 | *Sphagnum*-associated fungi in response to fertilization

The parallel decline in *Sphagnum* cover and the total relative abundance of *Sphagnum*-associated fungi after N, and especially N+PK, additions begs the question of whether the decline is driven simply by loss of *Sphagnum* or by the nutrient treatments. The results of the hierarchical analysis support the overarching role of limiting nutrients on fungal communities rather than the loss of *Sphagnum* alone, consistent with the results observed in a bog-fen peatland complex in northeastern China (Cao et al., 2022). *Sphagnum*-associated fungi can be mutualists, symbionts or antagonists of *Sphagnum* mosses (Kostka et al., 2016). However, the relationship between species identity and its role is still unknown. Whether there is a species-specific association between certain *Sphagnum*-associated fungi and their hosts, and how it affects peatland C dynamics should be addressed, given the critical role of *Sphagnum* mosses as ecosystem engineers in northern peatlands (van Breemen, 1995). Surprisingly, *Polytrichum* cover showed the strongest effect on *Sphagnum*-associated fungal community. The higher *Polytrichum* cover under moderate rates of N addition supported the competitive advance of *Polytrichum* over *Sphagnum* mosses via allocating excess N to growth (Bu et al., 2011; Mitchell et al., 2002).

5 | CONCLUSIONS

The imbalance between N and P exerts uncertain influence on peatlands with naturally low nutrient input, especially on decomposers. We saw strong effects of high nutrient additions in restructuring peatland fungal communities, especially with the combination of N and PK, which is in line with the nature of previously demonstrated N and P co-limitation at Mer Bleue. The negative response of ErMF in relative abundance to N and PK additions is accompanied by increasing relative abundance of saprotrophs, especially lignocellulose-degrading fungi. This shift in the composition of functional guilds after fertilization could affect peatland C stocks via the superior capacity of lignocellulose-degrading fungi to decompose recalcitrant organic matter. Possibly owing to the strong fluctuations of water table and subsidence of peat surface after years of heavy fertilization, we generally do not observe a greater effect of nutrient addition in the acrotelm than the catotelm, in which the occasional exposure to oxygen may account for the modest abundance of ErMF. The persistence of nitrophobic fungi under N addition may point to their sensitivity to high C allocation instead of nutrient supply, suggesting the need to explore fungal community response to nutrient hotspots of different sizes. Given the overarching role of limiting nutrients in structuring fungal community, it is likely that nutrient addition rather than decline in *Sphagnum* cover accounts for the drastic decline in the relative abundance of *Sphagnum*-associated fungi.

AUTHOR CONTRIBUTIONS

Meng Wang: Conceptualization (lead); methodology (lead); writing – original draft (lead); formal analysis (lead); writing – review and editing (equal); funding acquisition (lead). **Louis J. Lamit**: Conceptualization (supporting); formal analysis (supporting); writing – review and editing (equal). **Erik A. Lilleskov**: Conceptualization (supporting); formal analysis (supporting); methodology (supporting); writing – review and editing (equal); funding acquisition (lead). **Nathan Basiliko**: Formal analysis (supporting); writing – review and editing (equal). **Tim Moore**: Writing – review and editing (equal); funding acquisition (supporting). **Jill Bubier**: Writing – review and editing (equal); funding acquisition (supporting). **Galen Guo**: Formal analysis (supporting); methodology (supporting); writing – review and editing (equal). **Sari Juutinen**: Writing – review and editing (equal); funding acquisition (supporting). **Tuula Larmola**: Writing – review and editing equal); funding acquisition (supporting).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Sequence data are accessible via the National Center for Biotechnology Information (PRJNA1059234). The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.kh18932hd.

REFERENCES

Ackerman, D., Millet, D. B., & Chen, X. (2019). Global estimates of inorganic nitrogen deposition across four decades. *Global Biogeochemical Cycles*, *33*(1), 100-107. https://doi.org/10.1029/2018gb005990

Allison, S. D., Hanson, C. A., & Treseder, K. K. (2007). Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. *Soil Biology and Biochemistry*, *39*(8), 1878-1887. https://doi.org/10.1016/j.soilbio.2007.02.001

Andersen, R., Chapman, S. J., & Artz, R. R. E. (2013). Microbial communities in natural and disturbed peatlands: A review. *Soil Biology and Biochemistry*, *57*, 979-994. https://doi.org/10.1016/j.soilbio.2012.10.003

Anderson, M. J., & Willis, T. J. (2003). Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology*, *84*(2), 511-525. https://doi.org/10.1890/0012-9658(2003)084[0511:CAOPCA]2.0.CO;2

Basiliko, N., Moore, T. R., Jeannotte, R., & Bubier, J. L. (2006). Nutrient input and carbon and microbial dynamics in an ombrotrophic bog. *Geomicrobiology Journal*, *23*(7), 531-543.

https://doi.org/10.1080/01490450600897278

Bengtsson, F., Rydin, H., & Hájek, T. (2018). Biochemical determinants of litter quality in 15 species of *Sphagnum*. *Plant and Soil*, *425*(1), 161-176. https://doi.org/10.1007/s11104-018-3579-8

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García, M., Ebersberger, I., de Sousa, F., Amend, A., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y. J. K., Sanli, K., Eriksson, K. M., Vik, U., … Nilsson, R. H. (2013). Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution*, *4*(10), 914-919. https://doi.org/10.1111/2041-210X.12073

Berg, B., & Matzner, E. (1997). Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. *Environmental Reviews*, *5*(1), 1-25. https://doi.org/10.1139/a96-017

Birnbaum, C., Wood, J., Lilleskov, E., Lamit, L. J., Shannon, J., Brewer, M., & Grover, S. (2023). Degradation reduces microbial richness and alters microbial functions in an Australian peatland. *Microbial Ecology*, *85*(3), 875-891. https://doi.org/10.1007/s00248-022-02071-z

Bowden, R. D., Wurzbacher, S. J., Washko, S. E., Wind, L., Rice, A. M., Coble, A. E., Baldauf, N., Johnson, B., Wang, J. J., Simpson, M., & Lajtha, K. (2019). Long-term nitrogen addition decreases organic matter decomposition and increases forest soil carbon. *Soil Science Society of America Journal*, *83*(S1), S82-S95. https://doi.org/10.2136/sssaj2018.08.0293

Bragazza, L., Buttler, A., Habermacher, J., Brancaleoni, L., Gerdol, R., Fritze, H., Hanajík, P., Laiho, R., & Johnson, D. (2012). High nitrogen deposition alters the decomposition of bog plant litter and reduces carbon accumulation. *Global Change Biology*, *18*(3), 1163-1172. https://doi.org/10.1111/j.1365-2486.2011.02585.x

Bridgham, S. D., Pastor, J., Janssens, J. A., Chapin, C., & Malterer, T. J. (1996). Multiple limiting gradients in peatlands: A call for a new paradigm. *Wetlands*, *16*(1), 45-65. https://doi.org/10.1007/BF03160645

Bu, Z.-J., Rydin, H., & Chen, X. (2011). Direct and interaction-mediated effects of environmental changes on peatland bryophytes. *Oecologia*, *166*(2), 555-563. https://doi.org/10.1007/s00442-010-1880-1

