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Fungal community composition and genetic potential regulate fine root decay in northern temperate forests

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Abstract

Understanding how genetic differences among soil microorganisms regulate spatial patterns in litter decay remains a persistent challenge in ecology. Despite fine root litter accounting for ~50% of total litter production in forest ecosystems, far less is known about the microbial decay of fine roots relative to aboveground litter. Here, we evaluated whether fine root decay occurred more rapidly where fungal communities have a greater genetic potential for litter decay. Additionally, we tested if linkages between decay and fungal genes can be adequately captured by delineating saprotrophic and ectomycorrhizal fungal functional groups based on whether they have genes encoding certain ligninolytic class II peroxidase enzymes, which oxidize lignin and polyphenolic compounds. To address these ideas, we used a litterbag study paired with fungal DNA barcoding to characterize fine root decay rates and fungal community composition at the landscape scale in northern temperate forests, and we estimated the genetic potential of fungal communities for litter decay using publicly available genomes. Fine root decay occurred more rapidly where fungal communities had a greater genetic potential for decay, especially of cellulose and hemicellulose. Fine root decay was positively correlated with ligninolytic saprotrophic fungi and negatively correlated with ECM fungi with ligninolytic peroxidases, likely because these saprotrophic and ectomycorrhizal functional groups had the highest and lowest genetic potentials for plant cell wall degradation, respectively. These fungal variables overwhelmed direct environmental controls, suggesting fungal community composition and genetic variation are primary controls over fine root decay in temperate forests at regional scales.

KEYWORDS
ectomycorrhizal fungi, genes, lignin, plant cell wall degrading enzymes, plant litter decomposition, saprotrophic fungi

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1 | INTRODUCTION

An important goal in ecology is to understand how genetic differences among microorganisms determine the functioning of communities and ecosystems (Fierer, 2017; Hall et al., 2018; Zak et al., 2006). For example, variation in the functional genes of ectomycorrhizal (ECM) fungi is related to tree growth, coupling turnover in fungal community composition to the functioning of terrestrial ecosystems (Anthony et al., 2022; Pellitier, Ibáñez, et al., 2021; Pellitier, Zak, et al., 2021). Fungi also differ substantially in their genetic potential for the decay of plant litter (Miyauchi et al., 2020; Riley et al., 2014; Ruiz-Dueñas et al., 2021), an ecosystem process that broadly controls the balance of carbon stored in soil and the atmosphere (Schlesinger & Bernhardt, 2013). However, it remains unclear whether differences in the genetic potential of soil microorganisms regulate patterns in plant litter decay across broad spatial scales (Bradford et al., 2021; Jansson & Hofmockel, 2018).

Fungal species are often categorized into functional groups (e.g., saprotrophic vs. ECM) based upon their trophic status and decay physiologies (Lindahl & Tunlid, 2015; Nguyen et al., 2016; Tanunchai et al., 2022; Zanne et al., 2020). Whereas ECM fungi generally have fewer genes encoding extracellular decay enzymes than free-living saprotrophic fungi (Kohler et al., 2015; Lindahl & Tunlid, 2015; Martino et al., 2018), both saprotrophic and ECM fungal species span broad continuums of genetic potentials for decay (Pellitier & Zak, 2018; Riley et al., 2014). Consequently, it remains an open question how to assign functional groups to capture fungal genetic variation that operates at ecosystem scales (Romero-Olivares et al., 2021; Talbot et al., 2015; Treseder et al., 2021). Because process-based ecosystem models of carbon cycling increasingly incorporate microbial functional groups (Bradford et al., 2021; Sulman et al., 2019; Wieder et al., 2018), delineating functional groups that adequately represent links between fungal genes and spatial patterns of litter decay has important implications for our understanding of the terrestrial carbon cycle.

The presence of genes encoding high redox-potential class II fungal peroxidases (hereafter "ligninolytic peroxidases"), which fully oxidize lignin and lignin-derived complex polyphenolic compounds in soil organic matter (Bödeker et al., 2014; Hofrichter, 2002; Kirk & Farrell, 1987), could functionally distinguish ecologically relevant groups within the broader classifications of saprotrophic and ECM fungi. Ligninolytic saprotrophic fungi extensively decay lignocellulose because they have large suites of genes coding for ligninolytic peroxidases, as well as hemicellulolytic and cellulolytic enzymes, whereas nonligninolytic saprotrophs lack the specific subgroups of class II peroxidase genes required to fully decay lignified compounds (Baldrian, 2008; Floudas et al., 2012; Riley et al., 2014). Similarly, only certain lineages of ECM fungi have retained ligninolytic peroxidases and other oxidative decay mechanisms (e.g., Fenton chemistry) during their evolution from saprotrophic ancestors (Bödeker et al., 2009; Miyauchi et al., 2020; Pellitier & Zak, 2018; Shah et al., 2016). The potent decay capacity of ligninolytic peroxidases suggests the presence or absence of these genes could delineate fungal functional groups that have ecosystem-level impacts, and this appears to be true for soil organic matter decay by saprotrophic and ECM fungi (Argiroff et al., 2022; Kyaschenko et al., 2017; Lindahl et al., 2021), as well as for plant growth responses to ECM fungal composition (Pellitier, Ibáñez, et al., 2021; Pellitier, Zak, et al., 2021; Pellitier & Zak, 2021a, 2021b). However, genetic differences among saprotrophic and ECM fungal functional groups with and without ligninolytic peroxidases have not been directly linked to landscape-level patterns in plant litter decay.

Fine root litter is produced by the senescence of the smallest absorptive roots of trees (McCormack et al., 2015; Xia et al., 2010). Approximately 50% of total plant litter production in forest ecosystems is comprised of fine root litter (Freschet et al., 2013), and compounds derived from the decay of fine root litter are a primary source of soil organic matter (Angst et al., 2021; Jackson et al., 2017; Rasse et al., 2005). Furthermore, fine root litter contains high concentrations of lignin (threefold greater than leaf litter; Xia et al., 2015), suggesting its decay should be particularly sensitive to whether fungi have the genetic capacity for extensive lignocellulose degradation. However, our understanding of plant litter decay and its controlling factors is based almost exclusively on the decay of aboveground litter (i.e., senesced leaves and coarse woody debris; Berg et al., 1993; Berg & McClaugherty, 2020; Bradford et al., 2016). Consequently, the ecological factors controlling fine root decay are an important gap in our understanding of the terrestrial carbon cycle (Berg & McClaugherty, 2020).

In this study, we evaluated whether the genetic decay potential of fungal communities regulates fine root decay at the landscape scale. We measured rates of fine root decay across 12 northern temperate forest ecosystems that span environmental gradients of soil inorganic nitrogen (N) availability, water content, temperature, and pH (Argiroff et al., 2022; Pellitier, Ibáñez, et al., 2021; Zak et al., 1989). We characterized fungal community composition in decaying fine root litter using DNA barcoding and litter bags, and we used publicly available genomes to estimate the genetic potential of fungal communities for litter decay by calculating community-weighted gene abundances for plant cell wall-degrading enzymes (PCWDE; Anthony et al., 2022). We reasoned that higher genetic decay potential of fungal communities is conferred primarily by greater abundances of ligninolytic saprotrophs, and that fungal communities with greater genetic potential for the metabolism of plant litter would decay fine root litter more rapidly. ECM fungi can either accelerate (Argiroff et al., 2022; Lindahl et al., 2021) or slow organic matter decay (Fernandez et al., 2020; Sterkenburg et al., 2018), and an additional objective of our study was to determine how ECM fungi with and without ligninolytic peroxidases influenced community-level genetic decay potential and the decay of fine root litter. Finally, because environmental conditions can alter decay by modifying microbial physiology and community composition (Bradford et al., 2021), we evaluated whether relationships between fine root decay and fungal communities were robust to the inclusion of environmental variables in statistical models. We found that fine root decay occurred more rapidly where fungal communities had a greater genetic potential

for decay, and that fine root decay was positively correlated with ligninolytic saprotrophic fungi and negatively correlated with ECM fungi with ligninolytic peroxidases. Collectively, our results provide evidence that the composition and genetic potential of fungal communities are primary controls over fine root decay, which is a poorly understood component of the terrestrial carbon cycle.

