INTRASPECIFIC PLANT GENETIC DIVERSITY IMPACTS ON SOIL MICROBIAL COMMUNITY COMPOSITION AND NUTRIENT CYCLING

by

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Abstract

In forests around the world, disturbances are common and recognized as important change agents shaping forest ecosystem dynamics. However, we still do not fully understand the influence of disturbance on forest *intra*specific diversity, as most research has emphasized *inter*specific diversity. Even less is known about the implications of diversity-related changes in forest stand composition and structure on belowground ecosystem function such as carbon and nutrient cycling.

At the Arlington Agricultural Experimental Research Station in Wisconsin, we investigated how disturbance-mediated shifts in the genetic composition of aspen stands affect soil microbial dynamics, and the functional implications of microbial changes on soil biogeochemical cycling. The aspen stands include 14 individual trembling aspen (*Populus tremuloides*) genotypes. Half of the stands were later thinned by 75% to simulate a disturbance and decrease competitive inhibition amongst the trees, resulting in different proportions of genotypes and changes in the genetic composition of the experimental aspen populations over time. We found that soil bacterial and fungal biomass did not differ between stem thinning treatments. Both intraspecific diversity and thinning treatment did not affect soil carbon and nitrogen accumulation, soil bacterial alpha and beta diversity, the relative abundance of major bacterial phyla (with a few exceptions) or bacterial functionality. Tree diversity, but not thinning treatment, affected the relative abundances of fungal phyla and functionality. Bacterial and fungal phyla and functional groups also affected soil organic carbon and nitrogen concentrations. With anthropogenically induced environmental changes on the rise, it is important we understand how changes in microbial biomass, community composition, and functional groups can affect biogeochemical processes.

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Table of Contents

Abstract i

Acknowledgements ii

Chapter 1: Intraspecific plant genetic diversity impacts on soil microbial community composition and nutrient cycling 1

Introduction 1

Methods 5

Bacterial Biomass 10

Bacterial Diversity 10

Bacteria Relative Abundance 11

Fungal Biomass 11

Fungal Diversity 11

Fungi Relative Abundance 12

Bacterial Functional Traits 12

Fungal Functional Traits 13

Aboveground relationship with soil carbon and nitrogen 13

Bacteria's relationship with soil carbon and nitrogen 13

Fungi's Relationship to soil carbon and nitrogen 14

Discussion 15

Soil carbon and nitrogen not affected by tree diversity and thinning treatment 15

Microbial biomass not affected by tree diversity and thinning treatment 16

Tree diversity affects fungi and bacteria diversity differently 17

Tree diversity, but not thinning treatment, affects bacteria and fungal phyla relative abundances and functional groups 18

Bacterial and fungal phyla and functional characteristics can explain some of the variations in soil carbon and nitrogen 19

Conclusion 22

Figures 23

Supplementary Figures 24

Tables 32

References 45

Chapter 1: Intraspecific plant genetic diversity impacts on soil microbial community composition and nutrient cycling

Introduction

In forests around the world, disturbances caused by natural events and human actions play key roles in shaping forest ecosystem dynamics through the shaping of forest composition and structure, including the diversity of trees. Therefore, forest managers are increasingly interested in adopting silvicultural methods that simulate these types of disturbances in order to enhance biodiversity and maximize long-term forest health and sustainability. Though much focus has been given to large-scale disturbances such as hurricanes and fire, researchers have begun to consider the positive effects of intermediate-level disturbances that alter forest stand dynamics on forest biodiversity (Molino and Sabatier, 2001) and subsequently, ecosystem stability.

The biodiversity-ecosystem function (BEF) theory posits that a positive correlation exists between species richness and ecosystem function (Doherty and Zedler, 2018; Schwartz et al., 2000). BEF studies have historically focused on *inter*specific diversity, or diversity between species (Bolnick et al., 2011; Crutsinger et al., 2006; Odling-Smee et al., 2013; Whitham et al., 2003). There is emerging evidence that the influence of genetic diversity *within* a species (*intra*specific diversity) on their surrounding environment is comparable to interspecific effects (Des Roches et al., 2018; Raffard et al., 2019).