Bubier, J. L., Moore, T. R., & Bledzki, L. A. (2007). Effects of nutrient addition on vegetation and carbon cycling in an ombrotrophic bog. *Global Change Biology*, *13*(6), 1168-1186. https://doi.org/10.1111/j.1365-2486.2007.01346.x

Bushnell, B., Rood, J., & Singer, E. (2017). BBMerge – Accurate paired shotgun read merging via overlap. *PLoS One*, *12*(10), e0185056. https://doi.org/10.1371/journal.pone.0185056

Cao, C., Huang, J., Ge, L., Li, T., Bu, Z.-J., Wang, S., Wang, Z., Liu, Z., Liu, S. and Wang, M. (2022). Does shift in vegetation abundance after nitrogen and phosphorus additions play a key role in regulating fungal community structure in a northern peatland? *Frontiers in Microbiology*, *13*, 920382. https://doi.org/10.3389/fmicb.2022.920382

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335-336. https://doi.org/10.1038/nmeth.f.303

Charman, D. (2002). *Peatlands and Environmental Change*. Wiley.

Coleman-Derr, D., Desgarennes, D., Fonseca-Garcia, C., Gross, S., Clingenpeel, S., Woyke, T., North, G., Visel, A., Partida-Martinez, L. P., & Tringe, S. G. (2016). Plant compartment and biogeography affect microbiome composition in cultivated and native *Agave* species. *New Phytologist*, *209*(2), 798-811. https://doi.org/10.1111/nph.13697

De Cáceres, M., & Legendre, P. (2009). Associations between species and groups of sites: indices and statistical inference. *Ecology*, *90*(12), 3566-3574. https://doi.org/10.1890/08-1823.1

Defrenne, C. E., Moore, J. A., Tucker, C. L., Lamit, L. J., Kane, E. S., Kolka, R. K., Chimner, R. A., Keller, J. K., & Lilleskov, E. A. (2023). Peat loss collocates with a threshold in plant–mycorrhizal associations in drained peatlands encroached by trees. *New Phytologist*, *240*(1), 412-425. https://doi.org/10.1111/nph.18954

Du, E., Terrer, C., Pellegrini, A. F. A., Ahlström, A., van Lissa, C. J., Zhao, X., Xia, N., Wu, X., & Jackson, R. B. (2020). Global patterns of terrestrial nitrogen and phosphorus limitation. *Nature Geoscience*, *13*, 221-226. https://doi.org/10.1038/s41561-019-0530-4

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, *10*(10), 996-998. https://doi.org/10.1038/nmeth.2604

Edgar, R. C. (2016). UCHIME2: improved chimera prediction for amplicon sequencing. *bioRxiv*, 074252. https://doi.org/10.1101/074252

Edgar, R. C., & Flyvbjerg, H. (2015). Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics*, *31*(21), 3476-3482. https://doi.org/10.1093/bioinformatics/btv401

Fehrer, J., Réblová, M., Bambasová, V., & Vohník, M. (2019). The root-symbiotic *Rhizoscyphus ericae* aggregate and *Hyaloscypha* (*Leotiomycetes*) are congeneric: Phylogenetic and experimental evidence. *Studies in Mycology*, *92*(1), 195-225. https://doi.org/10.1016/j.simyco.2018.10.004

Fenner, N., & Freeman, C. (2020). Woody litter protects peat carbon stocks during drought. *Nature Climate Change*, *10*(4), 363-369. https://doi.org/10.1038/s41558-020-0727-y

Fernandez, C. W., & Kennedy, P. G. (2016). Revisiting the ‘Gadgil effect’: do interguild fungal interactions control carbon cycling in forest soils? *New Phytologist*, *209*(4), 1382-1394. https://doi.org/10.1111/nph.13648

Fritz, C., van Dijk, G., Smolders, A. J. P., Pancotto, V. A., Elzenga, T., Roelofs, J. G. M., & Grootjans, A. P. (2012). Nutrient additions in pristine Patagonian *Sphagnum* bog vegetation: can phosphorus addition alleviate (the effects of) increased nitrogen loads. *Plant Biology*, *14*(3), 491-499. https://doi.org/10.1111/j.1438-8677.2011.00527.x

Gadgil, R. L., & Gadgil, P. D. (1975). Suppression of litter decomposition by mycorrhizal roots of *Pinus radiata*. New Zealand Journal of Forestry Science, *5*, 33-41.

Guo, G. (2015). *Chronic N and nutrient deposition impact on diversity and abundance of bacteria, fungi, and* CH_4 *cycling prokaryotes at a northern peat bog*. (Master of Science). Laurentian University, Sudbury, Canada.

Hill, B. H., Elonen, C. M., Jicha, T. M., Kolka, R. K., Lehto, L. L. P., Sebestyen, S. D., & Seifert-Monson, L. R. (2014). Ecoenzymatic stoichiometry and microbial processing of organic matter in northern bogs and fens reveals a common P-limitation between peatland types. *Biogeochemistry*, *120*(1), 203-224. https://doi.org/10.1007/s10533-014-9991-0

Hodge, A. (2004). The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytologist*, *162*(1), 9-24. https://doi.org/10.1111/j.1469-8137.2004.01015.x

Hoosbeek, M. R., van Breemen, N., Vasander, H., Buttler, A., & Berendse, F.

(2002). Potassium limits potential growth of bog vegetation under elevated atmospheric CO_2 and N deposition. *Global Change Biology*, *8*(11), 1130-1138. https://doi.org/10.1046/j.1365-2486.2002.00535.x

Hupperts, S. F., & Lilleskov, E. A. (2022). Predictors of taxonomic and functional composition of black spruce seedling ectomycorrhizal fungal communities along peatland drainage gradients. *Mycorrhiza*, *32*(1), 67-81. https://doi.org/10.1007/s00572-021-01060-3

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

Juutinen, S., Bubier, J., & Moore, T. (2010). Responses of vegetation and ecosystem CO_2 exchange to 9 years of nutrient addition at Mer Bleue bog. *Ecosystems*, *13*(6), 874-887. https://doi.org/10.1007/s10021-010-9361-2

Juutinen, S., Moore, T. R., Bubier, J. L., Arnkil, S., Humphreys, E., Marincak, B., Roy, C., & Larmola, T. (2018). Long-term nutrient addition increased CH4 emission from a bog through direct and indirect effects. *Scientific Reports*, *8*(1), 3838. https://doi.org/10.1038/s41598-018-22210-2

Kiheri, H., Velmala, S., Pennanen, T., Timonen, S., Sietiö, O.-M., Fritze, H., Heinonsalo, J., van Dijk, N., Dise N., & Larmola, T. (2020). Fungal colonization patterns and enzymatic activities of peatland ericaceous plants following long-term nutrient addition. *Soil Biology and Biochemistry*, 147, 107833. https://doi.org/10.1016/j.soilbio.2020.107833

Kostka, J. E., Weston, D. J., Glass, J. B., Lilleskov, E. A., Shaw, A. J., &

Turetsky, M. R. (2016). The *Sphagnum* microbiome: new insights from an ancient plant lineage. *New Phytologist*, *211*(1), 57-64. https://doi.org/10.1111/nph.13993

