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**Title:** Decay by ectomycorrhizal fungi couples soil organic matter to nitrogen availability

**Running Title:** ECM decay decreases soil C storage

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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.

## 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 (Kyaschenko *et al.* 2017; Averill & Waring 2018), 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 sub-alpine 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 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 (Fig. 1). Senesced fine roots comprise ~50% of plant litter entering forest soils (Freschet *et al.* 2013) and, due to high lignin concentrations (Xia *et al.* 2015; Sun *et al.* 2018), 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 (Xia *et al.* 2018; Argiroff *et al.* 2019), and enhancing soil C storage (Zak *et al.* 2008; Chen *et al.* 2018). If this fungal mechanism operates across natural soil N gradients in temperate forests, it could cause SOM to increase with increasing inorganic N availability (Fig. 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 (Fig. 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 (Pellitier & Zak 2018; Miyauchi *et al.* 2020). 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 *et al.* 2021a,c), suggesting high inorganic N availability could enhance soil C storage by reducing the decay of existing lignin-derived SOM by ECM fungi with peroxidases (Fig. 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 (Phillips *et al.* 2013; Averill *et al.* 2018).

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 (Fig. 1). We characterized fungal community composition, lignin-derived SOM, and soil C storage across a natural gradient of soil inorganic N availability in northern broadleaf temperate forests (Zak *et al.* 1989; Pellitier *et al.* 2021a,b,c), in which microsite differences in topography create an inorganic N availability gradient by influencing water availability and nutrient retention (Zak *et al.* 1989; Zak and Pregitzer 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 (Fig. 1; *Saprotroph Mechanism*) or ECM fungi with peroxidases in soil (Fig. 1; *ECM Mechanism*). These fungal responses should cause lignin-derived SOM – and overall soil C – to be positively correlated with inorganic N availability (Fig. 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 (Thomas *et al.* 2012; Argiroff *et al.* 2019), and we targeted ECM fungi with peroxidases in soil because ECM fungi primarily decay compounds in existing SOM (Fig.

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 (Supplemental Information, Fig. 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 minimize differences in the biochemistry of litter inputs. Plots were adjacent to previously studied ECM communities in *Q. rubra* root tips (Pellitier *et al.* 2021a,b,c). Our plots ranged in mineralization rates from 0.08 to 1.19 $\mu$g N $g^{-1}$ · $d^{-1}$ (Fig. 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 (Zak *et al.* 1989; Zak & Pregitzer 1990; Pellitier et al. 2021b).

*Soil and SOM characteristics*

In May of 2019, we obtained 6 soil cores (2.5 cm diameter x 10 cm depth) within each plot (Oe and A horizons, excluding Oi) evenly spaced around a 1-m radius from the plot center. We transported cores on ice to the University of Michigan, sieved field-moist soil through 2-mm mesh, removed fine roots, and homogenized the sieved soil by plot (6 cores homogenized per plot x 6 plots x 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 mineralization 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 Analyzer (SEAL Analytical). Laboratory N mineralization measurements are strongly correlated with *in situ* net N mineralization 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 (Fig. 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; Grandy *et al.* 2007, 2009; Pold *et al.* 2017; Argiroff *et al.* 2019). We determined soil C and N from ground soil using a CN analyzer (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 deionized 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 characterize fungal communities in decaying fine root litter (Sun *et al.* 2018; Argiroff *et al.* 2019). 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 site. We chose the diameter cutoff of $\leq 0.5$ 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 (Xia *et al.* 2010; McCormack *et al.* 2015). For each site, we placed ~3 g of fine root litter into each of twelve 12-cm x 12-cm nylon mesh bags (opening size 53 $\mu$m), which admit fungal hyphae but prevent fine root ingrowth (Hobbie *et al.* 2010; Li *et al.* 2015; Sun *et al.* 2018). We sterilized 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 colonized from adjacent soil after deployment. In May of 2019 (during soil collection), we placed two litterbags horizontally near the center 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 (Fig. S3). After 13 months of decay (July of 2020), we retrieved the litter bags, transported them on ice to the laboratory, and homogenized 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 hours to determine moisture content and mineral content, respectively, and mass loss was calculated to determine decay rates.