2 | MATERIALS AND METHODS

2.1 | Site descriptions

Our study was conducted in 72 plots distributed across 12 forest sites (six plots per site) in northern Lower Michigan, USA (Figure S1; Argiroff et al., 2022). Plots were 2-m diameter circles located in even-aged (~100 year-old) second-growth northern hardwood forests of *Quercus rubra* co-occurring with *Acer rubrum* and *A. saccharum*; these plots were located near previously-characterized ECM fungal communities in *Q. rubra* root tips (Pellitier, Ibáñez, et al., 2021; Pellitier & Zak, 2021a; Pellitier, Zak, et al., 2021). Climatic and most edaphic characteristics were similar among sites due to relatively close proximity (separated by <50 km) and uniform soil texture (>85% sand; Zak et al., 1989; Zak & Pregitzer, 1990). However, microsite differences in topography have produced variation in nutrient retention and microclimates among and within sites, which have resulted in seasonally and interannually consistent differences in soil inorganic N availability, water content, temperature, and pH within and among sites (Figure S2; Argiroff et al., 2022; Pellitier, Ibáñez, et al., 2021; Zak et al., 1989; Zak & Pregitzer, 1990).

2.2 | Fine root decay

In May 2018, we collected five soil cores with a rectangular corer (12 × 12 cm) to a depth of 10 cm in a ~1.5 m radius within the canopy of five *Q. rubra* individuals at each site. We transported cores to the University of Michigan on ice and stored them at −20°C. We thawed the cores, rinsed roots free of adhering soil particles, and obtained fine roots ≤0.5 mm in diameter. Due to the well-recognized difficulty in distinguishing live and dead fine roots – which requires vital staining and the destructive examination of the root stele and cortex that would preclude accurate assessments of decay (Hobbie et al., 2010; Vogt & Persson, 1991) – we focused on avoiding roots that were obviously senesced and decaying (i.e., black in colour, shrivelled, or lacking structural integrity). This approach is consistent with other studies of fine root litter decay using litterbags, and results in the litter primarily comprising roots that were living at the time of collection (Hobbie et al., 2010; Sun et al., 2018; Xia et al., 2018). We dried the roots at 65°C for 48 h, combined them at the site level, and ground a subsample with a ball mill for the determination of initial fine root biochemistry. The diameter cutoff of ≤0.5 mm retained primarily first- through third-order fine roots, which belong to absorptive fine root modules that undergo rapid turnover and comprise most fine root litter produced by trees (McCormack et al., 2015; Xia et al., 2010). Furthermore, because *Q. rubra* occurs as dominant overstory trees in all sites, the roots we collected (which also contained a small and constant proportion of *A. rubrum* or *A. saccharum* roots) represent a large portion of fine root litter in these ecosystems.

We placed ~3 g dried fine root litter from the respective site into 12 × 12 cm nylon mesh litterbags (12 sites × 6 plots × 2 bags = 144 total litterbags) with 53 μm openings, which permit the ingrowth of fungal hyphae but exclude new fine roots (Hobbie et al., 2010; Li et al., 2015; Sun et al., 2018). We sterilized the litterbags using ethylene oxide (Cline & Zak, 2015), and we placed two litterbags per plot within the zone of greatest fine root density (~3 cm depth; Figure S3) to ensure the litterbags experienced environmental conditions in which the vast majority of fine root litter decays in these ecosystems (See et al., 2019). We deployed the litterbags in May 2019 to align with the onset of high plant and microbial activity at the beginning of the growing season (Zak et al., 1999). We collected the litterbags after 13 months (in July 2020), transported them on ice to the University of Michigan, homogenized the roots by plot, and weighed them. We obtained a subsample of the decaying roots for the immediate measurement of extracellular enzyme activities, and we stored a subsample at −80°C for characterization of fungal community composition. A subsample was oven-dried at 60°C to determine moisture content for mass loss measurements, a portion of which was ashed at 500°C for 6 h to measure mineral content. We calculated fine root decay as the proportion of initial ash-free dry mass lost over the 13-month field incubation.

2.3 | Fungal DNA barcoding

We isolated and purified DNA from 0.15 g (three 0.05 g subsamples) of decaying fine roots from each plot (n = 72) using the DNeasy Plant Mini Kit and DNeasy PowerClean CleanUp kit (Qiagen) following modified manufacturer's protocols (Supporting Information Methods; Argiroff et al., 2022). We amplified the ITS2 region of the universal fungal DNA barcode (i.e., the ribosomal internal transcribed spacer; Nilsson, Anslan, et al., 2019; Nilsson, Larsson, et al., 2019; Schoch et al., 2012) using PCR with ITS4-Fun/5.8 S-Fun primers (Table S1 and Supporting Information Methods; Pellitier et al., 2019; Taylor et al., 2016), and amplicons were sequenced using MiSeq 2 × 250 bp with v2 chemistry (Illumina) by the University of Michigan Microbiome Core. We determined the copy number of fungal ITS amplicons in decaying fine root litter using quantitative PCR (qPCR) and the ITS1F/5.8 S primers – a well-established primer set for ITS qPCR (Entwistle, Romanowicz, et al., 2018; Entwistle, Zak, et al., 2018; Fierer et al., 2005) – and used these data to estimate the absolute abundance of fungal functional groups (see Fungal functional groups).

We obtained high quality fungal sequences and determined 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) paired with cutadapt (Martin, 2011). We analysed

only forward reads, which has been shown to be more effective at accurately representing fungal community composition than joining forward and reverse ITS reads (Pauvert et al., 2019; Taylor et al., 2016). We assigned taxonomic classifications to sequences with the UNITE database (version 8.2 for fungi, release date 04.02.2020; Kõljalg et al., 2013; Nilsson, Larsson, et al., 2019) using the naïve Bayesian classifier (Wang et al., 2007) implemented in DADA2. We did not subsample sequence counts to avoid data loss and uncertainty (McMurdie & Holmes, 2013, 2014).

2.4 | Genetic potential for decay

To understand if fine root decay was related to the genetic decay potential of fungal communities, we first estimated genomic decay traits for abundant genera (present in ≥5 plots and representing ≥0.1% of sequences) assigned to functional groups (see *Fungal functional groups* below) in decaying fine root litter using sequenced genomes in the MycoCosm genome portal (Grigoriev et al., 2014) following Anthony et al. (2022). For each genome, we obtained gene copy numbers for 58 plant cell wall degrading enzyme (PCWDE) gene families (Ruiz-Dueñas et al., 2021) annotated using the CAZy database (Table S2; Levasseur et al., 2013; Lombard et al., 2014). We then calculated copy numbers of each PCWDE family per 10,000 genes in the genome to correct for differences in genome size (Anthony et al., 2022; Romero-Olivares et al., 2021; Treseder & Lennon, 2015). A total of 27 of 50 abundant genera belonging to four functional groups (ligninolytic saprotrophs, nonligninolytic saprotrophs, ECM with ligninolytic peroxidases, and ECM fungi without ligninolytic peroxidases) matched at least one sequenced genome and were assigned decay traits, which accounted for 81% of sequences assigned to the four focal functional groups and 55% of sequences overall (Table S3). ECM fungi with uncertain decay capacity, by definition, did not have sequenced genomes available. We inferred the community-level genetic potential for plant litter decay in each plot by averaging the genes for each PCWDE per genome at the genus level, and subsequently calculating community-weighted mean gene copies for each PCWDE based on the relative abundance of each genus (Anthony et al., 2022).

2.5 | Fungal functional groups

We assigned functional groups to all classified genera present in ≥5 plots and representing ≥0.1% of sequences (i.e., abundant genera; Table S4) using the approach described by Argiroff et al. (2022). Specifically, we identified ECM fungal genera with peroxidases that potentially decay lignin-derived polyphenolic soil organic matter (hereafter, "ECM fungi with ligninolytic peroxidases") by using the literature and publicly available sequenced genomes to determine if species within each genus possess class II peroxidase genes that are putatively ligninolytic (Bödeker et al., 2009; Kohler et al., 2015; Miyauchi et al., 2020; Nagy et al., 2016). We identified ligninolytic saprotrophic fungal genera using FUNGuild (Nguyen et al., 2016) and literature (Entwistle, Zak, & Argiroff, 2018; Ruiz-Dueñas et al., 2021). Importantly, the specific subgroups of class II peroxidases with the capacity to oxidize lignin and lignin-derived polyphenolic compounds in soil organic matter evolved only within the most recent common ancestor of Auriculariales and more recently diverging orders of Agaricomycetes (phylum Basidiomycota; Floudas et al., 2012; Nagy et al., 2016; Ruiz-Dueñas et al., 2021). Consequently, any class II peroxidases in Ascomycota and basal lineages of Agaricomycetes are nonligninolytic generic class II peroxidases; thus, we designated these genera as "ECM fungi without ligninolytic peroxidases" and "nonligninolytic saprotrophs". Furthermore, ECM fungi that have evolved within brown-rot Agaricomycete lineages, which have lost peroxidases (e.g., Boletales; Kohler et al., 2015; Pellitier & Zak, 2018), were also classified as "ECM without ligninolytic peroxidases", as were Agaricomycete lineages that possess genes that likely encode nonligninolytic generic class II peroxidases (e.g., *Laccaria* and *Amanita*; Ruiz-Dueñas et al., 2021). Other Agaricomycete ECM genera without available sequenced genomes were classified as "ECM fungi with uncertain decay capacity". "Other mycorrhizas" and "fungi with other or uncertain ecology" were identified with FUNGuild and literature (Martino et al., 2018; Seitzman et al., 2011; Smith & Read, 2010), but were excluded from analyses because they together comprised a much smaller proportion of fungal sequences (<4%). Absolute abundances of these functional groups were calculated by multiplying relative abundances (i.e., the proportion of fungal sequences) by ITS copy number as quantified by qPCR (sensu Clemmensen et al., 2015) and subsequently used in all analyses.