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13), 1-26. https://doi.org/10.18637/jss.v082.i13

Lai, J., Zou, Y., Zhang, J., & Peres-Neto, P. R. (2022). Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca.hp R package. *Methods in Ecology and Evolution*, *13*(4), 782-788. https://doi.org/10.1111/2041-210X.13800

Lamit, L. J., Romanowicz, K. J., Potvin, L. R., Lennon, J. T., Tringe, S. G., Chimner, R. A., Kolka, R. K., Kane, E. S., & Lilleskov, E. A. (2021). Peatland microbial community responses to plant functional group and drought are depth-dependent. *Molecular Ecology*, *30*(20), 5119-5136. https://doi.org/10.1111/mec.16125

Lamit, L. J., Romanowicz, K. J., Potvin, L. R., Rivers, A. R., Singh, K., Lennon, J. T., Tringe, S. G., Kane, E. S., & Lilleskov, E. A. (2017). Patterns and drivers of fungal community depth stratification in *Sphagnum* peat. *FEMS Microbiology Ecology*, *93*(7), fix082. https://doi.org/10.1093/femsec/fix082

Larmola, T., Bubier, J. L., Kobyljanec, C., Basiliko, N., Juutinen, S., Humphreys, E., Preston, M., & Moore, T. R. (2013). Vegetation feedbacks of nutrient addition lead to a weaker carbon sink in an ombrotrophic bog. *Global Change Biology*, *19*(12), 3729-3739. https://doi.org/10.1111/gcb.12328

Lems, K. (1956). Ecological study of the peat bogs of Eastern North America: III. Notes ofn the behavior of *Chamaedaphne calyculata*. *Canadian Journal of Botany*, *34*(1), 197-207. https://doi.org/10.1139/b56-018

Lenth, R. (2021). emmeans: Estimated marginal means, aka least-squares means. R package version 1.7.0. https://CRAN.R-project.org/package=emmeans

Li, T., Bu, Z., Liu, W., Zhang, M., Peng, C., Zhu, Q., Shi, S., Wang, M. (2019). Weakening of the ‘enzymatic latch’ mechanism following long-term fertilization in a minerotrophic peatland. *Soil Biology and Biochemistry*, *136*, 107528. https://doi.org/10.1016/j.soilbio.2019.107528

Li, T., Peng, C., Bu, Z., Zhu, Q., Song, H., Guo, X., & Wang, M. (2021). Woody plants reduce the sensitivity of soil extracellular enzyme activity to nutrient enrichment in wetlands: A meta-analysis. *Soil Biology and Biochemistry*, *159*, 108280. https://doi.org/10.1016/j.soilbio.2021.108280

Lilleskov, E. A., Fahey, T. J., Horton, T. R., & Lovett, G. M. (2002). Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. *Ecology*, *83*(1), 104-115. https://doi.org/10.1890/0012-9658(2002)083[0104:BEFCCO]2.0.CO;2

Lilleskov, E. A., Fahey, T. J., & Lovett, G. M. (2001). Ectomycorrhizal fungal aboveground community change over an atmospheric nitrogen deposition gradient. *Ecological Applications*, *11*(2), 397-410. https://doi.org/10.1890/1051-0761(2001)011[0397:EFACCO]2.0.CO;2

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 Ecology*, *4*(2), 174-183. https://doi.org/10.1016/j.funeco.2010.09.008

Lilleskov, E. A., Kuyper, T. W., Bidartondo, M. I., & Hobbie, E. A. (2024). Chapter 6 - Impacts of nitrogen deposition on forest mycorrhizal communities. In E. Du & W. de Vries (Eds.), *Atmospheric Nitrogen Deposition to Global Forests* (pp. 95-118). Academic Press.

Limpens, J., Granath, G., Gunnarsson, U., Aerts, R., Bayley, S., Bragazza, L., Bubier, J., Buttler, A., van den Berg, L. J. L., Francez, A. J., Gerdol, R., Grosvernier, P., Heijmans, M. M. P. D., Hoosbeek, M. R., Hotes, S., Ilomets, M., Leith, I., Mitchell, E. A. D., Moore, T., … Xu, B. (2011). Climatic modifiers of the response to nitrogen deposition in peat-forming *Sphagnum* mosses: a meta-analysis. *New Phytologist*, *191*(2), 496-507. https://doi.org/10.1111/j.1469-8137.2011.03680.x

Lin, X., Tfaily, M. M., Green, S. J., Steinweg, J. M., Chanton, P., Imvittaya, A., Chanton, J. P., Cooper, W., Schadt, C., & Kostka, J. E. (2014a). Microbial metabolic potential for carbon degradation and nutrient (nitrogen and phosphorus) acquisition in an ombrotrophic peatland. *Applied and Environmental Microbiology*, *80*(11), 3531-3540. https://doi.org/10.1128/aem.00206-14

Lin, X., Tfaily, M. M., Steinweg, J. M., Chanton, P., Esson, K., Yang, Z. K., Chanton, J. P., Cooper, W., Schadt, C., & Kostka, J. E. (2014b). Microbial community stratification linked to utilization of carbohydrates and phosphorus limitation in a boreal peatland at Marcell Experimental Forest, Minnesota, USA. *Applied and Environmental Microbiology*, *80*(11), 3518-3530. https://doi.org/10.1128/aem.00205-14

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

Liu, Y., Shi, G., Mao, L., Cheng, G., Jiang, S., Ma, X., An, L., Du, G., Johnson, N. C., & Feng, H. (2012). Direct and indirect influences of 8 yr of nitrogen and phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. *New Phytologist*, *194*(2), 523-535. https://doi.org/10.1111/j.1469-8137.2012.04050.x

Loisel, J., Yu, Z., Beilman, D. W., Camill, P., Alm, J., Amesbury, M. J., Anderson, D., Andersson, S., Bochicchio, C., Barber, K., Belyea, L. R., Bunbury, J., Chambers, F. M., Charman, D. J., De Vleeschouwer, F., Fiałkiewicz-Kozieł, B., Finkelstein, S. A., Gałka, M., Garneau, M., … Zhou, W. (2014). A database and synthesis of northern peatland soil properties and Holocene carbon and nitrogen accumulation. *Holocene*, *24*(9), 1028-1042. https://doi.org/10.1177/0959683614538073

MacDonald, A. J., Kirkpatrick, A. H., Hester, A. J., & Sydes, C. (1995). Regeneration by natural layering of heather (*Calluna vulgaris*): Frequency and characteristics in upland Britain. *Journal of Applied Ecology*, *32*(1), 85-99. https://doi.org/10.2307/2404418

Martino, E., Morin, E., Grelet, G.-A., Kuo, A., Kohler, A., Daghino, S., Barry, K. W., Cichocki, N., Clum, A., Dockter, R. B., Hainaut, M., Kuo, R. C., LaButti, K., Lindahl, B. D., Lindquist, E. A., Lipzen, A., Khouja, H.-R., Magnuson, J., Murat, C., ... Perotto, S. (2018). Comparative genomics and transcriptomics depict ericoid mycorrhizal fungi as versatile saprotrophs and plant mutualists. *New Phytologist*, *217*(3), 1213-1229. https://doi.org/10.1111/nph.14974