*Fungal community composition*

We characterized 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 (Schoch *et al.* 2012; Nilsson *et al.* 2019a). 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; Taylor *et al.* 2016; Pellitier *et al.* 2019). PCR libraries were normalized, purified, and sequenced using MiSeq 2x250 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' (Rosen *et al.* 2012; Callahan *et al.* 2016) 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 *et al.* 2018b).

We classified sequences using the naïve Bayesian classifier (Wang *et al.* 2007) and the UNITE database (Kõljalg *et al.* 2013; Nilsson *et al.* 2019b). 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 *et al.* 2018b; Ruiz-Dueñas *et al.* 2020). 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; Kohler *et al.* 2015; Nagy *et al.* 2016; De Crop *et al.* 2017; Miyauchi *et al.* 2020). 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 dichotomizing ECM genera into those with and 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 (Smith & Read 2008; Seitzman *et al.* 2011; Martino *et al.* 2018). Functional group abundances are relative to the total fungal community unless otherwise specified.

*Statistical analyses*

We used generalized additive mixed models (GAMM) in the package 'mgcv', which accommodate complex nonlinear 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 $\geq 0.95$ 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 $\geq 5$ plots and accounting for $\geq 0.1\%$ 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 (Fig. S4). Given this within-site variation and strong heterogeneity in SOM and fungal community composition at fine spatial scales (Taylor *et al.* 2014; Bogar *et al.* 2019), 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 mineralization or SOM biochemistry, likely from sampling error (Fig. 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 (Fig. S5), and were dominated by *Russula*, *Piloderma*, and *Cortinarius* (Fig. S6). Ligninolytic saprotrophs (32%) were most abundant in decaying fine root litter (Fig. S5) and were dominated by *Mycena*, *Gymnopus*, and *Trechispora* (Fig. 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; Fig. 2a) and decaying fine root litter ($R^2_{adj.}$ = 0.516, $P_{adj.}$ < 0.001; Fig. 2b; Table S2). ECM root tips (Pellitier *et al.* 2021b), fine root biomass (Fig. S7), and ITS copy number also declined with increasing inorganic N availability (Fig. 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; Fig. S9). The relative abundance of ligninolytic saprotrophs in soil increased as inorganic N availability increased ($R^2_{adj.}$ = 0.064, $P_{adj.}$ = 0.034; Fig. 2a), whereas ligninolytic saprotrophs in decaying fine root litter were not influenced by inorganic N availability ($P_{adj.}$ = 0.34; Fig. 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; Fig. 2a),

whereas this functional group in decaying fine root litter did not respond to inorganic N availability ($P_{adj.}$ = 0.12; Fig. 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; Fig. 2a) and decaying fine roots ($R^2_{adj.}$ = 0.189, $P_{adj.}$ < 0.001; Fig. 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 (Fig. 3a). Similarly, most ECM genera with peroxidases in decaying fine root litter significantly declined as inorganic N supply increased (Fig. 3b). ECM genera without peroxidases exhibited mixed responses to the inorganic N availability gradient in both soil and decaying fine root litter (Fig. 3). Non-ligninolytic saprotrophic genera in soil generally increased as inorganic N availability increased (Fig. 3a), but few ligninolytic saprotrophs responded significantly to inorganic N availability (Fig. 3).

*Links between fungal communities, SOM, and soil C*

Lignin-derived SOM ($P$ = 0.039; Fig. 4a) and soil C ($P$ = 0.019; Fig. 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; Fig. 4c), as well as the relative abundance of fungi with other or uncertain ecology ($P$ = 0.034; Fig. 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; Fig. 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 (Fig. S11), increased as inorganic N availability increased ($R^2_{adj.}$ = 0.085, $P_{adj.}$ = 0.045; Fig. 5a). Soil C was strongly positively correlated with lignin-derived SOM ($R^2_{adj.}$ = 0.407, $P_{adj.}$ < 0.001; Fig. 5b; Table S9). Consequently, soil C increased with increasing inorganic N availability ($R^2_{adj.}$ = 0.069, $P_{adj.}$ = 0.016; Fig. 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 (Fig. S12).