Changes in the diversity of plants could potentially lead to changes in microbial communities as a result of diverse aboveground litterfall and root exudates, affecting ecosystem functions such as carbon and nutrient cycling (Chen et al., 2019). Researchers have already

documented the effects of plant genotype on the soil microbiome. In a *Populus* common garden experiment, plant genotype alone was found to be responsible for up to 70% of the variation in the soil microbial community composition (Schweitzer et al., 2008a).

In this study, we investigated how disturbance-mediated shifts in the genetic composition of aspen in experimental stands affects soil microbial dynamics, and the functional implications of microbial changes on soil biogeochemical cycling. Trembling aspen (*Populus tremuloides*) is the most genetically diverse and widespread tree species in North America (Callahan et al., 2013). It plays a crucial role in sustaining wildlife biodiversity and ecosystem functioning across its range (Lindroth & St Clair, 2013; Rogers et al., 2020). Therefore, it is the premier model system for demonstrating the ecological consequences of intraspecific diversity.

The first goal of this study was to explore relationships between above- and belowground biodiversity in experimental stands of varying genotypic diversity in response to a thinning treatment. Past research in experimental aspen stands in Wisconsin by our collaborators in the Departments of Forest and Wildlife Ecology and Entomology demonstrated that following an intermediate-level disturbance (heavy thinning), the genetic compositions of the experimental aspen populations have followed different trajectories (Cope et al., 2021). In the disturbed populations, the slow growing genotypes with higher levels of chemical defense compounds were subjected to high competitive pressure and subsequently experienced high mortality. This resulted in faster growing genotypes becoming dominant. In comparison, the undisturbed populations maintain greater uniform genetic diversity (Cope et al., 2021).

These disturbance-mediated changes have been shown to influence litter decomposition and litter microbial biomass from multiple aspen genotypes (Kasmerchak, unpublished data).

However, it remains to be seen if the same competitive environment and stand-level dynamics apply to belowground ecosystem processes.

Multiple studies have shown that alterations in aboveground biodiversity affect belowground ecosystem functioning (Bardgett et al., 2005; Wardle et al., 2004; Zak et al., 2003). More recently, changes in plant intraspecific diversity can have wide-ranging implications for ecosystem-level processes, influencing soil microbial community composition in deciduous forest stands (Schweitzer et al., 2008b) as well as perennial and annually tilled cropping systems (Aira et al., 2010; Ulbrich et al., 2021). Furthermore, microbial diversity has been shown to undergo negative shifts after disturbances such as permafrost thaw (Seitz et al., 2021), induced soil degradation (Chaer et al., 2009), and forest conversion to agriculture (Borneman and Triplett, 1997). Changes in microbial biomass have also been observed due to land use change (Díaz-Vallejo et al., 2021). All in all, there is significant evidence that plant genotype and disturbance are predictive factors for soil microbial community change, but there is a paucity of information on soil microbial community response to change, how this community affects biogeochemical processes, and how these feedbacks ultimately drive ecosystem services provided by soil biota. As shifts in soil biodiversity can influence up to 50% of the variation in an ecosystem's multifunctionality (Delgado-Baquerizo et al., 2016; Wagg et al., 2014), it is vital that we understand what drives it.