Mitchell, E. A. D., Buttler, A., Grosvernier, P., Rydin, H., Siegenthaler, A., & Gobat, J. M. (2002). Contrasted effects of increased N and CO_2 supply on two keystone species in peatland restoration and implications for global change. *Journal of Ecology*, *90*(3), 529-533. https://doi.org/10.1046/j.1365-2745.2002.00679.x

Moore, T. R., Knorr, K.-H., Thompson, L., Roy, C., & Bubier, J. L. (2019). The effect of long-term fertilization on peat in an ombrotrophic bog. *Geoderma*, *343*, 176-186. https://doi.org/10.1016/j.geoderma.2019.02.034

Muff, S., Nilsen, E. B., O'Hara, R. B., & Nater, C. R. (2022). Rewriting results sections in the language of evidence. *Trends in Ecology & Evolution*, *37*(3), 203-210. https://doi.org/10.1016/j.tree.2021.10.009

Murphy, M. T. (2009). *Getting to the root of the matter: variations in vascular root biomass and production in peatlands and responses to global* *change*. (Ph.D.). McGill University, Montreal, Canada.

Netherway, T., Bengtsson, J., Buegger, F., Fritscher, J., Oja, J., Pritsch, K., Hildebrand, F., Krab, E. J., & Bahram, M. (2024). Pervasive associations between dark septate endophytic fungi with tree root and soil microbiomes across Europe. *Nature Communications*, *15*(1), 159. https://doi.org/10.1038/s41467-023-44172-4

Nilsson, R. H., Tedersoo, L., Ryberg, M., Kristiansson, E., Hartmann, M., Unterseher, M., Porter, T. M., Bengtsson-Palme, J., Walker, D. M., De Sousa, F., Gamper, H. A., Larsson, E., Larsson, K.-H., Kõljalg, U., Edgar, R. C., & Abarenkov, K. (2015). A comprehensive, automatically updated fungal its sequence dataset for reference-based chimera control in environmental sequencing efforts. *Microbes and Environments*, *30*(2), 145-150. https://doi.org/10.1264/jsme2.me14121

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., Boucher, O., Godderis, Y., Hinsinger, P., Llusia, J., Nardin, E., Vicca, S., Obersteiner, M., & Janssens, I. A. (2013). Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. *Nature Communications*, *4*, 2934. https://doi.org/10.1038/Ncomms3934

Pipes, G. T., & Yavitt, J. B. (2022). Biochemical components of *Sphagnum* and persistence in peat soil. *Canadian Journal of Soil Science*, *102*(3), 785-795. https://doi.org/10.1139/cjss-2021-0137

Põlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B. D., Clemmensen, K. E., Kauserud, H., Nguyen, N., Kjøller, R., Bates, S. T., Baldrian, P., Frøslev, T. G., Adojaan, K., Vizzini, A., Suija, A., Pfister, D., Baral, H.-O., Järv, H., Madrid, H., Nordén, J., ... Tedersoo, L. (2020). FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Diversity*, *105*(1), 1-16. https://doi.org/10.1007/s13225-020-00466-2

Poznanovic, S. K., Lilleskov, E. A., & Webster, C. R. (2015). Sharing rotting wood in the shade: ectomycorrhizal communities of co-occurring birch and hemlock seedlings. *Mycorrhiza*, *25*(2), 153-164. https://doi.org/10.1007/s00572-014-0597-0

Prescott, C. E., Grayston, S. J., Helmisaari, H.-S., Kaštovská, E., Körner, C., Lambers, H., Meier, I. C., Millard, P., & Ostonen, I. (2020). Surplus carbon drives allocation and plant–soil interactions. *Trends in Ecology & Evolution*, *35*(12), 1110-1118. https://doi.org/10.1016/j.tree.2020.08.007

Putz, F. E., Canham, C. D., & Ollinger, S. V. (2024). Belowground exploration by trees and shrubs. *Plant Ecology*, *225*(6), 605-610. https://doi.org/10.1007/s11258-024-01416-7

R Core Team. (2021). *R: A language and environment for statistical computing*. R foundation for Statistical Computing.

Rydin, H., & Jeglum, J. K. (2013). *The biology of peatlands* (2nd ed.). Oxford University Press.

Schillereff, D. N., Chiverrell, R. C., Sjöström, J. K., Kylander, M. E., Boyle, J. F., Davies, J. A. C., Toberman, H., & Tipping, E. (2021). Phosphorus supply affects long-term carbon accumulation in mid-latitude ombrotrophic peatlands. *Communications Earth & Environment*, *2*(1), 241. https://doi.org/10.1038/s43247-021-00316-2

Sheppard, L. J., Leith, I. D., Mizunuma, T., Leeson, S., Kivimaki, S., Neil Cape, J., van Dijk, N., Leaver, D., Sutton, M. A., Fowler, D., Van den Berg, L. J., Crossley, A., Field, C., & Smart, S. (2014). Inertia in an ombrotrophic bog ecosystem in response to 9 years' realistic perturbation by wet deposition of nitrogen, separated by form. *Global Change Biology*, *20*(2), 566-580. https://doi.org/10.1111/gcb.12357

Treseder, K. K., Berlemont, R., Allison, S. D., & Martiny, A. C. (2018). Nitrogen enrichment shifts functional genes related to nitrogen and carbon acquisition in the fungal community. *Soil Biology and Biochemistry*, *123*, 87-96. https://doi.org/10.1016/j.soilbio.2018.05.014

van Breemen, N. (1995). How *Sphagnum* bogs down other plants. *Trends in Ecology & Evolution*, *10*(7), 270-275. https://doi.org/10.1016/0169-5347(95)90007-1

Verbeke, B. A., Lamit, L. J., Lilleskov, E. A., Hodgkins, S. B., Basiliko, N., Kane, E. S., Andersen, R., Artz, R. R. E., Benavides, J. C., Benscoter, B. W., Borken, W., Bragazza, L., Brandt, S. M., Bräuer, S. L., Carson, M. A., Charman, D., Chen, X., Clarkson, B. R., Cobb, A. R., ... Chanton, J. P. (2022). Latitude, elevation, and mean annual temperature predict peat organic matter chemistry at a global scale. *Global Biogeochemical Cycles*, *36*(2), e2021GB007057. https://doi.org/10.1029/2021GB007057

Vesala, R., Kiheri, H., Hobbie, E. A., van Dijk, N., Dise, N., & Larmola, T. (2021). Atmospheric nitrogen enrichment changes nutrient stoichiometry and reduces fungal N supply to peatland ericoid mycorrhizal shrubs. *Science of the Total Environment*, *794*, 148737. https://doi.org/10.1016/j.scitotenv.2021.148737

Von Post, L. (1922). Sveriges geologiska undersöknings torvinventering och några av dess hittills vunna resultat. *Svenska Mosskulturföreningens Tidskrift*, *1*, 1-27.