## 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 (Figs. 4-5). Although ligninolytic genera (*e.g.*, *Mycena*) were abundant in root litter (Figs. 2b and S5-S6; Tables S1 and S7), which is common for fungal communities decaying fine roots (Philpott *et al.* 2017; Kohout *et al.* 2018; Argiroff *et al.* 2019), we found no evidence that these fungi responded to inorganic N availability (Figs. 2-3). Furthermore, lignin-derived SOM and soil C storage were not significantly related to the relative abundance of ligninolytic saprotrophic fungi (Fig. 4) or fine root mass loss (Fig. S12). These observations were inconsistent with the *Saprotroph Mechanism* (Fig. 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 (Figs. 2-3). This response was nearly uniform across ECM genera with peroxidases, including *Cortinarius*, *Piloderma*, and *Russula* (Fig. 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 (Fig. 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 (Phillips *et al.* 2013; Averill *et al.* 2018), 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 (Treseder *et al.* 2006; Baldrian 2009; Lindahl & Tunlid 2015), many conceptualizations assume ECM fungi selectively liberate N from SOM (Orwin *et al.* 2011; Smith & Wan 2019; Fernandez *et al.* 2020). 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 oxidize lignin-derived compounds to $CO_2$ (Kirk & Farrell 1987; Hofrichter 2002; 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 (Fig. 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 (Rineau *et al.* 2012; Shah *et al.* 2016; Pellitier & Zak 2018). Accordingly, we observed no correlation between soil C and ECM fungi without peroxidases (Fig. 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 (Fig. 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 *et al.* 2021b), 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 (Fig. 6). Plants associate with ECM mutualists that optimize the organic N acquisition return of their photosynthate investment (Hortal *et al.* 2017; Bogar *et al.* 2019). Specifically, investment in ECM symbionts with energetically costly organic N acquisition capacities is beneficial to plant hosts when inorganic N is scarce, favoring 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 *et al.* 2021c). 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 *et al.* 2021a,b), and our current findings suggest this enhanced decay, in turn, reduces lignin-derived SOM and soil C storage (Fig. 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 (Fig. 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 (Fig. 6; Clemmensen *et al.* 2015). This occurs because declines in tree productivity and pH favor 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 (Fig. 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 (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 (Fig. 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 (Fig. 5a), these putative differences in fine root litter inputs strengthen our conclusion that decay by ECM fungi with peroxidases regulate SOM.

Although our results are based on correlations between fungal community composition and SOM, we used these approaches to test specific *a priori* hypotheses (Fig. 1; Prosser 2020; Lindahl *et al.* 2021). Nonetheless, experimental approaches will be required to verify the *ECM Mechanism* (Fig. 1). For example, trenching experimentally excludes ECM fungi to isolate their role in SOM dynamics (Gadgil & Gadgil 1971; Averill & Hawkes 2016; Sterkenburg *et al.* 2018). However, given the long-term limitations of this approach (Fernandez & Kennedy 2016), a combination of laboratory manipulations, field observations, and modeling will be required to verify the quantitative importance of ECM fungi for SOM dynamics across inorganic N availability gradients (Zak *et al.* 2019b; Bradford *et al.* 2021). An additional mechanism through which anthropogenic N deposition slows lignin decay is by reducing the expression of saprotrophic peroxidase genes (Entwistle *et al.* 2018a; Zak *et al.* 2019a), 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 (Fig. 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 (Fig. 6). We emphasize 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 (Fig. 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 (Lilleskov *et al.* 2002; Bödeker *et al.* 2014) 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.