The second focus of this study is to evaluate if there are any associated changes in microbial community composition following the introduced disturbance, and the subsequent implications for soil biogeochemistry. The functional traits approach has garnered popularity in the field of microbial ecology in place of the more traditional metrics of taxonomy and community composition (Green et al., 2008). Functional traits can provide valuable insights into

a species' role in an ecosystem (Louca et al., 2016), making them useful for understanding microbial responses to environmental change and the subsequent implications for critical soil processes (Blagodatskaya et al., 2021; Fierer, 2017; Green et al., 2008; Romillac and Santorufo, 2021). Bacterial functional groups aid carbon and nutrient cycling through processes such as the breakdown of organic compounds, nitrogen fixation, nitrification, denitrification, mineralization, and release into the atmosphere. For example, cellulolytic microorganisms play an important role in the soil ecosystem by breaking down cellulose, the most abundant carbohydrate produced by plants. Nitrifying bacteria, on the other hand, convert ammonia into nitrate. Meanwhile, fungi can be divided into functional groups based on their trophic strategies as saprotrophs, symbiotrophs, and pathotrophs (Jastrow et al., 2007; Štursová et al., 2012; Treseder and Lennon, 2015). Saprotrophs contribute to carbon dynamics by breaking down decaying organic matter, while ectomycorrhizal fungi are known to promote nitrogen uptake by plants (Chalot and Brun, 1998).

This study provides a unique opportunity to explore how disturbance-mediated changes in intraspecific diversity affect microbial biomass, community composition, and functional groups and its implications on soil biogeochemical processes. I hypothesized that soil microbial community composition, diversity, and function would differ in the disturbed aspen populations from the undisturbed stands in response to changes in forest genetic structure. I also expected that soil carbon and nitrogen concentrations would be related to microbial functional traits. Understanding the relative contributions of bacterial and fungal functional groups to carbon and nitrogen cycling, as well as their resilience in relation to a rapidly changing environment, is important to fully assess their impact on belowground ecosystem dynamics in forests now and in the future.

Methods

Study site description and history

The experimental aspen populations were established at the Arlington Agricultural Research Station, College of Agricultural and Life Sciences, University of Wisconsin-Madison in Arlington, WI (43.3°N, 89°W) in Fall 2010 (Cope et al., 2021). Prior to aspen tree planting, the site was planted with hybrid poplar (*Populus nigra* x *P. Maximowiczii)*. There were 18 stands with 64 individual trees each, representing 16 genotypes collected from natural aspen stands throughout Wisconsin. Further analysis later revealed that only 15 unique genotypes exist, 14 of which were represented by the 18 stands (C. Cole, K. Mock, R. Lindroth, unpublished data). The trees were planted at a density of 40,000 stems ha^{-1} (0.5 m x 0.5 m spacing) in all four 2 x 2-meter quadrants within each plot. Non-experimental trees bordered each plot. The soils in the area are within the Huntsville series (Cumulic Hapludolls) formed in paleo floodplains capped with loess or wind-blown silt.

In Spring 2014, half of the experimental stands were heavily thinned to simulate an intermediate-level disturbance. Three of four replicates per genotype in each population were removed, resulting in a reduction of tree density by 75% to 10,000 trees ha^{-1}. Following disturbance, the genotypic composition of the stands diverged, resulting in differential selection of fast- and slow-growing genotypes in the unthinned and thinned stands respectively (Cope et al., 2021). It is also important to note that this divergence occurred over a period of 5 years, which is a small fraction of the standard lifespan of an aspen tree. This highlights the important role disturbance plays in shifting the expression of stand-level plant traits and C allocation strategies (Cope et al., 2021; Kruger et al., 2020).

Stand-level genetic diversity

Stand-level genetic diversity was measured based on the number of aspen genotypes and the relative frequency of each genotype. We used the vegan package in R studio (Oksanen et al., 2022) to estimate stand-level genetic diversity based on the Shannon Diversity Index (Shannon and Weaver, 1949), which takes into account the proportion of species in a particular community and the relative frequency of each species, based on the number of aspen genotypes and the relative frequency of each genotype.

Soil carbon and nitrogen measurements

Soil samples were taken from the surface horizon (0-10cm) of four subplots from each of the 18 plots (72 samples total) in 2019, air-dried, and pulverized in a SpexMill 8000D. Soil total carbon and nitrogen were determined by combustion and gas chromatography on a Flash 2000 Elemental Analyzer. Samples that contained inorganic carbon as determined by the acid drop test (Nelson and Sommers, 1983) were acid fumigated for 12 hours to remove carbonates (Harris et al., 2001). The elemental analyzer was used to run the fumigated samples, and the residual C is taken to represent organic C. Soil carbon and nitrogen stocks (Mg/ha) were calculated using plot-level soil bulk density.