2.6 | Enzymatic potential for decay

We used ~0.5 g of decaying root litter and previously described methods (Cline & Zak, 2015; Saiya-Cork et al., 2002) to measure the potential activity of five extracellular enzymes involved in litter decay. We assayed β-1,4-glucosidase and cellobiohydrolase to determine the enzymatic potential for cellulose decay, phenol oxidase and peroxidase to determine the potential for lignin decay, and N-acetyl-β-glucosaminidase, which is involved in the turnover and remodelling of fungal biomass (Baldrian, 2008). We used 200 μM methylumbelliferyl-linked substrates to fluorometrically determine potential activities of β-1,4-glucosidase, cellobiohydrolase, and N-acetyl-β-glucosaminidase. We used 25-mM L-dihydroxyphenylalanine to measure phenol oxidase and peroxidase activities based on absorbance.

2.7 | Environmental variables

We collected six 2.5-cm diameter soil cores at each plot to a depth of 10 cm in May 2019 and transported the cores on ice to the University of Michigan. We passed the soil through a 2-mm sieve, removed visible fine roots by hand, and homogenized the

soil by plot, resulting in 72 soil samples. Two 30 g subsamples were used for 28-day net N mineralization assays (Vitousek et al., 1982; Zak et al., 1989) to determine soil inorganic N availability, which reflects in situ inorganic N availability (Zak et al., 1989; Zak & Pregitzer, 1990) and N in root litter and soil organic matter (Figure S4). A subsample was oven-dried at 105°C for 24 h, ground to a fine powder in a ball mill, and used to determine soil C and N with a CN analyser (LECO). Sieved soil was air-dried for the determination of soil pH in slurries of 30 g of soil and 30 mL deionized water. We characterized the biochemistry of initial (i.e., undecayed) fine root litter using pyrolysis gas chromatography–mass spectrometry (py-GC/MS; Supporting Information Methods; Pold et al., 2017) to determine the relative abundance of aromatics, lignin, lipids, N-bearing, phenols, polysaccharides, proteins, and unknown origin.

We measured the mean of hourly soil volumetric water content and temperature during the growing season (1 May to 31 October 2019, and 1 May to 31 June 2020) using a Micro Station data logger (ONSET) at each site. We interpolated values for each plot by regressing plot-level measurements ($n = 4$) taken through the period of decay with hand-held probes against nearest logger values. Water content and temperature values were averaged across the growing season to obtain a single value per plot.

2.8 | Statistical analysis

We used the TITAN2 package (Baker & King, 2010) to identify PCWDE gene families whose estimated community-weighted mean abundances were associated with more rapid fine root decay. We treated PCWDE gene families as species abundances and fine root decay rates as an environmental gradient. PCWDE gene families were considered significantly associated with rapid fine root decay if they had purity and reliability values ≥0.95 and positive Z-scores (Baker & King, 2010). We used one-way ANOVA and Tukey's HSD to test differences in the summed gene counts of PWDEs among fungal functional groups. We used generalized additive mixed models (GAMMs) with a Gaussian error family to test the relationships between fine root decay and fungal genetic potential or community composition. GAMMs, which accommodate complex nonlinear relationships that may arise in ecological data sets, were implemented in the R package mgcv (Wood, 2011). We also included a spatial correlation structure to account for the fact that plots within a site were closer together than plots between sites, effectively accounting for geographic distance among plots as a random effect in the models. We elected to keep plots separate in all analyses to avoid data loss and mean inference fallacies caused by data aggregation at the site level (Bradford et al., 2021), and because fine root decay rates and environmental conditions varied considerably both within and among sites (Figures S2 and S5). Due to significant correlations between environmental variables (Table S5), we used PCA to obtain composite environmental axes (Figure S6) that were subsequently used in GAMM models to avoid variance inflation caused by colinear predictor variables (sensu Kyaschenko et al., 2017). We removed two plots from all analyses, one in which mass loss was over four standard deviations greater than the mean root decay rate and one for which the net N mineralization assay failed, for a total $n = 70$ plots. We used the "gam.check" function to ensure residuals were normally distributed and met the assumption of homoscedasticity. GAMMs were visualized using partial regression plots, which show the effect of a predictor variable on the response variable after accounting for the other predictors in the model (e.g., Anthony et al., 2022; Steidinger et al., 2020). We accepted statistical significance at $\alpha = 0.05$, and we performed all statistical analyses using R version 4.1.2 (R Core Team, 2021) and RStudio version 2021.9.0+351 (RStudio Team, 2021), with the phyloseq (McMurdie & Holmes, 2013), ShortRead (Morgan et al., 2009), biostrings (Pagès et al., 2020), and tidyverse (Wickham et al., 2019) packages.