Wang, H., Richardson, C. J., & Ho, M. (2015). Dual controls on carbon loss during drought in peatlands. *Nature Climate Change*, *5*(6), 584-587. https://doi.org/10.1038/nclimate2643

Wang, M., Lamit, L. J., Lilleskov, E. A., Basiliko, N., Moore, T. R., Bubier, J. L, Guo, G., Juutinen, S., & Larmola, T. (2024). Peatland fungal community responses to nutrient enrichment: a story beyond nitrogen. *Dryad*. Version 2. https://doi.org/10.5061/dryad.kh18932hd

Wang, M., Larmola, T., Murphy, M. T., Moore, T. R., & Bubier, J. L. (2016).

Stoichiometric response of shrubs and mosses to long-term nutrient (N, P and K) addition in an ombrotrophic peatland. *Plant and Soil*, *400*(1), 403-416. https://doi.org/10.1007/s11104-015-2744-6

Wang, M., Tian, J., Bu, Z., Lamit, L. J., Chen, H., Zhu, Q., & Peng, C. (2019). Structural and functional differentiation of the microbial community in the surface and subsurface peat of two minerotrophic fens in China. *Plant and Soil*, *437*(1-2), 21-40. https://doi.org/10.1007/s11104-019-03962-w

Wang, R., Goll, D., Balkanski, Y., Hauglustaine, D., Boucher, O., Ciais, P., Janssens, I., Penuelas, J., Guenet, B., Sardans, J., Bopp, L., Vuichard, N., Zhou, F., Li, B., Piao, S., Peng, S., Huang, Y., & Tao, S. (2017). Global forest carbon uptake due to nitrogen and phosphorus deposition from 1850 to 2100. *Global Change Biology*, *23*(11), 4854-4872. https://doi.org/10.1111/gcb.13766

Watmough, S., Gilbert-Parkes, S., Basiliko, N., Lamit, L. J., Lilleskov, E. A., Andersen, R., del Aguila-Pasquel, J., Artz, R. E., Benscoter, B. W., Borken, W., Bragazza, L., Brandt, S. M., Bräuer, S. L., Carson, M. A., Chen, X., Chimner, R. A., Clarkson, B. R., Cobb, A. R., Enriquez, A. S., ... Zahn, G. (2022). Variation in carbon and nitrogen concentrations among peatland categories at the global scale. *PLoS One*, *17*(11), e0275149. https://doi.org/10.1371/journal.pone.0275149

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. Innis, D. Gelfand, J. Sninsky & T. White (Eds.), *PCR protocols: a guide to methods and applications* (pp. 315-322). Academic Press.

Wiedermann, M. M., Kane, E. S., Potvin, L. R., & Lilleskov, E. A. (2017). Interactive plant functional group and water table effects on decomposition and extracellular enzyme activity in *Sphagnum* peatlands. *Soil Biology and Biochemistry*, *108*, 1-8.

https://doi.org/10.1016/j.soilbio.2017.01.008

Yin, T., Feng, M., Qiu, C., & Peng, S. (2022). Biological nitrogen fixation and nitrogen accumulation in peatlands. *Frontiers in Earth Science*, *10*, 670867. https://doi.org/10.3389/feart.2022.670867

Zak, D. R., Argiroff, W. A., Freedman, Z. B., Upchurch, R. A., Entwistle, E. M., & Romanowicz, K. J. (2019). Anthropogenic N deposition, fungal gene expression, and an increasing soil carbon sink in the Northern Hemisphere. *Ecology*, *100*(10), e02804. https://doi.org/10.1002/ecy.2804

Živković, T., Helbig, M., & Moore, T. R. (2022). Seasonal and spatial variability of biological N_2 fixation in a cool temperate bog. *Journal of Geophysical Research: Biogeosciences*, *127*(2), e2021JG006481. https://doi.org/10.1029/2021JG006481

Table 1 Fertilization experiment set-up.

		Addition rate			Cumulative addition to time of sampling in 2014		
		N	P	K	N	P	K
Treatment	Start year	($g\ m^{-2}\ yr^{-1}$)			($g\ m^{-2}$)		
Experiment 1							
C1	2000	0	0	0	0	0	0
PK	2000	0	5	6.3	0	75	95
1.6N	2000	1.6	0	0	24	0	0
1.6N+PK	2000	1.6	5	6.3	24	75	95
3.2N+PK	2001	3.2	5	6.3	44.8	70	88
6.4N+PK	2001	6.4	5	6.3	89.6	70	88
Experiment 2							
C2	2005	0	0	0	0	0	0
3.2N	2005	3.2	0	0	32	0	0
6.4N	2005	6.4	0	0	64	0	0

Table 2 PERMANOVA results of fungal community composition under different fertilization treatments and sampling depths.

Analysis*	Treatment			Depth			Treatment × Depth			Core		
	(*df*	*F*	*p*)	(*df*	*F*	*p*)	(*df*	*F*	*p*)	(*df*	*F*	*p*)
Experiment 1												
PERMANOVA mixed model	5, 12.57[#]	2.2	**0.001**	2, 18	5.72	**0.001**	10, 18	1.03	0.38	12, 18	2.03	**0.001**
PERMDISP	5, 42	0.31	0.95	2, 45	3.36	0.052	17, 30	9.83	0.21	17, 30	2.65	0.65
Experiment 2												
PERMANOVA mixed model	2, 6.13	1.26	**0.032**	2, 10	4.46	**0.0002**	4, 10	1.06	0.37	6, 10	3.05	**0.0001**
PERMDISP	2, 22	0.11	0.91	2, 22	2.42	0.14	8, 16	4.07	0.14	8, 16	2.59	0.45
All												
PERMANOVA mixed model	8, 18.6	2.05	**0.0001**	2, 28	8.24	**0.0001**	16, 28	1.12	0.077	18, 28	2.3	**0.0001**
PERMDISP	8, 64	0.84	0.75	2, 70	5.12	**0.013**	26, 46	10.95	0.083	26, 46	2.85	0.57

* PERMANOVA mixed models include treatment, depth and treatment × depth as fixed factors, and individual core as a random factor. PERMDISP is run to examine the differences in dispersion among treatments, depths, or all unique treatment × depth combination. Results with moderate ($p < 0.05$) to very strong evidence ($p < 0.001$) of difference among treatments and depth are highlighted in bold.

[#] The degrees of freedom for the nominator and denominator, respectively.

Table 3 Alpha diversity indices (mean ± SE) under different fertilization treatments and sampling depths.