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## Figure Legends

**Fig. 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.

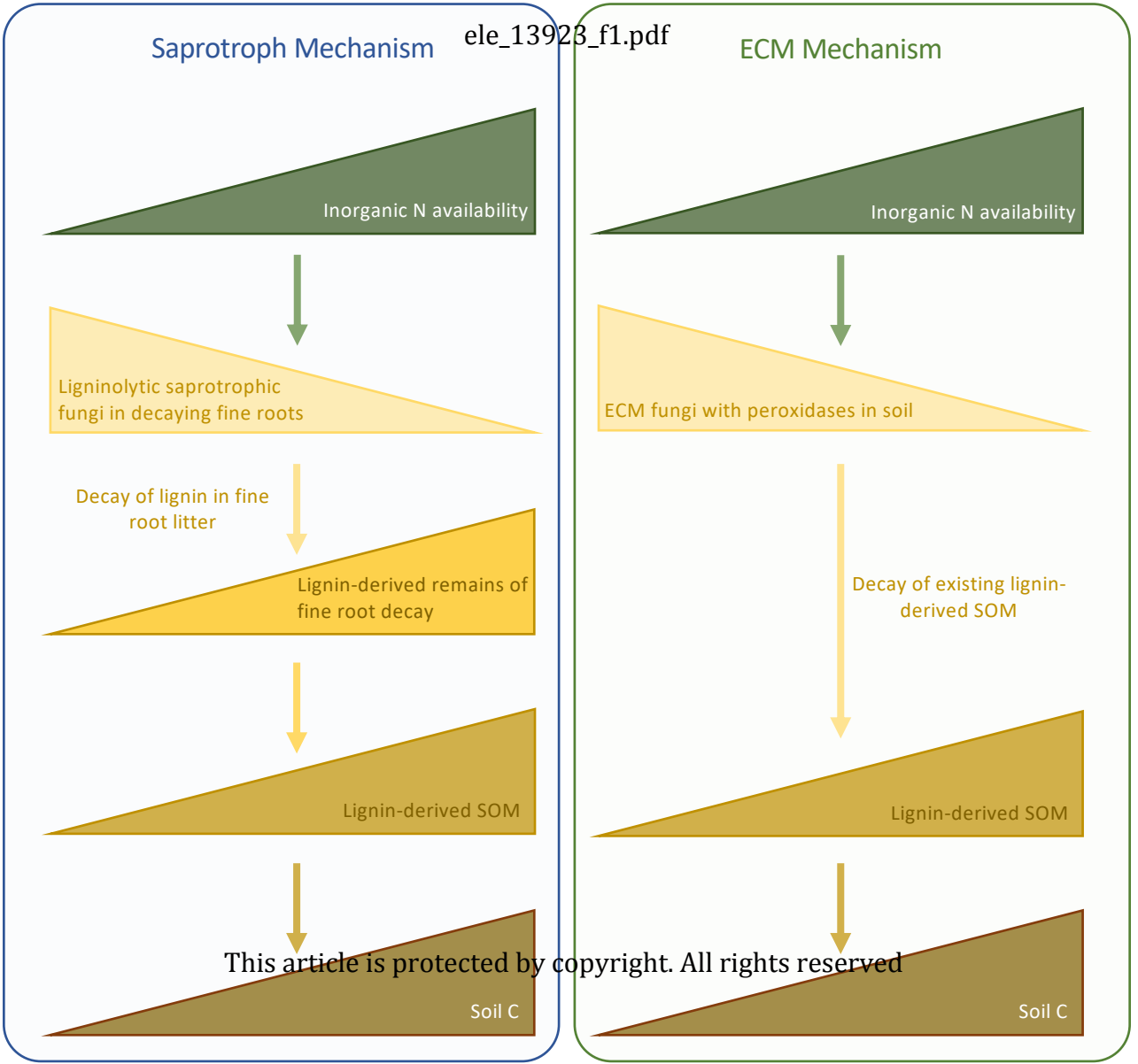
**Fig. 2:** Fungal functional group responses in soil (a) and decaying fine root litter (b) across the soil inorganic N availability gradient. Each plot (and each color) represents a separate GAMM with the relative abundance of a functional group as the dependent variable and soil inorganic N availability 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.