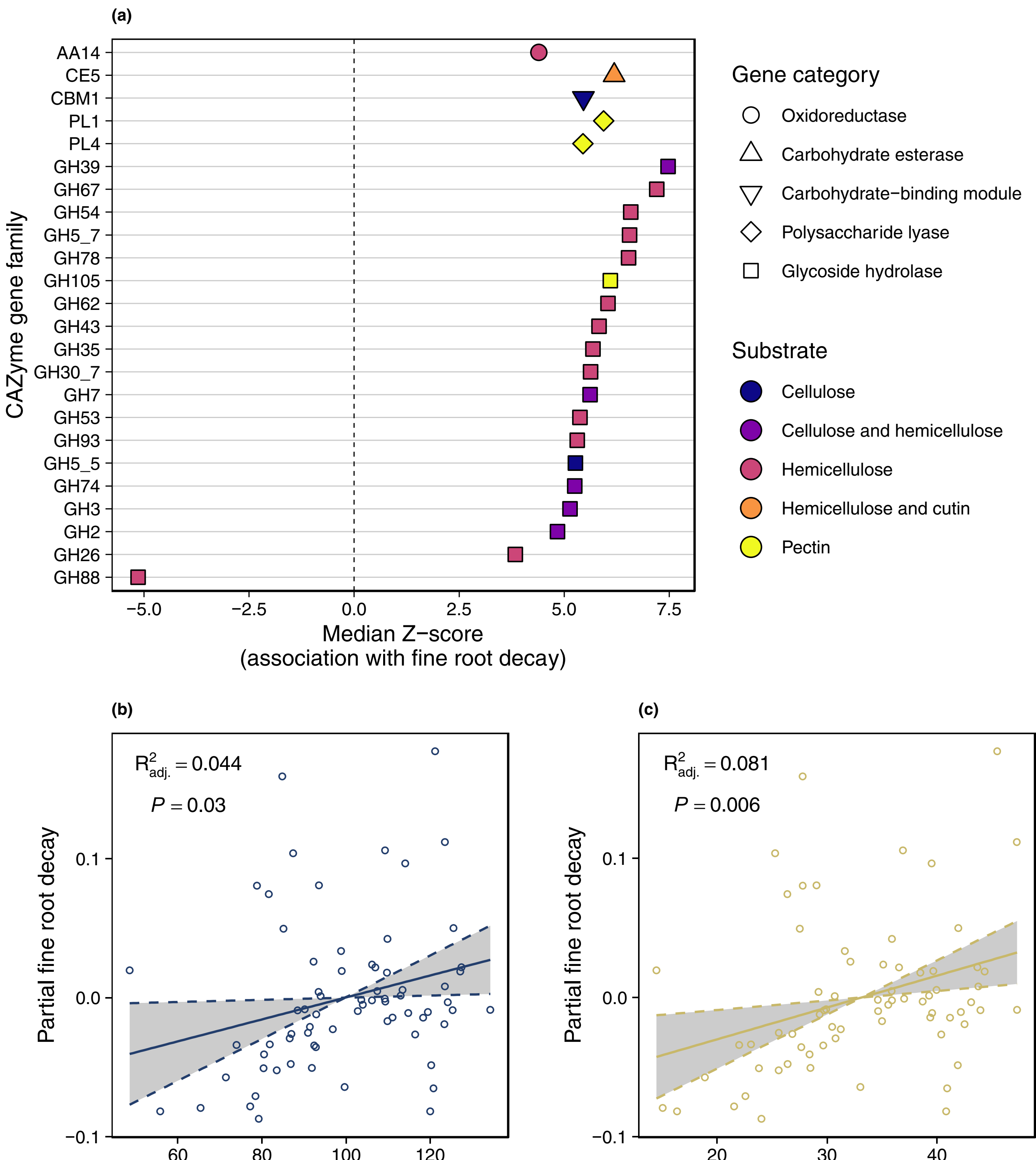
3 | RESULTS

3.1 | Environmental covariation

Soil inorganic N availability (Pearson's $r = .903$) and soil pH ($r = .840$) were strongly positively correlated with PCA axis 1 (PC1), whereas soil temperature was strongly negatively correlated with PC1 ($r = -.822$; Figure S6 and Table S5). Soil water content was strongly positively correlated with PC2 ($r = .914$). Consequently, increasing values of PC1 primarily represented an environmental axis along which inorganic N and soil pH increase and soil temperature decreases, whereas increasing values of PC2 primarily represented plots with greater water content (Figure S6).

3.2 | Genetic decay capacity and fine root decay

Twenty-four of the 58 gene families we evaluated were significantly related to fine root decay based on TITAN analysis, 23 of which were associated with more rapid decay rates (Figure 1a). Nearly 87% of the rapid decay-associated gene families were involved in the degradation of hemicellulose, cellulose, or both (Figure 1a). We used two separate GAMMs to determine if fine root decay was predicted by the community-level abundance of all PCWDEs or rapid-decay PCWDEs. Fine root decay increased with an increasing community-level abundance of PCWDEs ($R^2_{adj.} = .044$, $p = .03$; Figure 1b and Table S6), although this relationship did not remain significant when we re-ran the model including environmental axes PC1 and PC2 as additional predictors ($p = .087$; Table S6) possibly due to weak covariance between summed PCWDE genes and PC1 (Pearson's $r = .34$, $p = .003$). In the second GAMM using PCWDEs specifically associated with rapid root decay, fine root decay was positively correlated with the community-level abundance of rapid decay PCWDEs ($F = 8.15$, $R^2_{adj.} = .081$, $p = .006$; Figure 1c and Table S6). This response was robust to the inclusion of environmental variables in the model ($p = .014$; Table S6).



3.3 | Genetic decay capacity and fungal functional groups

Fungal communities in decaying fine root litter consisted primarily of ligninolytic saprotrophic genera (33% of fungal sequences) such as *Mycena* and other Basidiomycota, followed by nonligninolytic saprotrophs (15%), ECM fungi with ligninolytic peroxidases (15%), ECM fungi without ligninolytic peroxidases (4%), and ECM fungi with uncertain decay capacity (3%; Figures S7–S9 and Tables S7–S9; see Table S9 for a summary of sequencing effort). We used ANOVA to compare genome-level differences in genetic potential for litter decay between fungal functional groups (Figure 2a). Gene copies belonging to PCWDEs were most abundant in genomes of lignin-degrading saprotrophic fungi, followed by nonligninolytic

FIGURE 1 Association between the community-weighted mean values of plant cell wall degrading enzyme (PCWDE) gene families and fine root decay determined by TITAN analysis (a). A total of 23 of 24 statistically significant gene families were associated with more rapid fine root decay. Points indicate median Z-scores (across 1000 bootstrap replicates), which represent the magnitude of the change in the community-weighted mean of a gene family across a gradient of slow- to rapid fine root decay. Positive Z-scores indicate associations with rapid decay, and negative Z-scores indicate associations with slower decay. We considered responses statistically significant if both purity and reliability were ≥0.95. Panels b and c show partial regression plots of the relationships between fine root decay and the summed abundance of all 58 PCWDE community-weighted mean abundances (b) and the summed abundance of PCWDE gene families associated with rapid decay (c). These relationships were evaluated using GAMMs with either all or rapid decay associated PCWDEs as predictors. The models explained 4% (all PCWDEs) and 8% (rapid decay PCWDEs) of the variance in fine root decay ($R^2_{adj.} = .044$ and $R^2_{adj.} = .081$), although the relationship between fine root decay and all PCWDEs did not remain significant when environmental axes PC1 and PC2 were included as additional predictors ($p = .087$; Table S6). Partial regressions show the effect of a predictor variable after accounting for all other variables in the model, including the spatial component. We accounted for potential spatial autocorrelation using a spatial correlation structure in GAMM models that incorporates the geographic coordinates of each plot ($n = 70$).

fungal saprotrophs, and both ECM functional groups ($p < .05$; Figure 2a). Gene copies belonging to PCWDEs specifically associated with rapid fine root decay were also most abundant in genomes of lignin-degrading saprotrophic fungi, followed by nonligninolytic fungal saprotrophs, and both ECM functional groups ($p < .05$; Figure 2a).

We then used two separate GAMMs with five fungal functional groups as predictor variables and either the summed community-weighted mean abundance of all PCWDEs or rapid decay-associated PCWDEs to understand which fungal functional groups determined community-level genetic decay potential (Figure 2b,c). The summed community-weighted mean abundance of all PCWDEs was positively correlated with the abundance of lignin-degrading saprotrophic fungi and negatively correlated with the abundance of ECM fungi with ligninolytic peroxidases, together accounting for 56% of the variance in genetic decay capacity ($R^2_{adj.} = .556$, $p < .001$; Figure 2b and Table S10). Similarly, the summed abundance of PCWDEs associated with rapid fine root decay was positively correlated with lignin-degrading saprotrophic fungi and negatively correlated with ECM fungi with ligninolytic peroxidases, accounting for 62% of the variance in genetic decay capacity ($R^2_{adj.} = .622$, $p < .001$; Figure 2c and Table S10). Neither community-level estimate of genetic decay potential was related to nonligninolytic saprotrophs, ECM fungi without ligninolytic peroxidases, or ECM fungi with uncertain decay capacity ($p > .05$; Figures 2b,c, and Table S10). These relationships were robust to the inclusion of environmental variables in the models (Table S10).

3.4 | Enzyme activities and fungal functional groups

We assayed the potential activity of cellulolytic and ligninolytic enzymes, as well as N-acetyl-β-glucosaminidase, to understand whether differences in the activity of these decay enzymes were related to fungal composition (Figures S10 and S11 and Table S11). We used two separate GAMMs for each of the three enzyme activities, one using the five fungal functional groups as predictor variables and a second using the two environmental PCA axes (PC1 and PC2) as predictor variables for a total of six separate GAMMs. Cellulolytic enzyme activity was positively correlated with ECM fungi with ligninolytic peroxidases, and unimodally related to ECM fungi without ligninolytic peroxidases and with uncertain decay capacity ($p < .05$; Figure S10). Ligninolytic enzyme activity was not related to the abundance of fungal functional groups ($p > .05$; Figure S10). N-acetyl-β-glucosaminidase activity was positively correlated with the abundance of ligninolytic saprotrophs ($p < .001$) and ECM fungi without ligninolytic peroxidases ($p = .003$), and negatively correlated with the abundance of nonligninolytic saprotrophs (Figure S10 and Table S11). None of the enzyme activities were significantly correlated with the composite environmental PCA axes ($p > .05$; Figure S11 and Table S11).

3.5 | Fine root decay and fungal functional groups

We used a GAMM with fine root decay as the response variable and the four fungal functional groups as predictors to understand how fine root decay was related to fungal community composition (Figure 3). Fine root decay was positively correlated with the abundance of ligninolytic saprotrophic fungi ($R^2_{adj.} = .241$, $p < .001$; Figure 3a) and negatively correlated with the abundance of ECM fungi with ligninolytic peroxidases ($p = .005$; Figure 3c and Table S12). Fine root decay was negatively correlated with nonligninolytic saprotrophic fungi ($p = .007$; Figure 3b), and it was unrelated to ECM fungi without ligninolytic peroxidases ($p = .696$; Figure 3d and Table S12) and ECM fungi with uncertain decay capacity ($p = .199$; Figure 3e and Table S12). Additionally, these results were robust to the inclusion of environmental variables (i.e., PC1 and PC2) in the model (Table S13).