Treatments/Depths	**OTU richness**	**Shannon's diversity index**	**Pielou's evenness index**
Experiment 1			
C1	178 ± 26 A	3.3 ± 0.3 AB	0.65 ± 0.04 A
PK	172 ± 23 A	2.7 ± 0.2 A	0.54 ± 0.05 AB
1.6N	238 ± 37 AB	3.6 ± 0.2 ABC	0.67 ± 0.04 ABC
1.6N+PK	324 ± 57 C	4.5 ± 0.3 C	0.78 ± 0.04 BC
3.2N+PK	294 ± 46 BC	4.5 ± 0.3 BC	0.82 ± 0.06 BC
6.4N+PK	274 ± 42 ABC	4.7 ± 0.2 C	0.86 ± 0.03 C
10-20 cm	295 ± 26 b	3.7 ± 0.3	0.66 ± 0.04
30-40 cm	284 ± 32 b	3.9 ± 0.2	0.71 ± 0.03
60-70 cm	151 ± 12 a	3.9 ± 0.3	0.78 ± 0.05
Experiment 2			
C2	163 ± 23	3.5 ± 0.3	0.69 ± 0.05
3.2N	147 ± 16	3.6 ± 0.2	0.72 ± 0.04
6.4N	212 ± 34	3.8 ± 0.3	0.73 ± 0.05
10-20 cm	227 ± 30 b	3.7 ± 0.2	0.69 ± 0.03 ab
30-40 cm	156 ± 22 a	3.3 ± 0.3	0.65 ± 0.05 a
60-70 cm	134 ± 11 a	4.0 ± 0.2	0.73 ± 0.05 b

There is no evidence for an interactive effect between treatment and depth, therefore only the main effects are shown. Different upper-case letters indicate that there is evidence that the indices are different among treatments with all depths pooled. Different lower-case letters indicate that there is evidence ($p < 0.05$) that the indices are different among depths with all treatments pooled. Values that do not have letters indicate there is no evidence ($p > 0.1$) that the alpha diversity index is different among treatments or depths. Two sets of experiments are analyzed separately.

Table 4 Relative abundance (%) of putative nitrophobic and nitrophilic ectomycorrhizal fungi in response to the additions of N and/or PK.

Treatment	*Cenococcum*	*Cortinarius*	*Laccaria*	*Paxillus*	*Pseudotomentella*	*Suillus*	*Tomentella*
C1	0	0.123 BC	0	0	0	0	0
PK	0	0.002 A	0	0	0	0	0
1.6N	0	0.209 C	0	0	0	0.008	0
1.6NPK	0.001	0.007 A	0	0	0	0	0
3.2NPK	0.006	0.015 A	0	0	0.713	0.019	0
6.4NPK	0.016	0.005 A	0.017	0.010	0	0	0.092
C2	0	0	0	0	0	0	0
3.2N	0	0	0.004	0	0	0	0
6.4N	0.024	0.024	0.001	0	0	0	0
Response							
+N	↑	↑	↑	-	-	↑	-
+PK	-	↓	-	-	-	-	-
+NPK	↑	↓	↑	↑	↑	↑	↑
Strategy*	nitrophobic	nitrophobic	nitrophilic	nitrophilic	nitrophobic	nitrophobic	mixed

Symbols indicate the positive (↑), negative (↓) or neutral (-) effects of N, PK or NPK additions on the relative abundance of putative nitrophobic and nitrophilic ectomycorrhizal fungal species. Please note that the direction of response is based on the summary of trends instead of statistical analysis expect for *Cortinarius*, considering the limitation of small sample size. Different upper-case letters indicate that there is evidence that the relative abundance of *Cortinarius* is different among treatments with all depths pooled. *Different strategies are adopted from Lilleskov et al. (2001, 2002 and 2011).

Figure captions

Figure1 Relative abundance of dominant fungal orders for different treatments (a) and depths (b, Experiment 1; c, Experiment 2). Stacked bars are ordered by decreasing the number of total sequences per order (bottom to top) with all samples pooled. Abbreviations of treatments as described in Table 1.

Figure 2 Canonical analysis of principal coordinates (CAP) with fungal community composition among different treatments (a, with all depths pooled) and depths (b, with all treatments pooled). Samples of all treatments from the two experiments are pooled to visualize the pattern of vertical stratification. Abbreviations of treatments as described in Table 1.

Figure 3 Relative abundance of dominant fungal genera for different treatments (a) and depths (b, Experiment 1; c, Experiment 2). Stacked bars are ordered by decreasing the number of total sequences per genera (bottom to top) with all samples pooled. Abbreviations of treatments as describes in Table 1.

Figure 4 Relative abundance (mean ± SE) of ericoid mycorrhizal fungi, ectomycorrhizal fungi and *Sphagnum*-associated fungi under different treatments (a, d, g) and depths (b, c, e, f, h, i). There is very strong evidence ($p < 0.001$) for the interactive effects for ericoid mycorrhizal fungi in Experiment 1 (b) and ectomycorrhizal fungi in Experiment 2 (f), and only the main effects are shown for *Sphagnum*-associated fungi (Supplementary Table S5). Different upper-case letters indicate there is evidence ($p < 0.05$) that the relative abundance of fungal functional guild is different among treatments under the same depth. Different lower-case letters indicate there is evidence ($p < 0.05$) that the relative abundance of fungal functional guild is different among depths under the same treatment. Bars without letters indicate there is no evidence ($p > 0.1$) that the relative abundance of fungal functional guild is different among treatments or depths. Abbreviations of treatments as described in Table 1.

Figure 5 Relative abundance (mean ± SE) of lignocellulose-degrading fungi and saprotrophic fungi (includes only non-lignocellulose degrading saprotrophic fungi) under different treatments (a, d) and depths (b, c, Experiment 1; e, f, Experiment 2). Different upper-case letters indicate there is evidence ($p < 0.05$) that the relative abundance of saprotrophic fungi is different among treatments under the same depth. Bars without letters indicate there is no evidence ($p > 0.1$) that the relative abundance of fungal functional guild is different among treatments or depths. Abbreviations of treatments as described in Table 1.

Figure 6 Abundance (mean ± SE) of Ericaceae (a) and covers of *Sphagnum* (b) and *Polytrichum* (c) mosses in response to different treatments. Different upper-case letters indicate there is evidence ($p < 0.05$) that the relative abundance of fungal functional guild is different among treatments for each experiment separately. For Ericaceae, there is no evidence ($p > 0.1$) that treatments affect the abundance of Ericaceae in Experiment 1. Bars without letters indicate there is no evidence ($p > 0.1$) that the abundance of Ericaceae is different among treatments. Abbreviations of treatments as described in Table 1.

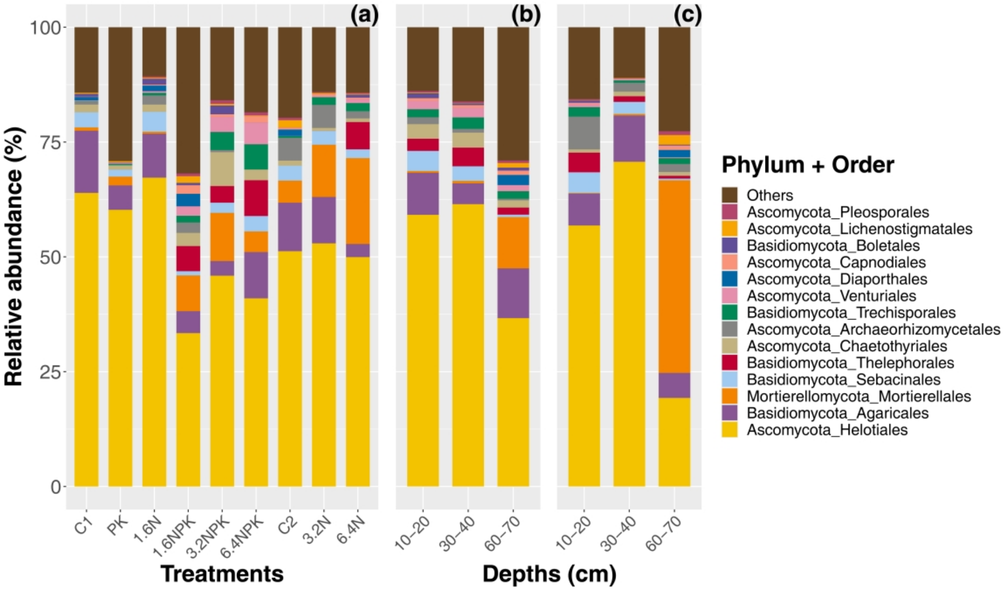

Figure1 Relative abundance of dominant fungal orders for different treatments (a) and depths (b, Experiment 1; c, Experiment 2). Stacked bars are ordered by decreasing the number of total sequences per order (bottom to top) with all samples pooled. Abbreviations of treatments as described in Table 1.