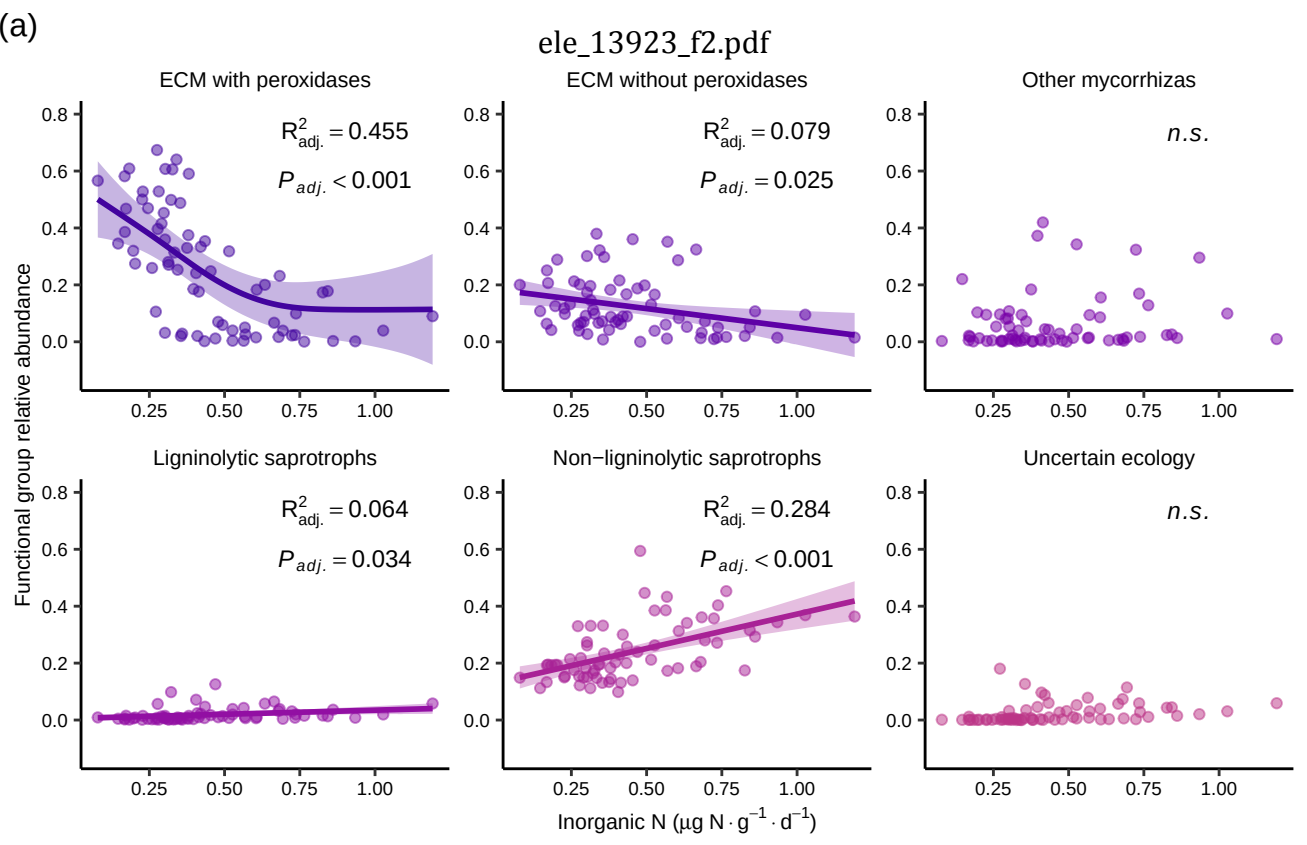
**Fig. 3:** Responses to inorganic N availability for individual genera in soil (a) and fine root litter (b) determined by TITAN analysis. ECM fungal genera with peroxidases nearly universally declined in relative abundance with increasing inorganic N availability. Bars display median Z-scores (across 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 booth purity and reliability $\geq 0.95$ 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.

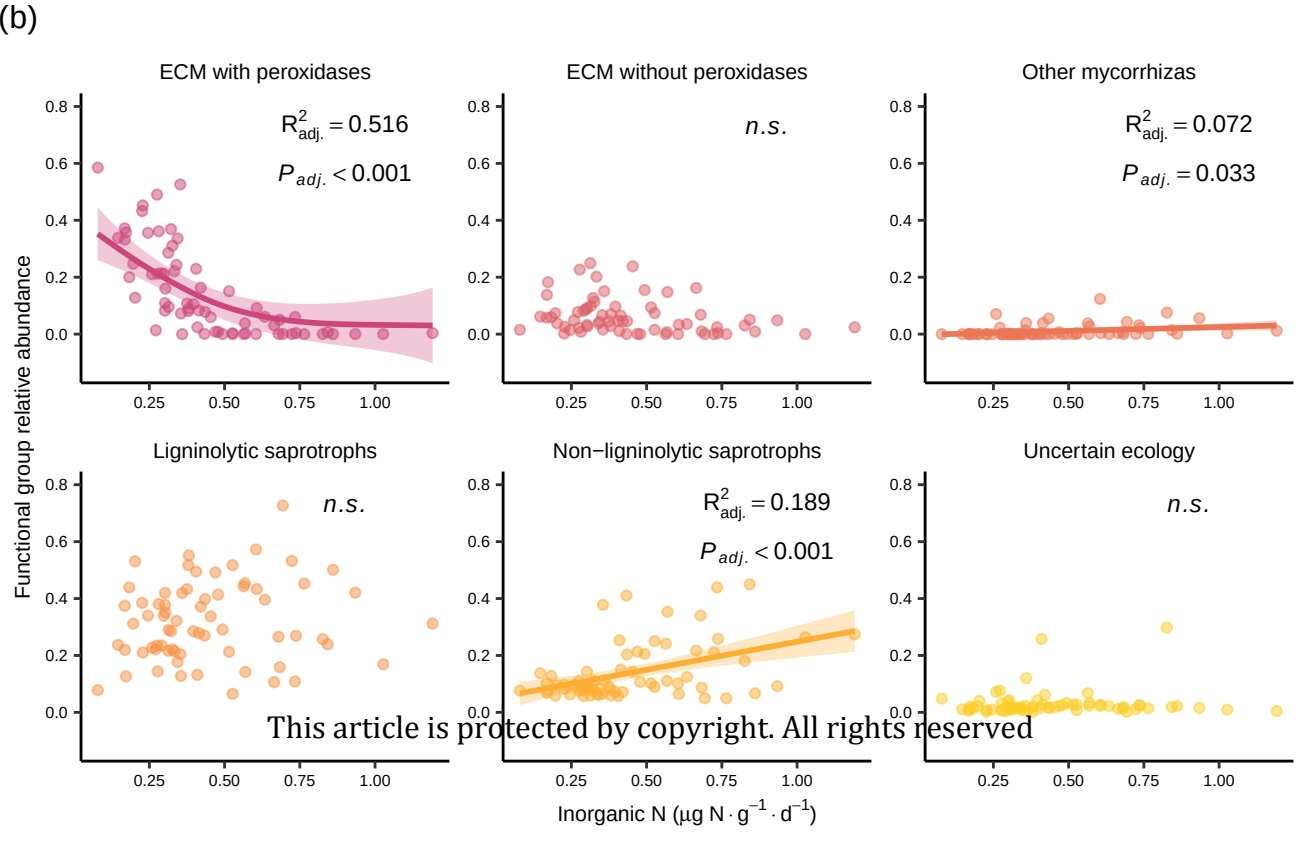
**Fig. 4:** Partial plots from multiple GAMM showing relationship between lignin-derived SOM and fungal functional groups in soil (a), soil C and fungal functional groups in soil (b), lignin-derived SOM and fungal functional groups in decaying fine roots (c), and soil C and fungal functional groups in 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 Fig. 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 visualization purposes only.

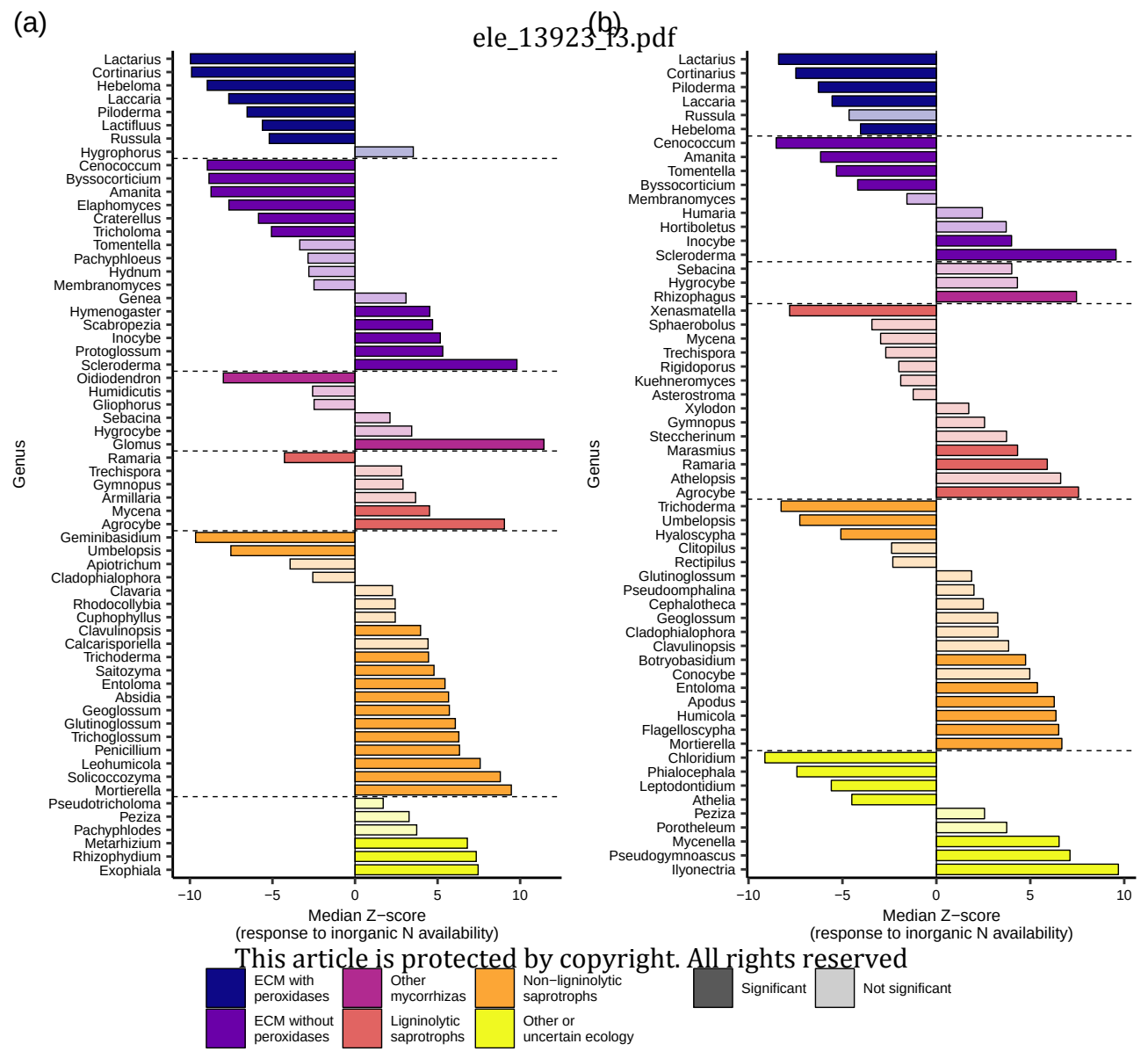
**Fig. 5:** Relationships between lignin-derived SOM and inorganic N availability (a), soil C and lignin-derived SOM (b), and soil C storage and inorganic N availability (c). Each plot (and each color) represents a separate GAMM with the relative abundance of a functional group as the dependent variable 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.

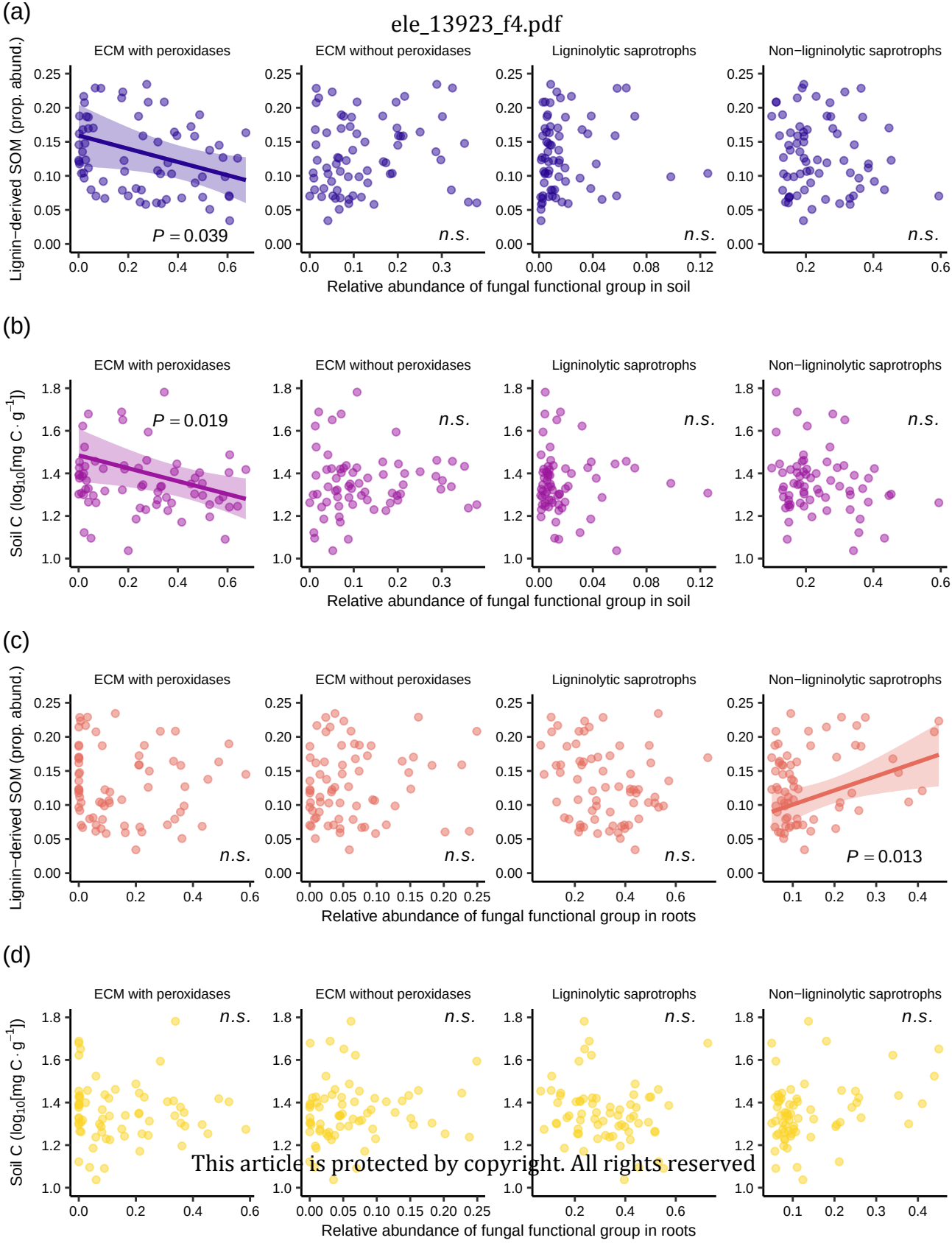
**Fig. 6:** Illustration of our proposed framework unifying relationships between N availability, ECM composition, and SOM across temperate and boreal ecosystems. As inorganic N availability decreases from high fertility to low fertility temperate forests, the abundance of ECM with peroxidases increases 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 favor stress-tolerant ericoid mycorrhizae over ECM taxa with peroxidases. Consequently, SOM increases as inorganic N declines after this point.