Soil potential nitrification rates were measured via a 13-day laboratory incubation (Robertson et al., 1999) to determine the amount of inorganic N accumulated over time. The samples were regularly checked during the incubation to adjust moisture content to keep samples at field capacity. At the end of the incubation, 50 mL of 2.0 *M* KCl was added to 10 g of each sample, and these samples were shaken for 70 minutes at 160 rpm. Extracts were filtered and frozen until analysis on a Lachat instrument for NH_4^+ and NO_3^- (Hart et al., 1994). Nitrification

and net mineralization rates were measured from the change in nitrate and ammonium concentrations during the incubation (Eq. 1 & 2), where f is final, 0 is initial, and T represents time:

$$N_{nitrified} = (Nitrate_f - Nitrate_0) / \mathrm{T}_{\mathrm{days}} \quad \text{(equation 1)}$$

$$N_{mineralization} = [(Nitrate_f + Ammonium_f)] - [(Nitrate_0 + Ammonium_0)] / \mathrm{T}_{\mathrm{days}} \quad \text{(equation 2)}$$

This approach measures potential rates under controlled conditions in the lab and are not necessarily representative of nitrogen availability in the field.

Phospholipid fatty acid analysis

To determine microbial biomass, soil samples from all subplots for each stand (72 samples total) were sent to Regen AgLab in Nebraska for phospholipid fatty acid (PLFA) analysis (Oates et al., 2017) using standard procedures and protocols for PLFA extraction and quantification (Balser et al., 2019; Bligh and Dyer, 1959). Although PLFA analysis does not provide taxonomic information at a species level, it does provide information in gross microbial functional groups, which are sensitive to land use and environmental changes (Smith et al., 2015).

We used distinct lipid biomarkers to differentiate microbial groups including: (1) the sum of 18:1 ω9c and 18:2 ω6,9c lipids as general fungal indicators, (2) the sum of monosaturated (excluding 16:1 ω5c) and cyclopropyl lipids at Gram-negative bacterial indicators, (3) anteiso-branched lipids as Gram-positive bacterial indicators, and (4) the sum of 15:0, 16:0, and 18:0 lipids as non-specific bacterial indicators. The biomass for different lipid biomarkers are expressed as nmol g soil^{-1}, which is used as an estimate of soil microbial biomass (Oates et al.,

2017). However, there are several limitations to this approach as the majority of biomarkers were obtained from pure cultures whilst environmental samples are inherently more complex (Willers et al., 2015). Additionally, the fatty acids of many microbial species remain unknown (Watzinger, 2015).

DNA extractions and processing

We extracted DNA for sequencing analysis using 0.25 g of frozen soil subsamples with the DNEasy PoweLyzer PowerSoil DNA extraction kit (QIAGEN, Germantown, MD). Extracted DNA was then amplified in triplicate with polymerase chain reaction (PCR), targeting the v4 region of the 16S rRNA gene with 515f and 806r primers (Walters et al., 2015) and the ITS2 gene region with 5.8S-Fun and ITS4-Fun primers (Taylor et al., 2016) with added barcodes and Illumina sequencing adapters (Kozich et al., 2013). We use the following parameters to carry out PCR in the thermocycler: 98^0C for 2 minutes, 98^0C for 10 seconds, 58^0C for 15 seconds, 72^0C for 10 seconds, repeat steps b-d 30 times, 72^0C for 2 minutes.

PCR amplicons were pooled, purified, and normalized using a SequalPrep Normalization Plate (ThermoFisher Scientific, Waltham, MA) and Wizard SV Gel and PCR Clean-Up System A9282 (Promega, Madison, WI). The pooled library was submitted to the UW-Madison Biotechnology Center for 2x300 PE Illumina MiSeq sequencing.

The 16S and ITS2 forward and reverse sequence reads were merged and quality filtered using the dada2 pipeline in R Studio. We implemented the dada2 denoise-paired algorithm to determine amplicon sequence variant level OTUs (Callahan et al., 2016). Taxonomies were assigned to the 16S reads with the aligned 515f-806r region of the 97% ID OTUs from the Silva database using a mothur classify.seqs knn method (version 138) (Quast et al., 2013). For the

ITS2 reads, we used the UNITE database (UNITE Community, 2023) as the reference database at 97% ID to assign taxonomy.

All bioinformatics were completed using R packages phyloseq (McMurdie and Holmes, 2013), ggplot (Wickham, 2016), and dplyr (Wickham et al., 2015). We did not average by site to represent the variability of our study area. To maintain data quality, we also only included samples with more than 200 sequences, resulting in 70 samples for 16S and 71 samples for ITS2. We acknowledge the potential limitations of DNA-based assessments of the soil microbiome, as extracellular DNA from dead microorganisms have been known to persist in soil for weeks to years, and can inflate the active microbial richness up to 55% (Carini et al., 2016). The abundance of extracellular DNA may obscure the soil microbial diversity estimates, contributing to the overestimation of the relative abundances of taxa that are key to ecosystem processes in our findings.

Functional traits

We defined a species' functional traits to be their ecological traits, which are functional assignments based on the life strategy, phenotypic, and quantitative genomic traits of a taxon from its nomenclature (Djemiel et al., 2022). We used the FAPROTAX database (Louca et al., 2016) to assign metabolic functions, ecological traits, or large functional groups relevant to bacteria and the FUNGuild database (Nguyen et al., 2016a) to assign ecological traits to fungi.

Statistical approach

To explore variability in bacterial and fungal composition, we compared data across all field samples using Bray-Curtis dissimilarities on Hellinger-transformed relative abundances

(Legendre and Gallagher, 2001). The community composition, color-coded by thinning treatment, was presented using NMDS ordinations. To test if treatment (thinned versus unthinned) and plot influenced soil microbial beta diversity, we performed a two-way PERMANOVA using the adonis function in the vegan R package (Oksanen et al., 2013).

We used linear mixed models to test whether thinning treatment has an effect on soil microbial alpha diversity and simple linear regression analyses to test whether tree Shannon diversity has an effect on soil microbial alpha diversity. Diversity indices (Shannon's Diversity and Shannon's Evenness) were estimated using the vegan package in R (Oksanen et al., 2013).

To test for the effect of stand thinning on soil carbon and nitrogen, relative abundance of individual bacteria and fungal phyla and of individual functional groups, and PLFA biomarker biomass, we constructed linear mixed models using treatment as a fixed effect and plot as a random effect using the lme4 package (Bates et al., 2015). To test how the plot-level genetic diversity of trees (tree diversity) affects soil carbon and nitrogen properties and bacteria and fungi phyla relative abundance, functional groups, and biomass, we did simple linear regression analyses. To control the expected proportion of false discoveries in multiple comparisons, we used the Benjamini-Hochberg procedure to calculate the False Discovery Rate (FDR) and p-value adjusted.

Data that did not fit assumptions for linear mixed models and simple linear regression analysis were transformed. We considered a significant effect when the p-value <0.05, unless otherwise noted.

Results

Bacterial Biomass

Tree diversity and thinning treatment were not correlated with Actinobacteria, Gram-positive, Gram-negative, or total bacterial biomass (Tables 1 & 2; Figure S1).

Bacterial Diversity

Bacterial evenness was found to be positively related to tree diversity (ß = 0.009, p-value = 0.061, R^2 = 0.051; Table 6, Figure S2b), but not treatment. Tree diversity and thinning treatment did not affect bacterial richness or Shannon Diversity (Table 6; Figure 2a). The bacterial NMDS (K = 2, Stress = 0.20; