LETTER

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Decay by ectomycorrhizal fungi couples soil organic matter to nitrogen availability

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Abstract

Interactions between soil nitrogen (N) availability, fungal community composition, and soil organic matter (SOM) regulate soil carbon (C) dynamics in many forest ecosystems, but context dependency in these relationships has precluded general predictive theory. We found that ectomycorrhizal (ECM) fungi with peroxidases decreased with increasing inorganic N availability across a natural inorganic N gradient in northern temperate forests, whereas ligninolytic fungal saprotrophs exhibited no response. Lignin-derived SOM and soil C were negatively correlated with ECM fungi with peroxidases and were positively correlated with inorganic N availability, suggesting decay of lignin-derived SOM by these ECM fungi reduced soil C storage. The correlations we observed link SOM decay in temperate forests to tradeoffs in tree N nutrition and ECM composition, and we propose SOM varies along a single continuum across temperate and boreal ecosystems depending upon how tree allocation to functionally distinct ECM taxa and environmental stress covary with soil N availability.

KEYWORDS
decomposition, fine roots, lignin, mycorrhizal fungi, nitrogen, plant–soil interactions, saprotrophic fungi, soil carbon

INTRODUCTION

Interactions between inorganic nitrogen (N) availability and fungal community composition are important controls over soil organic matter (SOM) dynamics in temperate and boreal forests (Averill & Waring, 2018; Kyaschenko et al., 2017), yet the context dependency of these relationships has precluded general theory to predict how SOM varies due to fungal responses to N availability. For example, SOM in many boreal and subalpine forests is negatively correlated with the abundance of ectomycorrhizal (ECM) fungi that decay SOM using class II peroxidases (hereafter, 'peroxidases'; Lindahl et al., 2021), and SOM either increases or decreases with soil N availability depending upon whether these ECM taxa decline or increase (Clemmensen et al., 2015, 2021). In contrast, SOM stocks decline with increasing N availability in fertile boreal forests because of an increase in ligninolytic saprotrophic fungi (Kyaschenko et al., 2017), which also decay lignified compounds in plant litter and SOM using peroxidases (Floudas et al., 2012). In a high-fertility temperate forest, SOM declined with increasing N availability due to greater decay by non-ligninolytic saprotrophic Ascomycete fungi (Mayer et al., 2021). However, relationships between soil inorganic N availability, fungal composition, and SOM remain poorly understood in temperate forests spanning low to intermediate fertility. Addressing this gap may provide a unified framework for predicting how fungal composition and inorganic N availability regulate SOM and the vast amounts of carbon (C) it stores across boreal and temperate forests (Jackson et al., 2017).

Nitrogen deposition experiments in temperate forests suggest increasing inorganic N availability across

natural gradients could enhance the accumulation of SOM by modifying the composition of saprotrophic fungi in decaying fine roots (Figure 1). Senesced fine roots comprise ~50% of plant litter entering forest soils (Freschet et al., 2013) and, due to high lignin concentrations (Sun et al., 2018; Xia et al., 2015), are the major source of lignin-derived polyphenolic SOM in many temperate forests (Thomas et al., 2012; Xia et al., 2015). Experimental N additions in these ecosystems reduce the abundance of ligninolytic saprotrophic fungi inhabiting decaying fine roots—possibly by decreasing their competitive ability—thereby slowing fine root decay, causing lignin-derived compounds to accumulate as SOM (Argiroff et al., 2019; Xia et al., 2018), and enhancing soil C storage (Chen et al., 2018; Zak et al., 2008). If this fungal mechanism operates across natural soil N gradients in temperate forests, it could cause SOM to increase with increasing inorganic N availability (Figure 1, *Saprotroph Mechanism*).

Increasing inorganic N availability could also promote soil C storage in temperate forests by altering the composition of ectomycorrhizal (ECM) fungal communities (Figure 1). Certain ECM lineages have retained peroxidases in their evolution from ligninolytic saprotrophic ancestors, which these ECM fungi likely use to obtain N organically bound in complex polyphenolic SOM (Miyauchi et al., 2020; Pellitier & Zak, 2018). Recent evidence revealed that the relative abundance of ECM fungi with peroxidases in root tips of temperate forest trees declined across a natural gradient of soil inorganic N availability (Pellitier, Zak, et al., 2021; Pellitier & Zak, 2021), suggesting high inorganic N availability could enhance soil C storage by reducing the decay of existing lignin-derived SOM by ECM fungi with peroxidases (Figure 1, *ECM Mechanism*). While ECM with peroxidases likely restrict SOM accumulation in boreal forests (Baskaran et al., 2017; Clemmensen et al., 2021; Lindahl et al., 2021), studies in temperate forests have not addressed the regulatory role of compositional variation within the ECM fungal community because they have primarily focused on how SOM varies between ecosystems dominated by either ECM or arbuscular mycorrhizal fungi (Averill et al., 2018; Phillips et al., 2013).

Here, we tested the hypothesis that soil C storage increases with soil inorganic N availability and that this response is linked to declines in saprotrophic as well as ECM fungi with the genetic potential to decay lignin and lignin-derived SOM (Figure 1). We characterised fungal community composition, lignin-derived SOM, and soil C storage across a natural gradient of soil inorganic N availability in northern broadleaf temperate forests (Pellitier, Ibáñez, et al., 2021; Pellitier & Zak, 2021; Pellitier, Zak, et al., 2021; Zak et al., 1989), in which microsite differences in topography create an inorganic N availability gradient by influencing water availability and nutrient retention (Zak et al., 1989; Zak & Pregitzer,
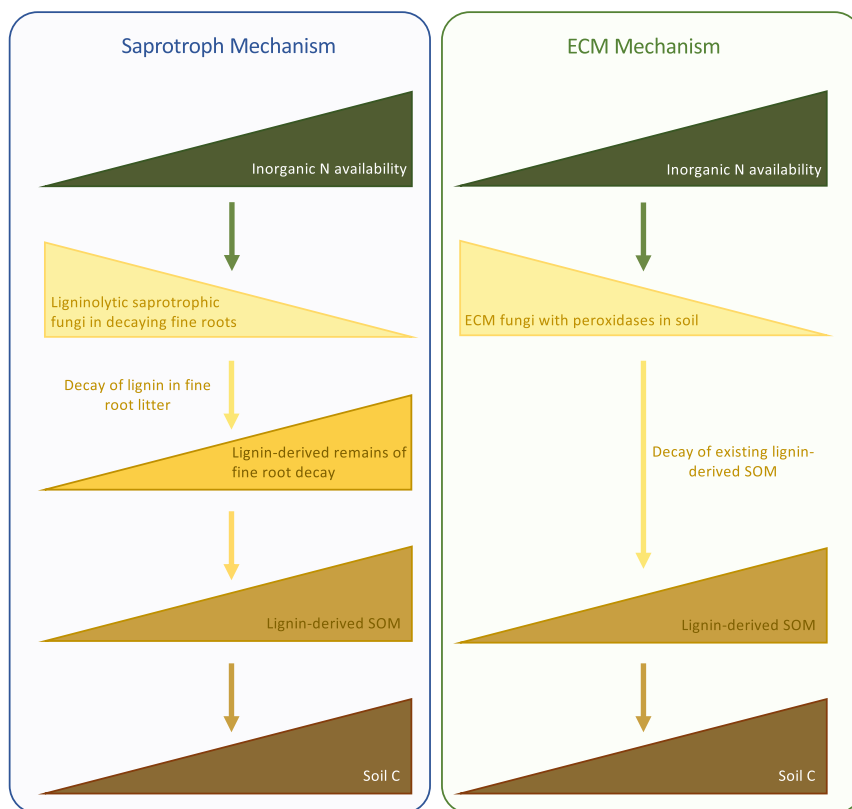
FIGURE 1 Two fungal mechanisms (*Saprotroph Mechanism* and *ECM Mechanism*) could cause naturally high inorganic N availability to suppress the decay of lignin and its derivatives, thereby promoting the accumulation of lignin-derived SOM and increasing soil C storage

1990). We predicted that the relative abundance of ligninolytic fungi in decaying fine root litter and ECM fungi with peroxidases in soil would decline with increasing soil inorganic N availability. We further reasoned that lignin-derived SOM and soil C storage would be negatively correlated with the relative abundance of ligninolytic fungi in decaying fine roots (Figure 1; *Saprotroph Mechanism*) or ECM fungi with peroxidases in soil (Figure 1; *ECM Mechanism*). These fungal responses should cause lignin-derived SOM—and overall soil C—to be positively correlated with inorganic N availability (Figure 1). We focused on ligninolytic fungi in decaying fine root litter because these fungi regulate the amount of lignin-derived fine root material entering SOM (Argiroff et al., 2019; Thomas et al., 2012), and we targeted ECM fungi with peroxidases in soil because ECM fungi primarily decay compounds in existing SOM (Figure 1; Pellitier & Zak, 2018; Sterkenburg et al., 2018). By addressing this knowledge gap and comparing our findings to studies from contrasting ecosystems, we aimed to generate a unified framework for predicting how fungal composition regulates the relationship between N availability and SOM across boreal and temperate forests.

MATERIALS AND METHODS

Site descriptions and study design

We established 72 circular plots (2-m diameter) randomly in 12 forest sites (6 plots per site) in northern Lower Michigan, USA (Figure S1). All plots were located in even-aged (~100 year-old) second-growth northern hardwood forests on uniformly sandy soils (~85% sand), and do not vary in climate due to close geographic proximity (separated by <50 km; Zak et al., 1989; Zak & Pregitzer, 1990). Plots were in mixed stands of *Quercus rubra* (red oak) and *Acer rubrum* (red maple), which co-occur across the inorganic N availability gradient, to minimise differences in the biochemistry of litter inputs. Plots were adjacent to previously studied ECM communities in *Q. rubra* root tips (Pellitier, Ibáñez, et al., 2021; Pellitier & Zak, 2021; Pellitier, Zak, et al., 2021). Our plots ranged in mineralisation rates from 0.08 to 1.19 µg N g^{-1} day^{-1} (Figure S4). These values captured the full gradient of inorganic N availability in the upper Lake States region and have remained seasonally and interannually stable since the 1980s (Pellitier, Ibáñez, et al., 2021; Zak et al., 1989; Zak & Pregitzer, 1990).

Soil and SOM characteristics

In May of 2019, we obtained 6 soil cores (2.5 cm diameter × 10 cm depth) within each plot (Oe and A horizons, excluding Oi) evenly spaced around a 1-m radius from the plot centre. We transported cores on ice to the University of Michigan, sieved field-moist soil through 2-mm mesh, removed fine roots, and homogenised the sieved soil by plot (6 cores homogenised per plot × 6 plots × 12 sites = 72 samples). We froze a subsample of fresh soil at −80°C for DNA isolation, and immediately used two 30 g subsamples of sieved field-moist soil for 28-day laboratory net N mineralisation assays to quantify inorganic N availability (Vitousek et al., 1982; Zak et al., 1989). We measured extractable inorganic N (NO_3^- and NH_4^+) pre- and post-incubation with an AQ2 Discrete Analyser (SEAL Analytical). Laboratory N mineralisation measurements are strongly correlated with in situ net N mineralisation across the forest ecosystems in this study system (Zak et al., 1989; Zak & Pregitzer, 1990) and are therefore a robust representation of inorganic N availability. Inorganic N availability is also correlated with fine root C/N and SOM C/N (Figure S2), and therefore reflects N availability more broadly.

We used a subsample of oven-dried ground soil to determine the relative abundance of lignin-derived SOM by pyrolysis gas chromatography-mass spectrometry (py-GC/MS) following previously described methods (Appendix S1; Argiroff et al., 2019; Grandy et al., 2007, 2009; Pold et al., 2017). We determined soil C and N from ground soil using a CN analyser (LECO). The remaining field-moist soil was air-dried at room temperature and used to determine soil pH with 30 g of air-dried soil in 1:1 slurries in deionised water. We interpolated hourly soil temperature and volumetric water content from May to October of 2019 at each plot by regressing handheld probe measurements against nearest hourly values from a Micro Station data logger (ONSET) at each site.

Decaying fine root litter

We used litterbags to characterise fungal communities in decaying fine root litter (Argiroff et al., 2019; Sun et al., 2018). In May of 2018, we collected soil at each site around 5 mature *Q. rubra* individuals, which occur in the overstory at all sites and therefore represent a large fraction of fine root litter in these forest ecosystems. We collected, rinsed, and dried fine roots ≤0.5 mm in diameter and composited them by the site. We chose the diameter cutoff of ≤0.5 mm because this retained approximately first- through third-order fine roots, which comprise the absorptive fine root modules that turn over rapidly and produce the majority of fine root litter (McCormack et al., 2015; Xia et al., 2010). For each site, we placed ~3 g of fine root litter into each of twelve 12-cm × 12-cm nylon mesh bags (opening size 53 µm), which admit fungal hyphae but prevent fine root ingrowth (Hobbie et al., 2010; Li et al., 2015; Sun et al., 2018). We sterilised litterbags and roots using ethylene oxide (Steris Corporation; Cline & Zak, 2015) to eliminate fungi without altering root biochemistry, and thus assumed any fungi in fine

root litter colonised from adjacent soil after deployment. In May of 2019 (during soil collection), we placed two litterbags horizontally near the centre of each plot and replaced overlying soil without disturbing its vertical distribution. Bags were located at the interface of the O and A horizons (depth of ~3 cm) within the dense mat of fine roots (Figure S3). After 13 months of decay (July of 2020), we retrieved the litter bags, transported them on ice to the laboratory, and homogenised roots by plot. Roots were weighed, a subsample was stored at −80°C for DNA isolation, an additional subsample was oven-dried to constant mass at 60°C and ashed at 500°C for 6 h to determine moisture content and mineral content, respectively, and mass loss was calculated to determine decay rates.

Fungal community composition

We characterised fungal communities inhabiting soil and decaying fine roots using the ITS2 region of the fungal nuclear ribosomal internal transcribed spacer (ITS) region, which is the universal fungal DNA barcode (Nilsson, Anslan, et al., 2019; Schoch et al., 2012). We isolated DNA from 0.15 g decaying fine roots and 1 g soil from each plot (Appendix S1). We targeted the ITS2 region using PCR amplification with ITS4-Fun/5.8S-Fun primers following previously published protocols (Appendix S1; Pellitier et al., 2019; Taylor et al., 2016). PCR libraries were normalised, purified, and sequenced using MiSeq 2 × 250 bp with v2 chemistry (Illumina). We obtained high quality sequences and calculated amplicon sequence variants (ASVs; Callahan et al., 2017; Pauvert et al., 2019) from forward reads using 'DADA2' (Callahan et al., 2016; Rosen et al., 2012) with 'cutadapt' (Martin, 2011). Additionally, we used quantitative real-time PCR (qPCR) of the ITS region with ITS1F and 5.8S primers to determine absolute fungal abundance in soil and decaying roots (Entwistle, Zak, et al., 2018).

We classified sequences using the naïve Bayesian classifier (Wang et al., 2007) and the UNITE database (Kõljalg et al., 2013; Nilsson, Larsson, et al., 2019). We removed genera present in fewer than 5 plots and accounting for <0.1% of sequences separately for the soil and fine roots datasets, leaving 72% of fungal reads from soil and 70% from decaying roots that were assigned to functional groups (Table S1). Ligninolytic saprotrophs were identified with FUNGuild (Nguyen et al., 2016) and literature (Entwistle, Zak, et al., 2018; Ruiz-Dueñas et al., 2021). We used literature to identify ECM genera containing species with class II peroxidases ('AA2' CAZymes; Levasseur et al., 2013; Lombard et al., 2014) in their genomes (Bödeker et al., 2009; De Crop et al., 2017; Kohler et al., 2015; Miyauchi et al., 2020; Nagy et al., 2016). We assumed all species in an ECM genus have peroxidases if these genes have been detected in the species with sequenced genomes belonging to that genus, which

may change as more ECM genomes are sequenced. Class II peroxidases are confined to Auriculariales and more recently diverging orders of Agaricomycetes (Floudas et al., 2012; Nagy et al., 2016). Thus, we assumed ECM and saprotrophic genera outside these lineages do not have peroxidases or strong ligninolytic capacity (hereafter 'ECM without peroxidases' and 'non-ligninolytic saprotrophs'). Remaining ECM genera were identified using FUNGuild. We acknowledge dichotomising ECM genera into those with or without peroxidases is a coarse approximation of oxidative decay capacity since there is considerable variation within genera in peroxidase gene copies (Miyauchi et al., 2020). However, it is currently unclear how variation in peroxidase gene copy number corresponds to in situ ECM decay activity (Pellitier & Zak, 2018), and we believe our current classification is an acceptable initial approximation of function pending experimental verification. We identified 'other mycorrhizas' and 'fungi with other or uncertain ecology' using FUNGuild and the literature (Martino et al., 2018; Seitzman et al., 2011; Smith & Read, 2008). Functional group abundances are relative to the total fungal community unless otherwise specified.

Statistical analyses

We used generalised additive mixed models (GAMM) in the package 'mgcv', which accommodate complex non-linear patterns (Wood, 2011), to test three sets of relationships. First, we evaluated the relationship between each fungal functional group and inorganic N availability for soil and decaying fine root litter. We corrected p-values for false discovery rate using the Benjamini–Hochberg false discovery rate correction (Benjamini & Hochberg, 1995) for these 12 individual GAMMs. In a complementary test of these patterns, we used the package 'TITAN2' (Baker & King, 2010) to test for fungal genera that significantly responded to the inorganic N supply gradient, based on Hellinger-transformed abundances (Legendre & Legendre, 2012). Genera with both purity and reliability ≥0.95 were considered significantly related to inorganic N availability (Baker & King, 2010). Second, we used multiple GAMM to understand if lignin-derived SOM and soil C were correlated with fungal functional groups. Each model had lignin-derived SOM or soil C as the independent variable, and the six fungal functional groups in soil or in decaying fine root litter as the predictor variables. Finally, we used separate GAMM to test whether lignin-derived SOM and soil C were correlated with inorganic N availability.

We performed all analyses using fungal genera present in ≥5 plots and accounting for ≥0.1% of sequences and elected not to subsample sequence counts to limit uncertainty and data loss (McMurdie & Holmes, 2013, 2014). We log_{10}-transformed soil C to obtain normally distributed residuals. Plots within each site varied

considerably in inorganic N availability, and there was substantial overlap in inorganic N among plots from different sites (Figure S4). Given this within-site variation and strong heterogeneity in SOM and fungal community composition at fine spatial scales (Bogar et al., 2019; Taylor et al., 2014), we quantified all variables at the plot level and treated these values separately. Because plots within the same site may be similar due to spatial proximity and unmeasured ecological processes, we accounted for spatial autocorrelation using a spatial correlation structure in all GAMM. Four plots had ecologically unrealistic values for net N mineralisation or SOM biochemistry, likely from sampling error (Figure S4). We removed these plots from all analyses. We accepted statistical significance at $\alpha = 0.05$. All analyses were performed in R version 4.0.2 (R Core Team, 2020) with RStudio version 1.4.869 (RStudio Team, 2020), using the packages 'ShortRead' (Morgan et al., 2009), 'Biostrings' (Pagès et al., 2020), 'phyloseq' (McMurdie & Holmes, 2013), and the 'tidyverse' (Wickham et al., 2019).

RESULTS

Fungal responses to inorganic N availability

ECM fungi with peroxidases (26% of sequences) were the most abundant functional group in soil (Figure S5), and were dominated by *Russula*, *Piloderma*, and *Cortinarius* (Figure S6). Ligninolytic saprotrophs (32%) were most abundant in decaying fine root litter (Figure S5) and were dominated by *Mycena*, *Gymnopus*, and *Trechispora* (Figure S6). The abundance of ECM with peroxidases decreased as soil inorganic N availability increased in both soil ($R^2_{adj.} = 0.455$, $p_{adj.} < 0.001$; Figure 2a) and decaying fine root litter ($R^2_{adj.} = 0.516$, $p_{adj.} < 0.001$; Figure 2b; Table S2). ECM root tips (Pellitier, Ibáñez, et al., 2021), fine root biomass (Figure S7), and ITS copy number also declined with increasing inorganic N availability (Figure S8). Thus, the absolute abundance of ECM fungi with peroxidases clearly declined with inorganic N availability. The proportion of taxa with peroxidases within the ECM fungal community also declined with increasing inorganic N availability, although this response was not significant after accounting for spatial autocorrelation ($p = 0.101$; Figure S9). The relative abundance of ligninolytic saprotrophs in soil increased as inorganic N availability increased ($R^2_{adj.} = 0.064$, $p_{adj.} = 0.034$; Figure 2a), whereas ligninolytic saprotrophs in decaying fine root litter were not influenced by inorganic N availability ($p_{adj.} = 0.34$; Figure 2b). The relative abundance of ECM fungi without peroxidases in soil also declined with increasing inorganic N availability ($R^2_{adj.} = 0.079$, $p_{adj.} = 0.025$; Figure 2a), whereas this functional group in decaying fine root litter did not respond to inorganic N availability ($p_{adj.} = 0.12$; Figure 2b).

The relative abundance of non-ligninolytic saprotrophic fungi increased with increasing inorganic N availability in soil ($R^2_{adj.} = 0.284$, $p_{adj.} < 0.001$; Figure 2a) and decaying fine roots ($R^2_{adj.} = 0.189$, $p_{adj.} < 0.001$; Figure 2b). The relationship between ECM fungi with peroxidases and inorganic N availability was robust to inclusion of soil pH, volumetric water content, and temperature in the GAMM (Table S3). Sequencing yield and taxonomic distributions are described in Tables S4–S7.

We found that 7 of 8 ECM genera in soil that possess peroxidases significantly declined in relative abundance as inorganic N availability increased (Figure 3a). Similarly, most ECM genera with peroxidases in decaying fine root litter significantly declined as inorganic N supply increased (Figure 3b). ECM genera without peroxidases exhibited mixed responses to the inorganic N availability gradient in both soil and decaying fine root litter (Figure 3). Non-ligninolytic saprotrophic genera in soil generally increased as inorganic N availability increased (Figure 3a), but few ligninolytic saprotrophs responded significantly to inorganic N availability (Figure 3).

Links between fungal communities, SOM, and soil C

Lignin-derived SOM ($p = 0.039$; Figure 4a) and soil C ($p = 0.019$; Figure 4b) were significantly negatively related to ECM fungi with peroxidases in soil (Table S8). Lignin-derived SOM was positively related to the relative abundance of non-ligninolytic saprotrophic fungi in decaying fine root litter ($p = 0.013$; Figure 4c), as well as the relative abundance of fungi with other or uncertain ecology ($p = 0.034$; Figure S10); however, the latter relationship was driven by two large outliers and was likely spurious. Lignin-derived SOM and soil C were not significantly related to the relative abundance of ligninolytic saprotrophs ($p > 0.05$; Figure 4 and Table S8).

Response of SOM biochemistry and soil C storage to inorganic N availability

The relative abundance of lignin-derived SOM, which accounted for 13% of SOM on average (Figure S11), increased as inorganic N availability increased ($R^2_{adj.} = 0.085$, $p_{adj.} = 0.045$; Figure 5a). Soil C was strongly positively correlated with lignin-derived SOM ($R^2_{adj.} = 0.407$, $p_{adj.} < 0.001$; Figure 5b; Table S9). Consequently, soil C increased with increasing inorganic N availability ($R^2_{adj.} = 0.069$, $p_{adj.} = 0.016$; Figure 5c). These relationships were robust to the inclusion of soil pH, volumetric water content, and temperature in the GAMM (Table S3). Furthermore, fine root mass loss was not correlated with inorganic N availability, lignin-derived SOM, or soil C storage (Figure S12).
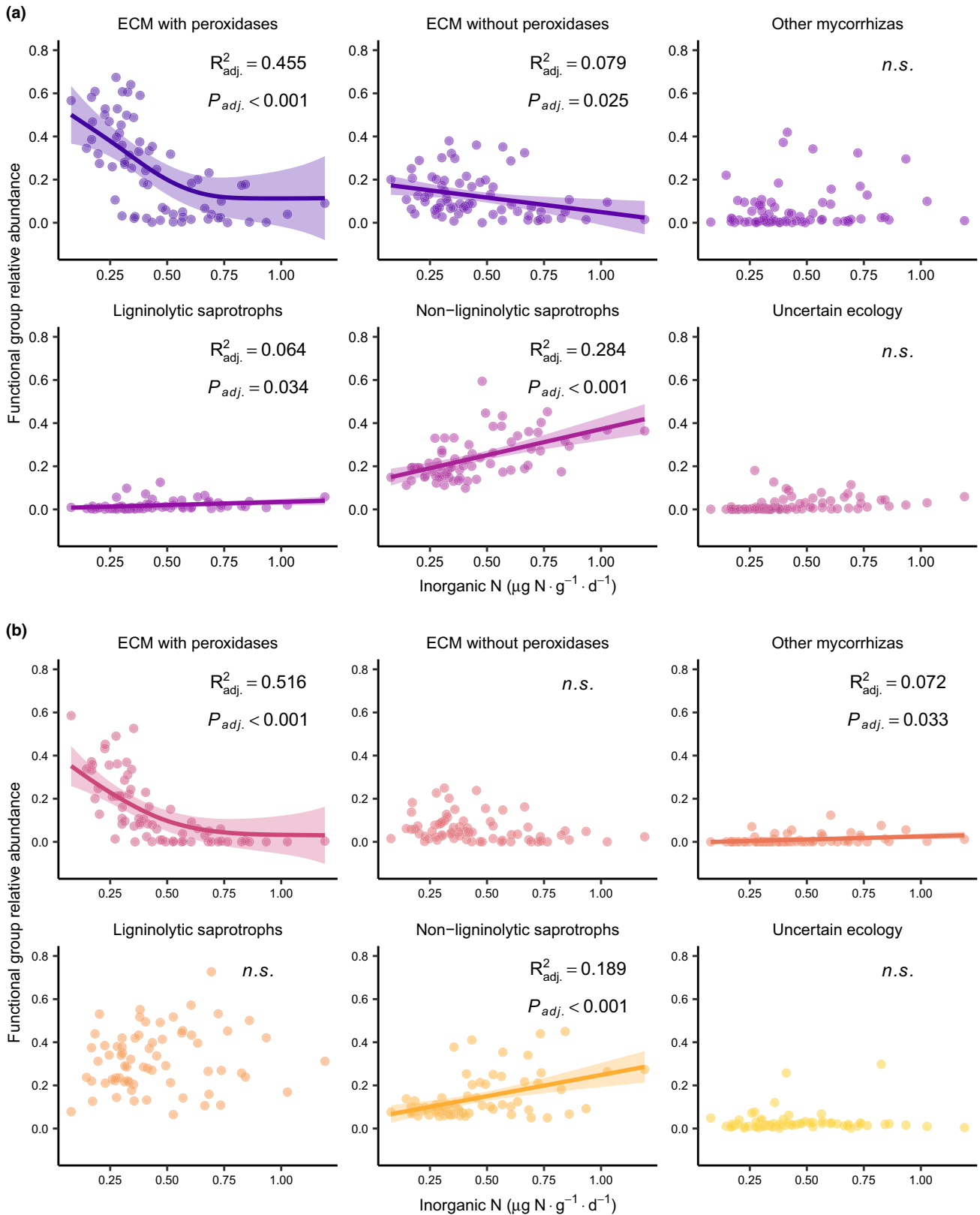
FIGURE 2 Fungal functional group responses in soil (a) and decaying fine root litter (b) across the soil inorganic N availability gradient. Each plot (and each colour) represents a separate GAMM with the relative abundance of a functional group as the dependent variable and soil inorganic N availability as the independent variable, from which trend lines, 95% confidence intervals, and R^2 were calculated. We corrected p-values for multiple tests using the Benjamini–Hochberg false discovery rate correction. We explicitly accounted for spatial autocorrelation in GAMM models with a spatial correlation structure based on the geographic coordinates of each plot ($n = 68$). $n.s.$, not significant
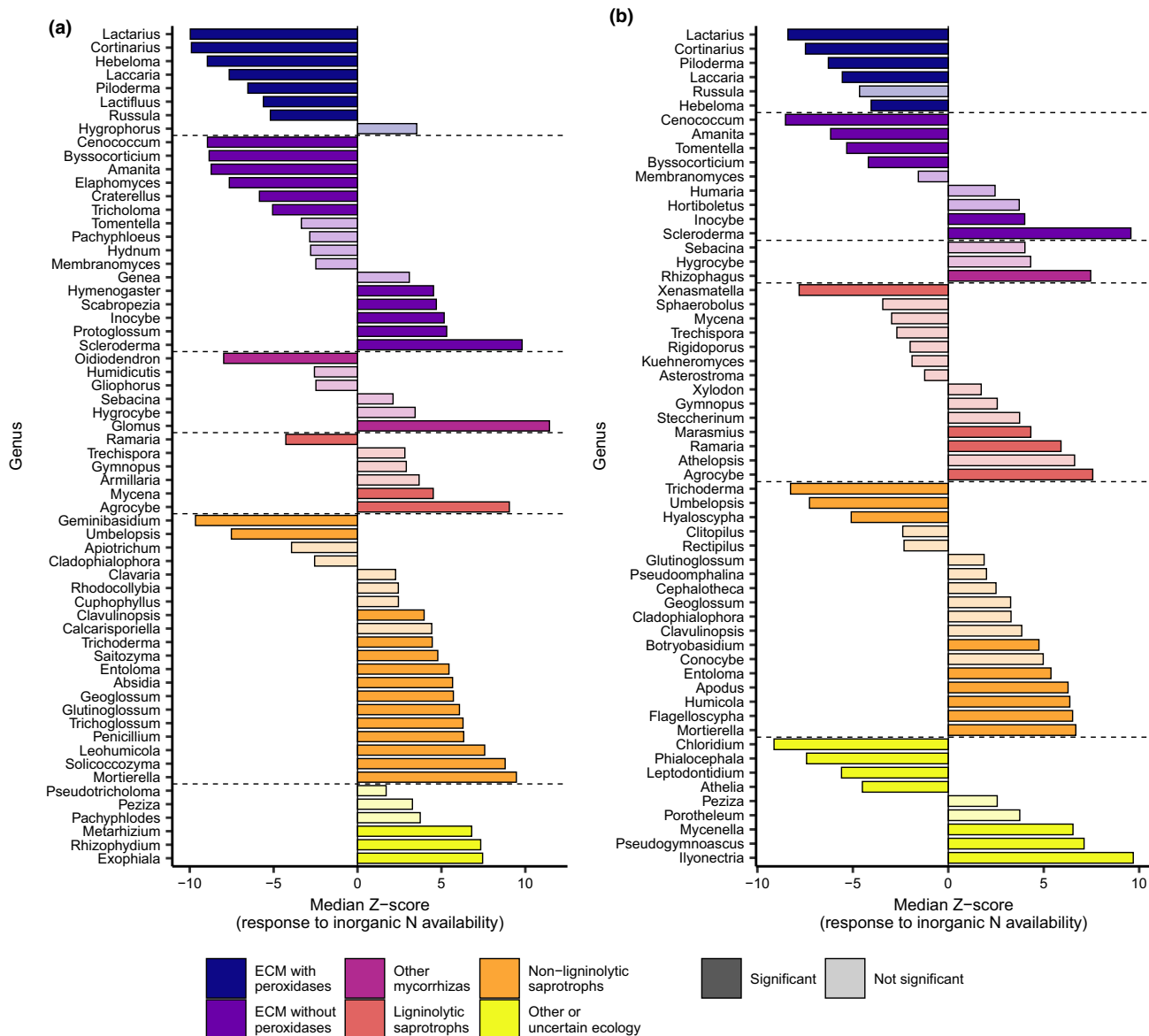
FIGURE 3 Responses to inorganic N availability for individual genera in soil (a) and fine root litter (b) determined by TITAN analysis. ECM fungal genera with peroxidases nearly universally declined in relative abundance with increasing inorganic N availability. Bars display median Z-scores (across 1000 bootstrap replicates), which represent the magnitude of the change in genus relative abundance across the gradient of inorganic N availability. Positive Z-scores indicate genera that increased with increasing inorganic N availability, whereas negative Z-scores indicate genera that decreased in relative abundance with increasing inorganic N availability. We considered responses with both purity and reliability ≥0.95 as statistically significant (solid bars), and <0.95 for either purity or reliability as not significant (faded bars). Genus abundances were Hellinger-transformed prior to TITAN analysis

DISCUSSION

## Turnover in ECM composition constrains SOM accumulation

Our findings suggest the accumulation of SOM in ECM-dominated temperate forests is restricted by the decay activity of ECM fungi with peroxidases (Figures 4 and 5). Although ligninolytic genera (e.g., *Mycena*) were abundant in root litter (Figure 2b and Figures S5 and S6; Tables S1 and S7), which is common for fungal communities decaying fine roots (Argiroff et al., 2019; Kohout et al.,

2018; Philpott et al., 2017), we found no evidence that these fungi responded to inorganic N availability (Figures 2 and 3). Furthermore, lignin-derived SOM and soil C storage were not significantly related to the relative abundance of ligninolytic saprotrophic fungi (Figure 4) or fine root mass loss (Figure S12). These observations were inconsistent with the *Saprotroph Mechanism* (Figure 1), plausibly because natural inorganic N gradients are more subtle than N deposition experiments. In contrast, the relative abundance of ECM fungi with peroxidases declined with increasing inorganic N availability (Figures 2 and 3). This response was nearly uniform across ECM genera
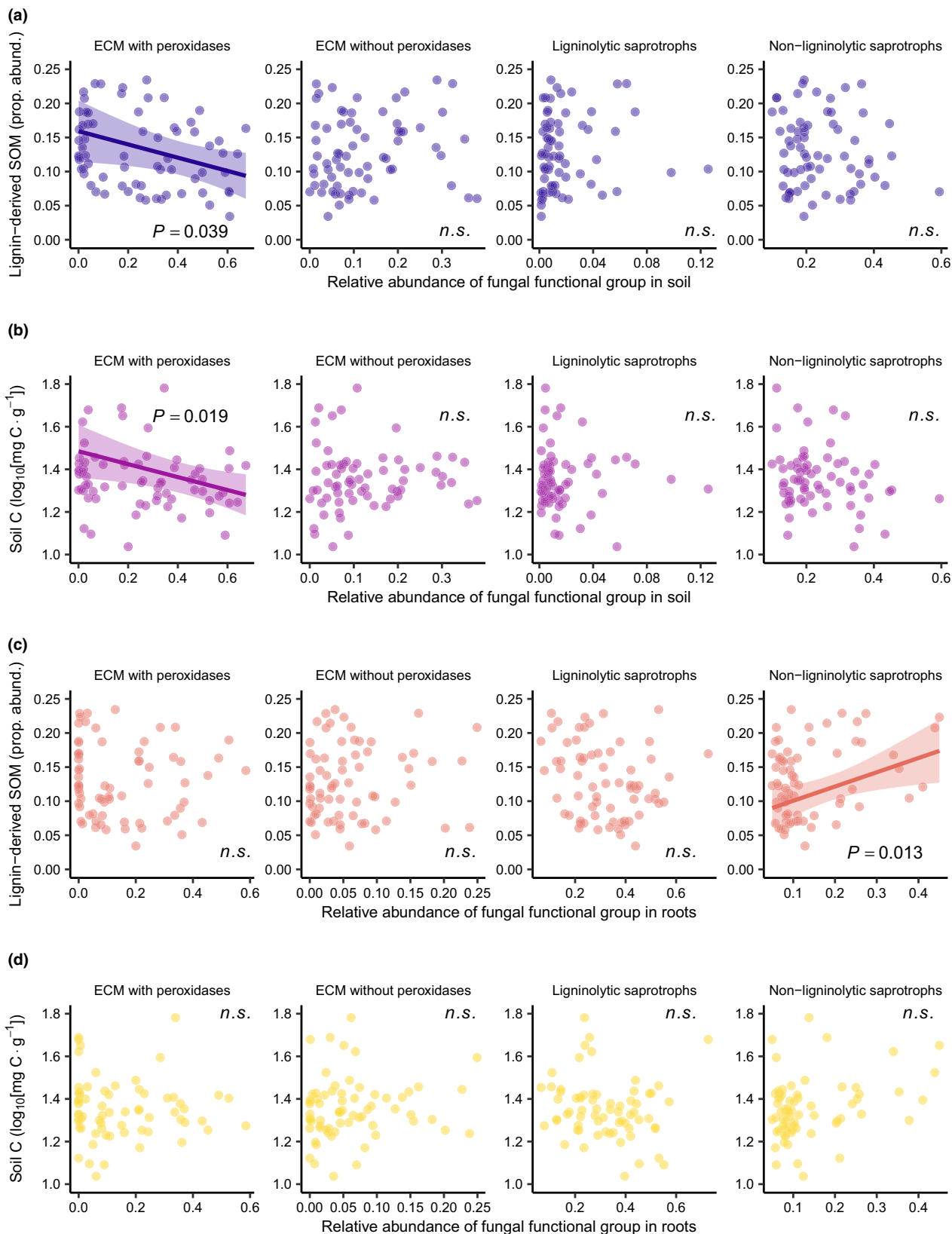
FIGURE 4 Partial plots from multiple GAMM showing relationship between lignin-derived SOM and fungal functional groups in soil (a), soil C and fungal functional groups in soil (b), lignin-derived SOM and fungal functional groups in decaying fine roots (c), and soil C and fungal functional groups in soil (d). Each lettered panel represents a separate multiple GAMM with all functional groups as predictor variables, from which p-values and R^2_{adj} were calculated. Other mycorrhizas and fungi with uncertain ecology were included in the four models but are not shown (see Figure S7). We explicitly accounted for spatial autocorrelation in GAMM models with a spatial correlation structure based on the geographic coordinates of each plot ($n = 68$). Trend lines and confidence intervals are for visualisation purposes only
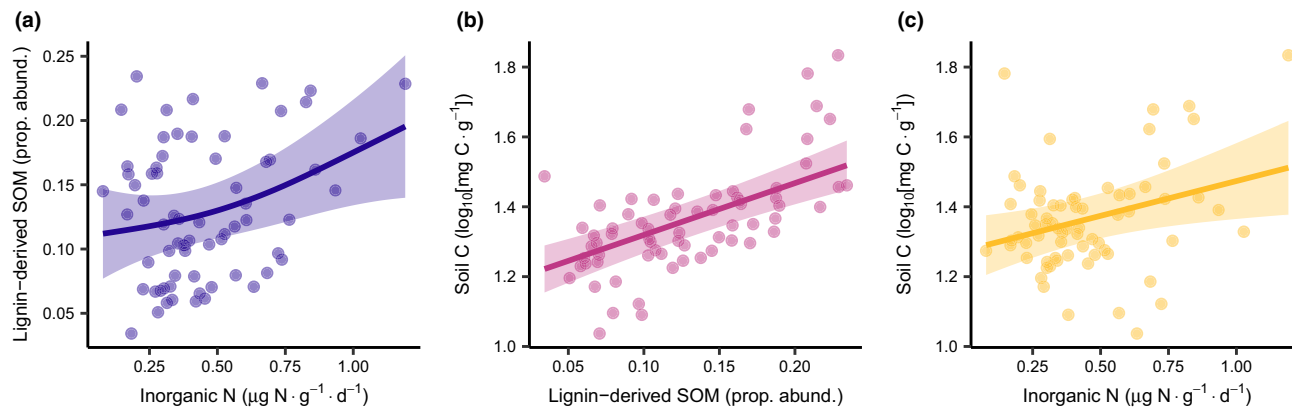
FIGURE 5 Relationships between lignin-derived SOM and inorganic N availability (a), soil C and lignin-derived SOM (b), and soil C storage and inorganic N availability (c). Each plot (and each colour) represents a separate GAMM with the relative abundance of a functional group as the dependent variable and soil inorganic N availability as the independent variable, from which trend lines, 95% confidence intervals, and R^2 were calculated. We explicitly accounted for spatial autocorrelation in GAMM models among plots ($n = 68$) using a spatial correlation structure

with peroxidases, including *Cortinarius*, *Piloderma*, and *Russula* (Figure 3, Tables S1 and S7; Miyauchi et al., 2020). Importantly, lignin-derived SOM and soil C storage were negatively related to the relative abundance of ECM fungi with peroxidases in soil, which is consistent with the prediction that naturally high inorganic N availability promotes soil C storage by reducing the decay of existing lignin-derived SOM by ECM fungi with peroxidases (Figure 1; *ECM Mechanism*). We caution that these relationships have relatively low explanatory power ($R^2 < 0.1$), suggesting other processes also contribute to differences in SOM in our study. Nonetheless, our findings highlight a surprising similarity between SOM in the mineral soil of temperate broadleaf forests and low fertility boreal ecosystems with large organic horizons, whereby soil C storage is reduced where ECM fungi that have retained greater oxidative decay capacities are more abundant (Clemmensen et al., 2015, 2021; Lindahl et al., 2021).

Studies of mycorrhizae and SOM in temperate forests have primarily focused on how soil C storage differs between ecosystems dominated by ECM or arbuscular mycorrhizae (Averill et al., 2018; Phillips et al., 2013), yet our observations suggest compositional turnover *within* ECM-dominated fungal communities is also an important control over SOM dynamics. Because ECM fungi do not assimilate or respire the organic C compounds they decay while acquiring organic N (Baldrian, 2009; Lindahl & Tunlid, 2015; Treseder et al., 2006), many conceptualisations assume ECM fungi selectively liberate N from SOM (Fernandez et al., 2020; Orwin et al., 2011; Smith & Wan, 2019). However, ECM lineages that have retained peroxidases from ligninolytic saprotrophic ancestors are unlikely to liberate N from SOM without also extensively decaying lignin-derived SOM, because peroxidases and ancillary enzymes fully and extracellularly oxidise lignin-derived compounds to CO_2 (Hofrichter, 2002; Kirk & Farrell, 1987; Pellitier & Zak, 2018). This prediction is consistent with our observation that lignin-derived

SOM and soil C storage were negatively correlated with the relative abundance of ECM fungi with peroxidases (Figure 4a,b). By contrast, ECM fungi that have evolved within Ascomycota or other non-ligninolytic saprotrophic lineages either have minimal decay capacity or use non-enzymatic Fenton chemistry to selectively acquire organic N (Pellitier & Zak, 2018; Rineau et al., 2012; Shah et al., 2016). Accordingly, we observed no correlation between soil C and ECM fungi without peroxidases (Figure 4). Thus, the effect of ECM fungi on soil C storage appears to depend on the decay traits of dominant ECM taxa.

The results of our study have important implications for how soil C storage in temperate forests may respond to environmental change. For example, rising atmospheric CO_2 stimulates plant growth (Campbell et al., 2017), and ectomycorrhizal plants may increase their investment in organic N acquisition by ECM mutualists to maintain this growth (Terrer et al., 2016). Our observation that certain ECM fungi may decrease soil C when acquiring N from SOM (Figure 4a,b) suggests elevated CO_2 could decrease soil C storage in temperate forests by increasing organic N acquisition. This prediction is consistent with recent evidence that elevated CO_2 decreases soil C in ecosystems dominated by plants associated with ECM fungi (Terrer et al., 2021), and our study provides a plausible mechanism to explain this pattern. However, just as functional variation among ECM communities alters the capacity for trees to obtain N from SOM under elevated CO_2 (Pellitier, Ibáñez, et al., 2021), our study suggests that not all ECM communities will similarly impact SOM as atmospheric CO_2 continues to increase. Assuming elevated CO_2 does not modify ECM composition, we propose that increased plant allocation to organic N acquisition in response to rising atmospheric CO_2 will reduce soil C storage where ECM communities are dominated by taxa with peroxidases but will not decrease soil C storage where ECM have selective or minimal decay capacity.

A unified framework linking ECM fungi, N availability, and SOM

We propose apparent context dependency in relationships between SOM, fungi, and N availability can be resolved into general predictive understanding if we consider how plant allocation to 'expensive' ECM decay capacities and environmental stress vary with N availability (Figure 6). Plants associate with ECM mutualists that optimise the organic N acquisition return of their photosynthate investment (Bogar et al., 2019; Hortal et al., 2017). Specifically, investment in ECM symbionts with energetically costly organic N acquisition capacities is beneficial to plant hosts where inorganic N is scarce, favouring ECM taxa with a greater genetic potential to obtain N from SOM using peroxidases and other oxidative enzymes (Baskaran et al., 2017; Defrenne et al., 2019; Pellitier & Zak, 2021). These decay traits enable ECM communities in soils with low inorganic N availability to more substantially supplement tree N nutrition with N from SOM across the ecosystems in our study (Pellitier, Ibáñez, et al., 2021; Pellitier, Zak, et al., 2021), and our current findings suggest this enhanced decay, in turn, reduces lignin-derived SOM and soil C storage (Figure 6). We propose that this nutritional tradeoff continues to operate in relatively fertile boreal forests, in which ECM fungi with peroxidases decline with increasing N availability and plausibly enable greater SOM decay by ligninolytic saprotrophs (Figure 6; Kyaschenko et al., 2017).

However, as N availability continues to decline from relatively fertile to low fertility boreal ecosystems, the relationship between ECM with peroxidases and fertility reverses (Figure 6; Clemmensen et al., 2015). This occurs because declines in tree productivity and pH favour stress-tolerant mycorrhizae belonging to Ascomycota over ECM fungi with peroxidases that require greater photosynthate allocation (Sterkenburg et al., 2015), causing soil C storage to increase with declining fertility (Clemmensen et al., 2015). Thus, we propose that the abundance of ECM with peroxidases restricts SOM accumulation across gradients of soil inorganic N availability, but that the direction of this relationship depends on the location along a broader continuum of N availability and photosynthate allocation that connects boreal and temperate ecosystems (Figure 6). Exceptions to this pattern could occur where narrower ranges of soil N availability in fertile temperate forests cause less variation in ECM abundance (Mayer et al., 2021) or where dramatic transitions in boreal and subarctic ecosystem types reverse the relationships between environmental stress, host allocation to ECM with greater decay potential, and N availability (Clemmensen et al., 2021).

Important considerations and conclusions

Aboveground productivity in the forests we studied is positively correlated with inorganic N availability
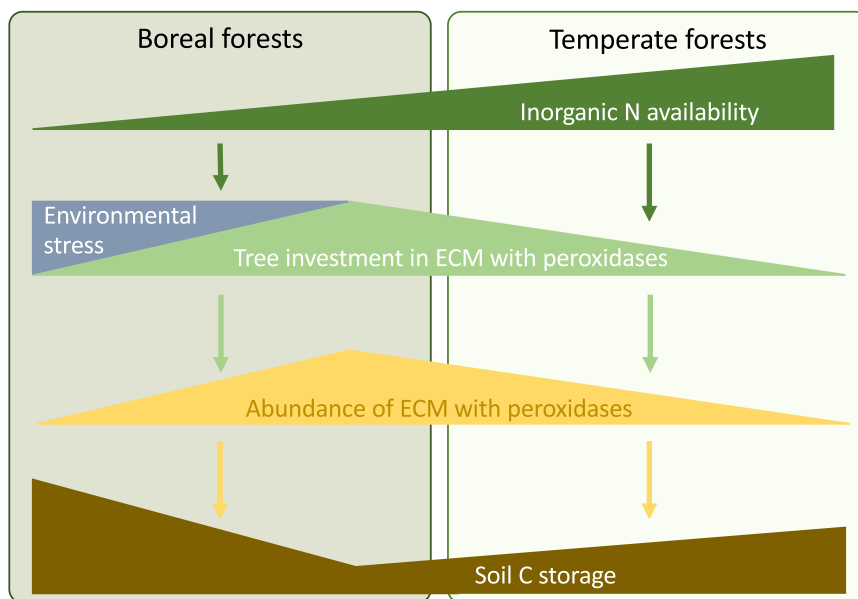
FIGURE 6   Illustration of our proposed framework unifying relationships between N availability, ECM composition, and SOM across temperate and boreal ecosystems. As inorganic N availability decreases from high fertility to low fertility temperate forests, the abundance of ECM with peroxidases increases due to increased photosynthate allocation to ECM with greater decay capacity. This shift in fungal composition increases the decay of lignin-derived SOM, thereby causing soil C storage to decline with decreasing inorganic N availability. This pattern continues into high-fertility boreal forests, until reduced productivity and increased environmental stress favour stress-tolerant ericoid mycorrhizae over ECM taxa with peroxidases. Consequently, SOM increases as inorganic N declines after this point

(Zak et al., 1989), suggesting higher aboveground litter production could cause greater soil C storage where inorganic N availability is also high. However, this explanation is unlikely because fine root biomass declined with inorganic N availability (Figure S7). Thus, total litter production (above- plus belowground) may be relatively even across the inorganic N availability gradient. Additionally, because fine root litter is the primary source of lignin-derived SOM in forest soils (Thomas et al., 2012; Xia et al., 2015), the amount of lignified plant material entering soil should be greatest at the low end of the inorganic N availability gradient. Because we observed the lowest amount of lignin-derived SOM in low inorganic N soils (Figure 5a), these putative differences in fine root litter inputs strengthen our conclusion that decay by ECM fungi with peroxidases regulates SOM.

Although our results are based on correlations between fungal community composition and SOM, we used these approaches to test specific *a priori* hypotheses (Figure 1; Lindahl et al., 2021; Prosser, 2020). Nonetheless, experimental approaches will be required to verify the *ECM Mechanism* (Figure 1). For example, trenching experimentally excludes ECM fungi to isolate their role in SOM dynamics (Averill & Hawkes, 2016; Gadgil & Gadgil, 1971; Sterkenburg et al., 2018). However, given the long-term limitations of this approach (Fernandez & Kennedy, 2016), a combination of laboratory manipulations, field observations, and modelling will be required to verify the quantitative importance of ECM fungi for SOM dynamics across inorganic N availability gradients (Bradford et al., 2021; Zak, Pellitier, et al., 2019). An additional mechanism through which anthropogenic N deposition slows lignin decay is by reducing the expression of saprotrophic peroxidase genes (Entwistle, Romanowicz, et al., 2018; Zak, Argiroff, et al., 2019), a response also observed in some ECM fungi (Bödeker et al., 2014). Accounting for this possibility across natural gradients of inorganic N availability should be another objective of future studies.

Together, our observations support the idea that soil C increases across a natural soil inorganic N availability gradient due to a decline in decay by ECM fungi with peroxidases (Figure 1, *ECM Mechanism*). Our study suggests ECM community composition and its turnover across soil N availability gradients is one control over SOM in temperate forests, and we propose that shifts in plant allocation to certain ECM fungi and environmental stress across these gradients place temperate and boreal ecosystems along a single continuum of soil N availability, ECM fungi, and SOM (Figure 6). We emphasise that decay by ECM fungi and its effect on soil C vary with ECM community composition and cannot be universally ascribed to all ECM communities (Figure 6). In addition to rising atmospheric CO_2, numerous additional drivers of environmental change alter the composition and activity of ECM communities, including anthropogenic N deposition (Bödeker et al., 2014; Lilleskov et al., 2002) and climatic shifts (Steidinger et al., 2020). Explicitly considering the direct decay of SOM by ECM fungi and the contingency of this process on ECM community composition may improve our ability to predict how ongoing environmental change impacts soil C storage.

AUTHOR CONTRIBUTIONS
WAA and DRZ designed the study; WAA, RAU, DRZ, and PTP performed field sampling; WAA, RAU, and JPB performed laboratory analyses; WAA analysed data and wrote the manuscript; PTP made additional conceptual contributions; all authors contributed to revisions.

PEER REVIEW
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DATA AVAILABILITY STATEMENT
Sequence data are available on Genbank (Project Accession PRJNA714922), and all other data and code are available on Github (https://github.com/ZakLab-Soils/ECM_SOM_RProject) and DRYAD https://doi.org/10.5061/dryad.zs7h44jb4.

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