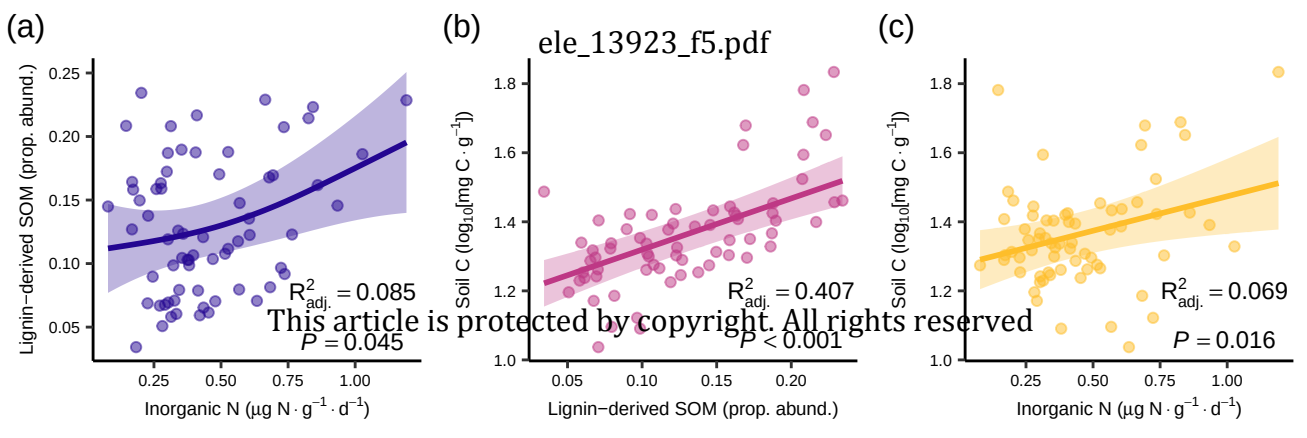









(a)
(b)
(c)
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Lignin−derived SOM (prop. abund.)
Inorganic N (μg N · g⁻¹ · d⁻¹)
Soil C (log10[mg C · g⁻¹])
Lignin−derived SOM (prop. abund.)
Soil C (log10[mg C · g⁻¹])
Inorganic N (μg N · g⁻¹ · d⁻¹)
R²adj. = 0.085
P = 0.045
R²adj. = 0.407
P < 0.001
R²adj. = 0.069
P = 0.016
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