3.6 | Fine root decay and the environment

To understand if fine root decay was related to environmental conditions, we used a GAMM with fine root decay as the response variable and the composite environmental PC1 and PC2 (Figure S6 and Table S5) as predictor variables. Fine root decay was unrelated to PC1 ($p = .58$; Figure 4a and Table S14), which represented a gradient

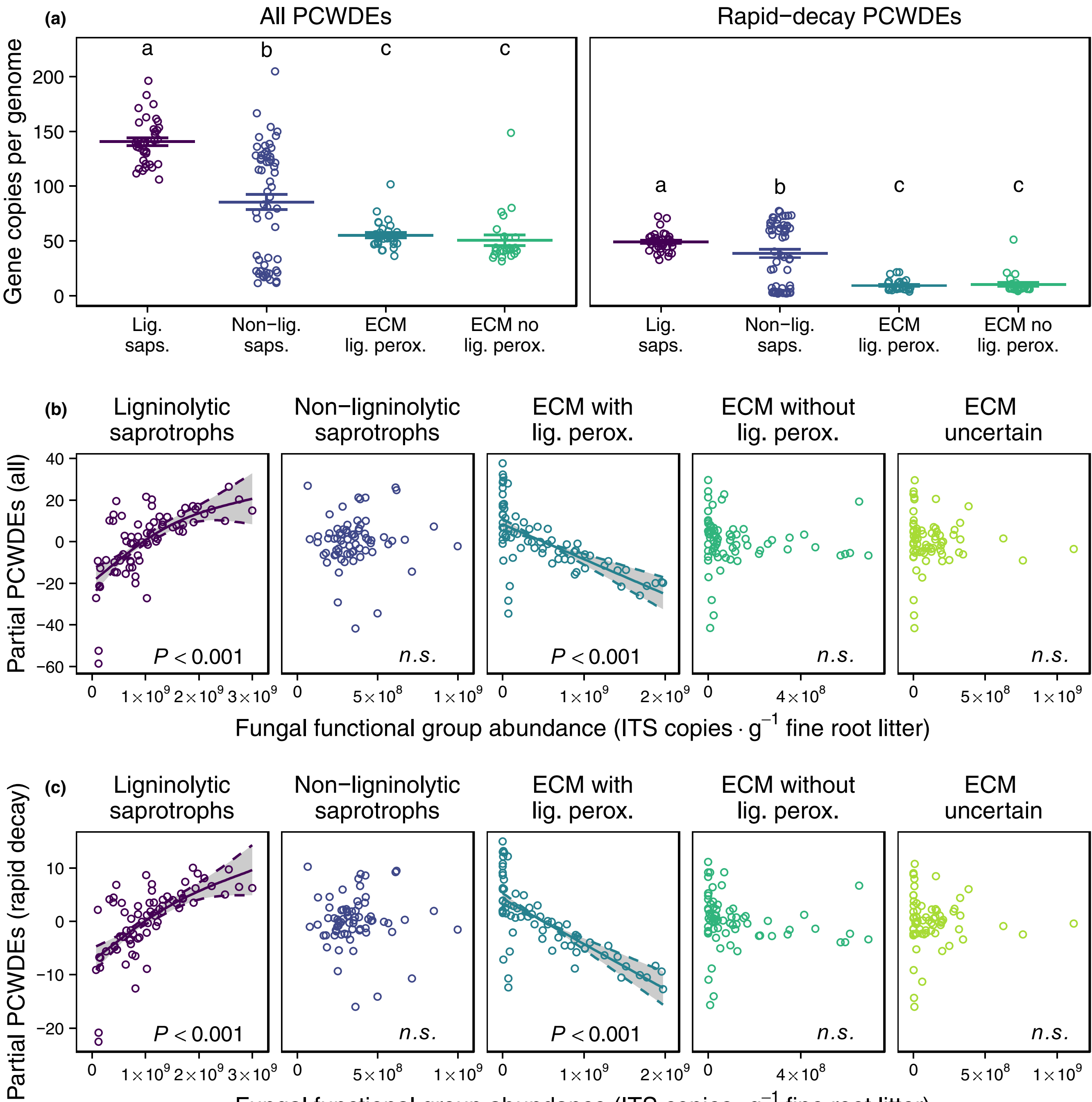


FIGURE 2 Total number of genes in all 58 PCWDEs or rapid root decay PCWDEs in the genomes of fungi belonging to different functional groups, presented as gene copies per 10,000 genes (a). Horizontal lines are means, and error bars represent ±1 SE; different lowercase letters represent significantly different mean gene counts based on Tukey's HSD ($p < .05$). Panels b and c show partial regression plots of the relationships between fungal functional groups and the community-weighted mean abundance all PCWDEs (b) and rapid decay associated PCWDE gene families (c). These relationships were evaluated using a multiple GAMM with four fungal functional groups as predictors. The models explained 57% of the variance in all PCWDE gene families ($R^2_{adj.}$ = 0571) and 64% of the variance in PCWDE gene families associated with rapid decay ($R^2_{adj.}$ = .638). Partial regressions show the effect of a predictor variable after accounting for all other variables in the model, including the spatial component. We accounted for potential spatial autocorrelation using a spatial correlation structure in GAMM models that incorporates the geographic coordinates of each plot (n = 70). ECM with lig. perox., ECM fungi with ligninolytic peroxidases; ECM without lig. perox., ECM fungi without ligninolytic peroxidases; ECM uncertain, ECM fungi with uncertain decay capacity; n.s., not significant ($p > .05$).

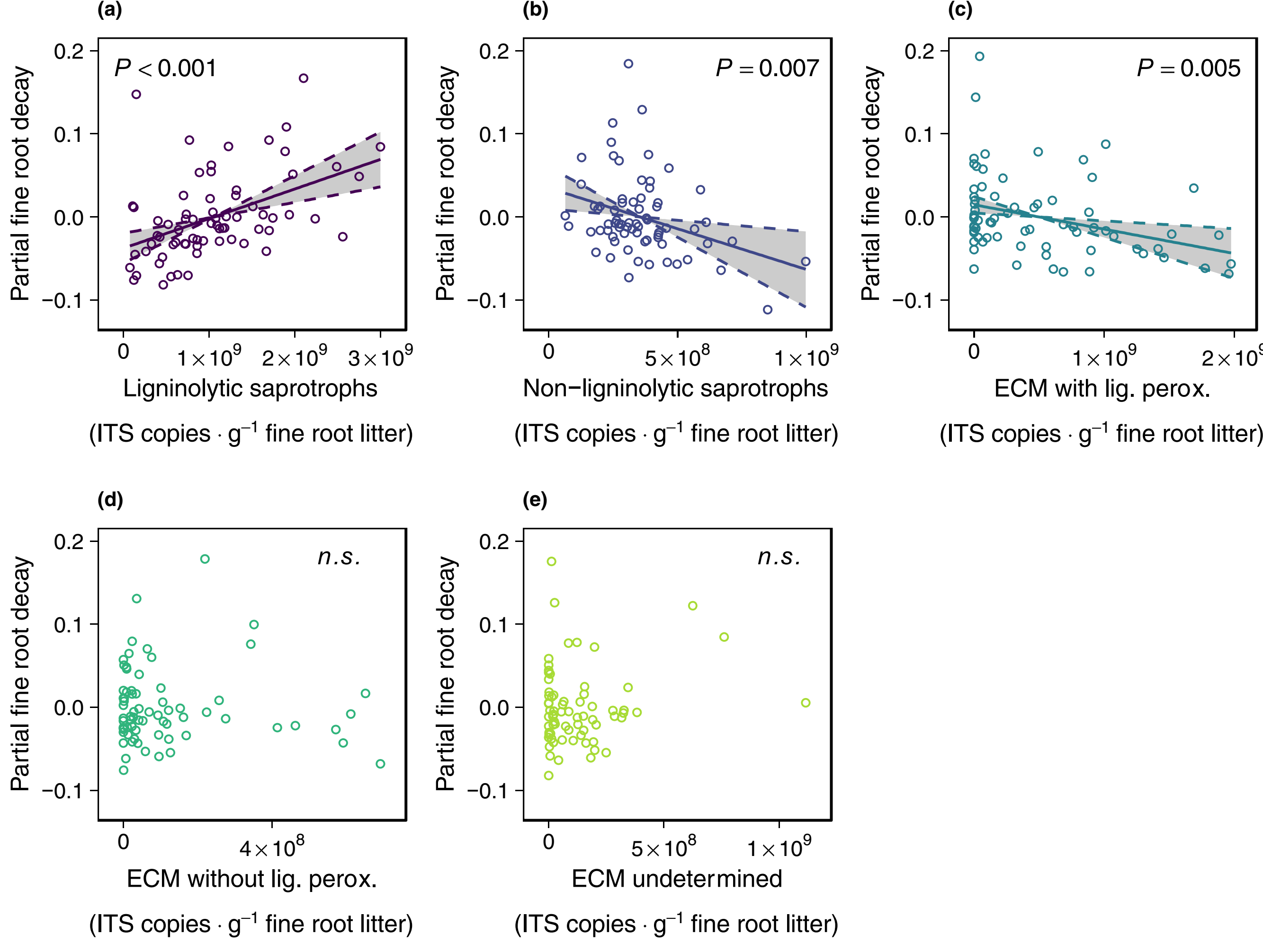


FIGURE 3 Partial regressions showing the relationships between fine root decay and the abundance of ligninolytic saprotrophic fungi (a), nonligninolytic fungi (b), ECM fungi with ligninolytic peroxidases (c), ECM fungi without ligninolytic peroxidases (d), and ECM fungi with uncertain decay capacity (e). These relationships were evaluated using one GAMM with the five fungal functional groups as s predictor variables. This model explained 24% of the variance in fine root decay ($R^2_{adj.}$ = .241). Partial regressions show the effect of a predictor variable after accounting for all other variables in the model, including the spatial component. We accounted for potential spatial autocorrelation in both models using a spatial correlation structure in GAMM models that incorporates the geographic coordinates of each plot (n = 70). n.s., not significant (p > .05).

along which inorganic N and soil pH increase and soil temperature decreased (Figure S6 and Table S5). Similarly, fine root decay was not significantly correlated with PC2 (p = .097; Figure 4b and Table S14), which primarily represented a gradient of increasing soil water content (Figure S6 and Table S5).

3.7 | Relationships between fungal functional groups and the environment

To understand whether environmental conditions may indirectly influence fine root decay by modifying fungal community composition, we used five separate GAMMs to predict the abundance of each fungal functional group using environmental PC1 (along which soil inorganic N and soil pH increased, and soil temperature decreased; Figure S6 and Table S5) and environmental PC2 (primarily representing increasing water availability) as predictor variables (Figure 5). The abundance of lignin-degrading saprotrophic fungi exhibited a unimodal relationship with PC1 and was negatively correlated with PC2 ($R^2_{adj.}$ = .287, p < .05; Figure 5 and Table S15). Nonligninolytic saprotrophs were positively correlated with PC1 ($R^2_{adj.}$ = .114, p = .005; Figure 5 and Table S15) but were unrelated to PC2 (p = .114). ECM fungi with ligninolytic peroxidases declined with increasing values of PC1 and were unimodally related to PC2 ($R^2_{adj.}$ = .554, p < .05; Figure 5 and Table S15). ECM fungi without ligninolytic peroxidases were negatively correlated with PC1 ($R^2_{adj.}$ = .191, p < .001; Figure 5 and Table S15) but unrelated to PC2 (p = .373), whereas ECM fungi with uncertain decay capacity were unrelated to both PC1 and PC2 (p > .05; Figure 5 and Table S15).

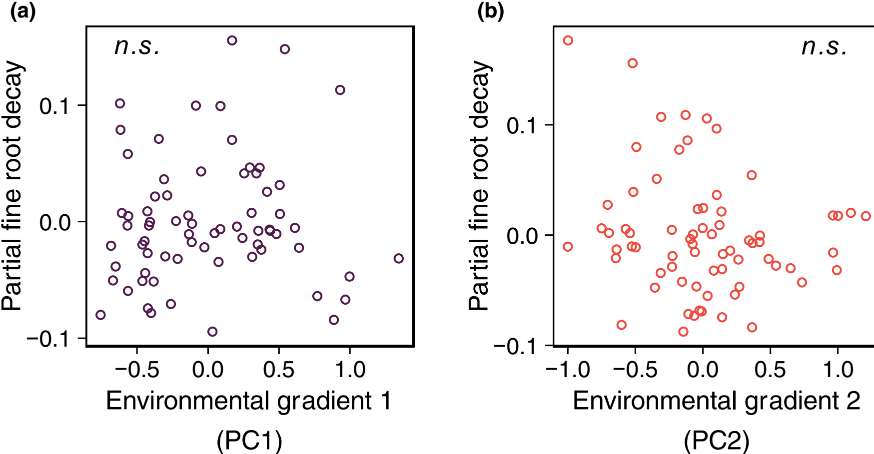


FIGURE 4 Partial regressions showing the relationships between fine root decay and environmental PC1 (a), and environmental PC2 (b). These relationships were evaluated using a GAMM model with only the environmental PCA axes as predictors. PC1 and PC2 are composite environmental axes obtained from a PCA of environmental variables intended to deal with multicollinearity among environmental variables (Figure S6 and Table S5). Soil inorganic N availability and soil pH were positively correlated with PC1, soil temperature was negatively correlated with PC1, and soil water content was positively correlated with PC2 (Figure S6 and Table S5). Partial regressions show the effect of a predictor variable after accounting for all other variables in the model, including the spatial component. We accounted for potential spatial autocorrelation in both models using a spatial correlation structure in GAMM models that incorporates the geographic coordinates of each plot ($n = 70$). n.s., not significant ($p > .05$).

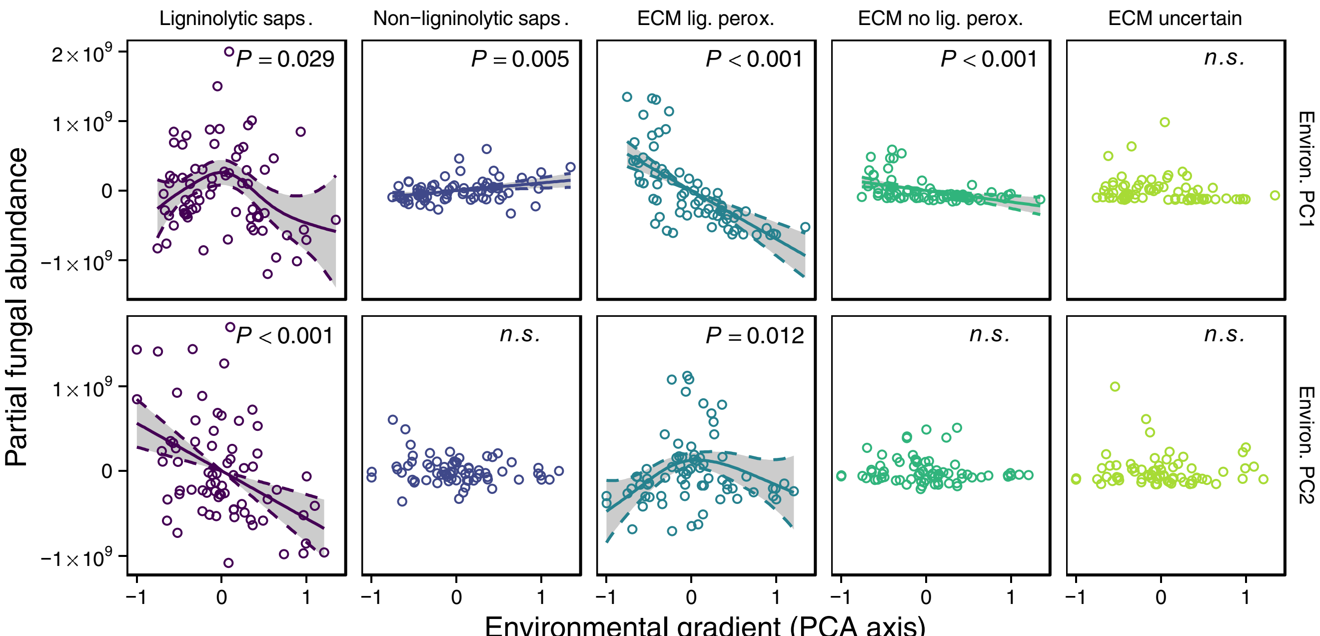


FIGURE 5 Partial regressions showing the relationships between the abundance of fungal functional groups and environmental variables. Each column represents a separate multiple GAMM with a functional group as the response variable and the two environmental composite PCA axes 1 and 2 (rows) as predictors to deal with multicollinearity between environmental variables. Increasing values of PC1 represented an environmental axis along which inorganic N and soil pH increased and soil temperature decreased, whereas increasing values of PC2 primarily represent plots with greater water content (Figure S6 and Table S5). The variance explained by the environment in each model is as follows: ligninolytic saprotrophs, 29% ($R^2_{adj.} = .287$); nonligninolytic saprotrophs, 11% ($R^2_{adj.} = .114$); ECM with ligninolytic peroxidases, 55% ($R^2_{adj.} = .554$; ECM without ligninolytic peroxidases, 19% ($R^2_{adj.} = .191$); ECM with uncertain decay capacity, 1.6% ($R^2_{adj.} = .016$). Partial regressions show the effect of a predictor variable after accounting for all other variables in the model, including the spatial component. We accounted for spatial autocorrelation using a spatial correlation structure that incorporates the location of each plot ($n = 70$). ECM lig. perox., ECM fungi with ligninolytic peroxidases; ECM no lig. perox.", ECM fungi without ligninolytic peroxidases; ECM uncertain, ECM fungi with uncertain decay capacity; *n.s.*, not significant ($p > .05$).

4 | DISCUSSION

Our study addresses a long-standing goal in microbial ecology, which is to understand how the composition of microbial communities and their genetic differences regulate ecosystem functioning (Fierer, 2017; Hall et al., 2018). Traditional ecological theory assumes environmental conditions and litter biochemistry control litter decay rates across broad spatial scales by uniformly regulating the physiology of microbial communities, irrespective of differences in microbial traits and interactions (Berg et al., 1993; Berg & McClaugherty, 2020; Bradford et al., 2016). This paradigm has been challenged by growing evidence that the decay of leaf litter and woody debris can respond to differences in microbial community composition in ways that are not predicted by the environment alone (Glassman et al., 2018; Maynard et al., 2018; Smith & Peay, 2021). However, the mechanisms through which microbial composition regulates litter decay have remained unclear, limiting our ability to accurately structure and parameterize biogeochemical models that predict how the terrestrial carbon cycle will respond to ongoing environmental change (Bradford et al., 2021). Our findings begin to address this knowledge gap by demonstrating that landscape-level patterns in fine root decay are correlated with the abundance of ligninolytic saprotrophic fungi, nonligninolytic fungi, and ECM fungi with ligninolytic peroxidases (Figure 3), and that these links between composition and function are underpinned by differences in the community-level genetic potential for litter decay (Figures 1 and 2).

Fine root decay was related to the genetic potential of fungal communities for plant cell wall degradation, occurring more rapidly where the community weighted mean abundance was greater for all PCWDE genes and rapid decay associated PCWDE genes (Figure 1b,c). Somewhat surprisingly – given the lignin-rich biochemistry of fine root litter (Figure S12; Xia et al., 2015) – the gene families associated with rapid root decay did not include peroxidases (CAZyme family “AA2”) and were instead primarily involved in the decay of cellulose and hemicellulose (Figure 1a). Cellulose and hemicellulose metabolism occurs more extensively than the oxidation of lignin in earlier stages of litter decay (Berg, 2014; Berg & McClaugherty, 2020; Talbot & Treseder, 2012), and fine roots decay relatively slowly (e.g., 60%–70% of initial fine root mass can remain after 3–6 years of decay; Sun et al., 2018; Xia et al., 2018). Thus, we observed the relatively early stages of this process (~13 months), plausibly explaining why genes involved in the depolymerization of cellulose and hemicellulose regulated fine root decay at the temporal scale of our study. However, peroxidases likely still enabled ligninolytic saprotrophic fungi to access cellulose and hemicelluloses occluded by lignin polymers (Baldrian, 2008; Talbot et al., 2012). Although verifying this dynamic would require characterizing changes in the biochemistry of decaying root litter, it would explain why decay was positively correlated with ligninolytic saprotrophic fungi (Figure 3a) and negatively correlated with nonligninolytic saprotrophic fungi (Figure 3b). Predicting community-level phenotypes of soil microbes from functional gene abundances has remained a persistent challenge (Jansson & Hofmockel, 2018), and it is important to recognize that our findings are based on estimates of functional gene abundances from genomes matching only a portion of fungal sequences (55%) at the genus level. Nonetheless, our study provides new evidence that fine root decay is coupled to the abundance of key cellulolytic and hemicellulolytic PCWDE genes in fungal communities.

Links between fine root decay and genetic potential (Figure 1) corresponded to differences in the abundance of fungal functional groups (Figures 2 and 3). We found that ligninolytic saprotrophic fungi, which are abundant members of fungal communities in decaying roots (Figures S7 and S8; Argiroff et al., 2019; Kohout et al., 2018; Philpott et al., 2018), had the most gene copies of all PCWDEs and those associated with rapid fine root decay in their genomes (Figure 2a). Although this finding aligns with previous genomic evidence (Floudas et al., 2012; Nagy et al., 2016; Riley et al., 2014), our results provide new insights into the consequences of these genomic differences at community and ecosystem levels. Specifically, the community-level genetic potential for fine root decay was greater where ligninolytic fungi were more abundant (Figure 2b,c), plausibly explaining the positive correlation between fine root decay and ligninolytic fungi we observed (Figure 3a). Similarly, ECM fungi with ligninolytic peroxidases had fewer copies of PCWDEs associated with rapid root decay in their genomes (Figure 2a), which is consistent with the large contractions in hydrolytic enzyme-encoding genes revealed by previous genomic analyses (Kohler et al., 2015; Martino et al., 2018; Miyauchi et al., 2020). We found that both the genetic potential for litter decay and rates of fine root decay were negatively correlated with the abundance of ECM fungi with ligninolytic peroxidases (Figures 2 and 3c), suggesting genomic characteristics of ECM fungi operate at community and ecosystem scales to influence landscape-level patterns in fine root decay.

The composition of ECM fungal communities may influence soil organic matter dynamics (Frey, 2019; Zak, Pellitier, et al., 2019), and our findings suggest functional differences among ECM lineages are important for fine root decay. ECM fungi can slow the decay of plant litter by competing with free-living saprotrophic fungi (Fernandez & Kennedy, 2016), which mediate the majority of litter decay (Lindahl & Tunlid, 2015; Starke et al., 2021). ECM fungi with greater decay capacity are thought to compete with saprotrophic fungi more extensively on lignin-rich litter (Fernandez et al., 2020; Smith & Wan, 2019), which may explain why fine root decay was negatively correlated with ECM fungi with ligninolytic peroxidases (Figure 3c) but was unrelated to ECM fungi without ligninolytic peroxidases (Figure 3d) and uncertain decay capacity (Figure 3e). Interestingly, we previously found that ECM fungi with ligninolytic peroxidases directly accelerated the decay of soil organic matter in these ecosystems (Argiroff et al., 2022). Consequently, ECM fungi with ligninolytic peroxidases appear to slow the early stages of root litter decay (Figure 3c) but accelerate the decay of older soil organic matter (Argiroff et al., 2022). Stage-dependent effects of ECM fungi on decay have been observed in boreal forests, and likely arise because ECM fungi inhibit the saprotroph-dominated early stages of litter decay but accelerate the decay of older soil organic matter that

yields little energy for saprotrophic growth (Sterkenburg et al., 2018). Our results suggest these dynamics control spatial variation in soil organic matter dynamics across temperate forest ecosystems.

The presence of ligninolytic peroxidases distinguished ecologically relevant groups within the broader classifications of saprotrophic and ECM fungi (Figures 2 and 3), which has important implications for incorporating gene-to-ecosystem mechanisms into process-based models of carbon cycling. Microbe-explicit processes can improve ecosystem carbon models, but distilling the immense taxonomic and functional diversity of soil microbial communities to support these models remains challenging (Bradford et al., 2021; Wieder et al., 2015, 2018). We found that the division of fungal genera into ligninolytic saprotrophs, nonligninolytic saprotrophs, ECM fungi with ligninolytic peroxidases, and ECM fungi without ligninolytic peroxidases, accounted for 43%–47% of the variation in genome-level PCWDE abundance (Figure 2a), 55%–62% of the community-level abundance of these PCWDEs (Figure 2b,c), and 24% of the variation in fine root decay (Figure 3). Moreover, community-level genetic variation and fine root decay were differentially correlated with ligninolytic saprotrophs, ECM with ligninolytic peroxidases, nonligninolytic saprotrophs, ECM fungi without ligninolytic peroxidases, and ECM fungi with uncertain decay capacity (Figures 2 and 3). Consequently, the abundances of these functional groups can be adequate proxies for mechanistic links between fungal genetic potential and the decay of fine root litter as fine root decay is incorporated into ecosystem carbon models.