363x214mm (300 x 300 DPI)

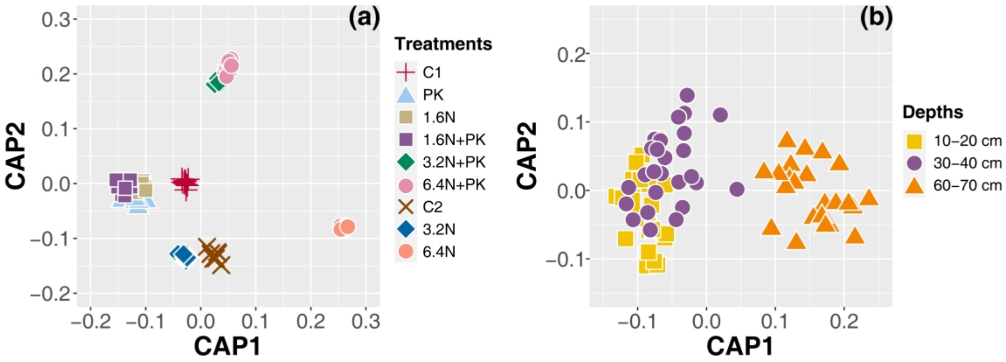

Figure 2 Canonical analysis of principal coordinates (CAP) with fungal community composition among different treatments (a, with all depths pooled) and depths (b, with all treatments pooled). Samples of all treatments from the two experiments are pooled to visualize the pattern of vertical stratification. Abbreviations of treatments as described in Table 1.

271x98mm (300 x 300 DPI)

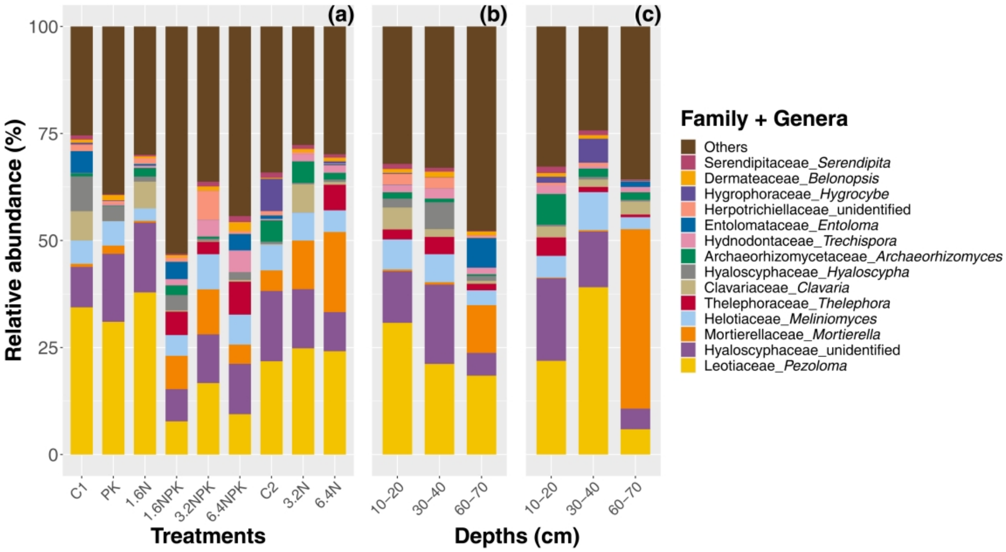

Figure 3 Relative abundance of dominant fungal genera for different treatments (a) and depths (b, Experiment 1; c, Experiment 2). Stacked bars are ordered by decreasing the number of total sequences per genera (bottom to top) with all samples pooled. Abbreviations of treatments as describes in Table 1.

389x214mm (300 x 300 DPI)

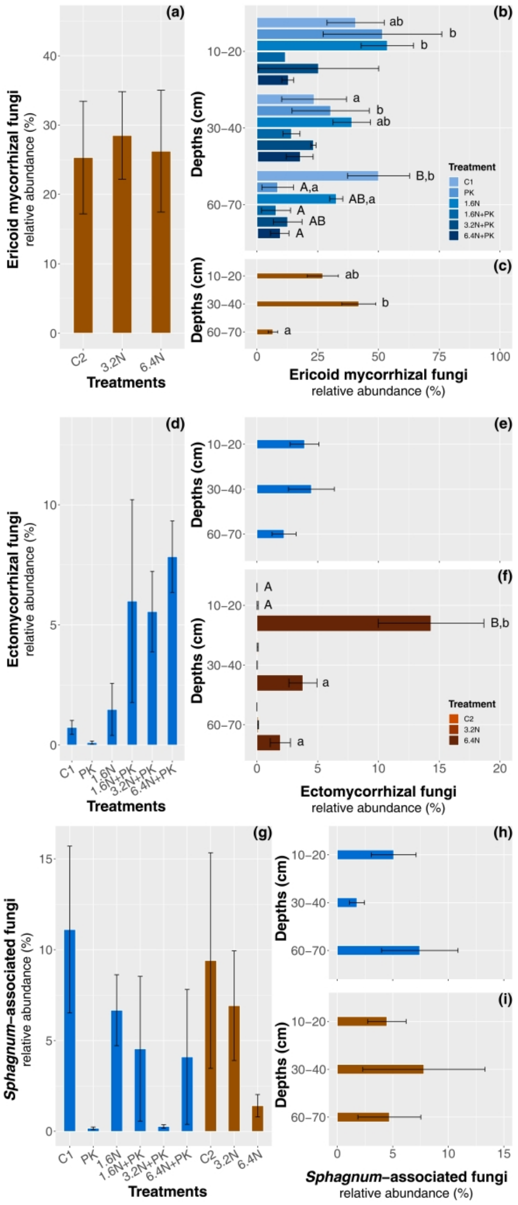

Figure 4 Relative abundance (mean ± SE) of ericoid mycorrhizal fungi, ectomycorrhizal fungi and Sphagnum-associated fungi under different treatments (a, d, g) and depths (b, c, e, f, h, i). There is very strong evidence ($p < 0.001$) for the interactive effects for ericoid mycorrhizal fungi in Experiment 1 (b) and ectomycorrhizal fungi in Experiment 2 (f), and only the main effects are shown for Sphagnum-associated fungi (Supplementary Table S5). Different upper-case letters indicate there is evidence ($p < 0.05$) that the relative abundance of fungal functional guild is different among treatments under the same depth. Different lower-case letters indicate there is evidence ($p < 0.05$) that the relative abundance of fungal functional guild is different among depths under the same treatment. Bars without letters indicate there is no evidence ($p > 0.1$) that the relative abundance of fungal functional guild is different among treatments or depths. Abbreviations of treatments as described in Table 1.

292x680mm (300 x 300 DPI)

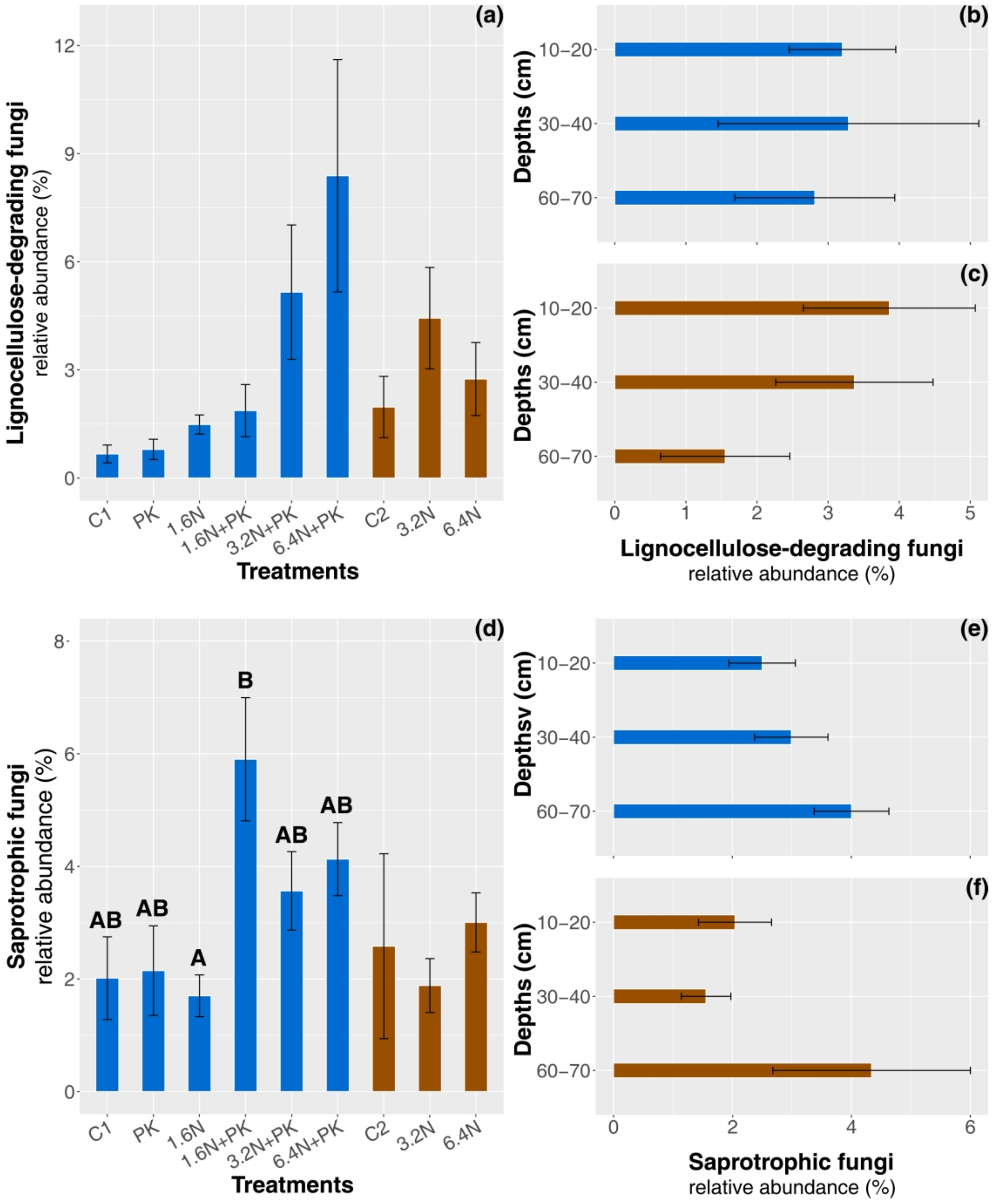

Figure 5 Relative abundance (mean ± SE) of lignocellulose-degrading fungi and saprotrophic fungi (includes only non-lignocellulose degrading saprotrophic fungi) under different treatments (a, d) and depths (b, c, Experiment 1; e, f, Experiment 2). Different upper-case letters indicate there is evidence ($p < 0.05$) that the relative abundance of saprotrophic fungi is different among treatments under the same depth. Bars without letters indicate there is no evidence ($p > 0.1$) that the relative abundance of fungal functional guild is different among treatments or depths. Abbreviations of treatments as described in Table 1.

361x438mm (300 x 300 DPI)

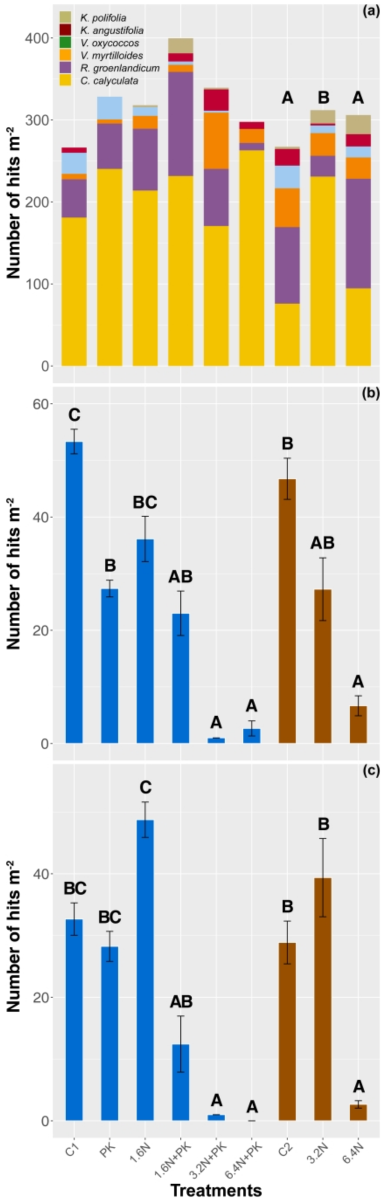

Figure 6 Abundance (mean ± SE) of Ericaceae (a) and covers of Sphagnum (b) and Polytrichum (c) mosses in response to different treatments. Different upper-case letters indicate there is evidence ($p < 0.05$) that the relative abundance of fungal functional guild is different among treatments for each experiment separately. For Ericaceae, there is no evidence ($p > 0.1$) that treatments affect the abundance of Ericaceae in Experiment 1. Bars without letters indicate there is no evidence ($p > 0.1$) that the abundance of Ericaceae is different among treatments. Abbreviations of treatments as described in Table 1.

186x582mm (300 x 300 DPI)