Extracellular enzyme activities provided additional insight into the activity of fungal communities in decaying fine roots (Figures S10 and S11 and Table S11). Somewhat surprisingly, cellulolytic and ligninolytic enzyme activities were not related to the abundance of either saprotrophic fungal functional group ($p > .05$; Figure S10). However, N-acetyl-β-glucosaminidase activity was strongly positively correlated with the relative abundance of ligninolytic saprotrophic fungi ($p < .001$, Figure S9). This chitin-depolymerizing enzyme is involved in the remodelling and recycling of fungal cell walls (Baldrian, 2008), and its correlation with ligninolytic saprotrophic fungi could reflect that this fungal functional group was undergoing more rapid growth and turnover than other groups. Enzyme activities are sometimes related to rates of decomposition and soil carbon cycling (Chen et al., 2018), but this is not always the case (Sorouri & Allison, 2022). Our findings suggest that the genetic potential for litter decay can be a better predictor of links between fungal community composition and fine root decay rates in the temperate forests in our study (Figures 1 and 3), but that extracellular enzyme activities also captured certain dynamics that genetic data did not, such as the possible rapid turnover of ligninolytic saprotrophic fungal biomass plausibly related to decay rates (Figure S10).

Environmental controls over fine root decay are often weaker and more variable than the decay of aboveground litter (Beidler & Pritchard, 2017; See et al., 2019; Wambsganss et al., 2021), and our results suggest these patterns may arise because fungal community composition and genetic variation overwhelm the direct effects of the environment on fine root decay. Unlike the abundance of fungal functional groups and genetic decay potential, which accounted for up to 24% of the variation in decay rates we observed (Figures 1 and 3), none of the environmental variables we measured directly predicted fine root decay (Figure 4a,b). We propose that the high lignin concentrations of fine roots (Figure S12) relative to leaf litter, which is a widely observed pattern across litter types (threefold higher lignin concentrations in root litter than for leaf litter; Xia et al., 2015), cause their decay to depend more heavily on the composition and genetic decay potential of fungal communities than environmental effects on overall community activity. Specifically, fine root decay was likely positively correlated with the abundance of ligninolytic fungi (Figure 3a) because they are the only microbes with the genetic capacity to simultaneously and completely decay lignin, cellulose, and hemicelluloses (Baldrian, 2008; Hofrichter, 2002; Kirk & Farrell, 1987). Similarly, fine root decay likely decreased with increasing abundances of ECM fungi with peroxidases (Figure 3c) because resource limitation of saprotrophic growth – and thus the suppressive effect of competition by ECM fungi – is greater for lignin-rich litter (Fernandez et al., 2020; Smith & Wan, 2019). Consequently, environmental effects on decay appear to be indirectly propagated through their influence on fungal composition, such as the negative effect of soil water content (PC2) on ligninolytic saprotrophs and the negative effect of soil inorganic N availability (PC1) on ECM fungi with peroxidases (Figure 5).

An important limitation of this study is that our results are based on correlations between fine root decay, genetic potential, fungal community composition, and the environment. For example, we cannot conclusively exclude the possibility that changes in fungal community composition were driven by succession during fine root decay (sensu Herzog et al., 2019; Kohout et al., 2021) rather than the causal driver of fine root decay rates. However, we tempered this limitation by addressing specific hypotheses (Lindahl et al., 2021; Prosser, 2020) based on prior experimental evidence that declines in the abundance of ligninolytic fungi and increases in nonligninolytic fungi can slow the decay of fine root litter and other lignin-rich substrates (Argiroff et al., 2019; Entwistle, Romanowicz, et al., 2018; Entwistle, Zak, & Argiroff, 2018). Nonetheless, it will be important to experimentally manipulate the composition of fungal communities to explicitly test its role in regulating fine root decay (Glassman et al., 2018; Smith & Peay, 2021; Sterkenburg et al., 2018). Furthermore, although we found that fungal genes and functional group abundances were strongly correlated with fine root decay, these fungal variables accounted for ≤24% of variation in decay rates (Figures 1 and 3). Differential expression of peroxidases and other genes encoding PCWDEs can also control decay rates (Entwistle, Romanowicz, et al., 2018; Zak, Argiroff, et al., 2019), and it is possible that accounting for variation in gene expression along with genetic potential and functional group abundances will increase explanatory power.

Additionally, although emerging methods to assess rates of fine root decay hold promise to improve our understanding of this process (Li et al., 2022), it remains nearly impossible to estimate fine root mass loss and simultaneously characterize microbial

communities without using a litterbag approach. This approach inherently disturbs or removes endophytic taxa of roots that are important initial inhabitants of decaying fine root litter (Kohout et al., 2018, 2021). The presence of fungal endophytes can modify community assembly and litter decay through priority effects (Cline et al., 2018). Because the composition of root endophytes varies among our study sites (Pellitier & Zak, 2021b), it is possible that we did not capture the full extent to which fungal community composition and genetic decay potential influences fine root decay. Similarly, the abundance of ECM fungi with ligninolytic peroxidases declined with inorganic N availability in these sites (Pellitier, Ibáñez, et al., 2021; Pellitier & Zak, 2021a; Pellitier, Zak, et al., 2021) in parallel with ECM fungi in soil and fine root litter (Argiroff et al., 2022), and this pattern suggests residual ECM fungal necromass in fine root litter also varied in its composition among plots in our study. Although ECM fungal necromass likely accounted for a small fraction of the first- through third-order roots used in our litterbag study, we cannot rule out the possibility that different compositions of ECM fungal necromass influenced the patterns of decay that we observed (Fernandez et al., 2016) and emphasize that experimentally evaluating this effect is an important area for future research.

Finally, we emphasize that our classification of ECM fungi with putatively ligninolytic peroxidases is an initial approximation of function pending additional genome sequencing and experimental verification. There is growing evidence that certain Agaricomycete lineages of ECM fungi (e.g., *Cortinarius*, *Hebeloma*, *Piloderma*, and *Russula*) possess multiple copies of class II peroxidase genes (Bödeker et al., 2009; Kohler et al., 2015; Miyauchi et al., 2020; Nagy et al., 2016) that are likely involved in organic matter decay based on in situ detection of gene transcripts and peroxidase activity (Bödeker et al., 2014; Sterkenburg et al., 2018), as well as negative relationships between the abundance of these peroxidase-containing lineages and soil organic matter stocks (Argiroff et al., 2022; Clemmensen et al., 2021; Lindahl et al., 2021). However, genera that we classified as ECM fungi with uncertain decay capacity may ultimately possess ligninolytic peroxidase once additional genome sequencing is performed and the functionality of peroxidases within ECM genomes is evaluated (Pellitier & Zak, 2018; Zak, Argiroff, et al., 2019; Zak, Pellitier, et al., 2019). There is a clear need to sequence genomes of under-sampled ECM lineages and experimentally test how the type and copy number of oxidative decay genes is related to the in situ activity of ECM fungi, with the potential to establish continuous relationships between decay and these traits rather than a dichotomous classification.

Together, the relationships we observed suggest variation in the abundance of ligninolytic saprotrophs and ECM fungi with ligninolytic peroxidases regulates spatial patterns in fine root decay by modifying the genetic potential of fungal communities for plant cell wall degradation (Figures 1–3). These results improve our understanding of a poorly resolved component of the terrestrial carbon cycle (Berg & McClaugherty, 2020) by supporting the idea that fungal community composition and genetic variation are important controls over fine root decay in northern temperate forests. A central goal in microbial ecology is to understand the mechanisms that couple microbial community composition to soil biogeochemistry (Fierer, 2017), and our work addresses this knowledge gap by demonstrating that shifts in fungal community composition control fine root decay by determining the abundance of key genes encoding cellulolytic and hemicellulolytic enzymes (Figures 1–3). Finally, models of soil carbon cycling increasingly include microbial functional groups, traits, and interactions (Bradford et al., 2021). We found that fungal composition and genetic variation overwhelmed the direct effects of the environment on fine root decay (Figure 4), and instead indirectly coupled environmental conditions to the decay of fine root litter (Figure 5). Thus, models based on fungal functional groups will be essential to understanding how ongoing environmental change modifies fine root decay, a globally important process that is central to the cycling and storage of carbon in soil.

AUTHOR CONTRIBUTIONS

William A. Argiroff and Donald R. Zak designed the study; William A. Argiroff, Rima A. Upchurch, Donald R. Zak, and Peter T. Pellitier set up the field experiment and performed field sampling; William A. Argiroff, Rima A. Upchurch, and Julia P. Belke performed laboratory analyses; William A. Argiroff analysed data and wrote the manuscript. All authors contributed to revisions.

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DATA AVAILABILITY STATEMENT

Sequence data have been made available on Genbank (project accession no. PRJNA714922), and all other data and code are available in DRYAD (https://doi.org/10.5061/dryad.vq83bk3xr) and Github (https://github.com/ZakLab-Soils/Root_Decay_RProject).

BENEFITS GENERATED

Benefits from this research accrue from the sharing of our data and results on public databases as described above.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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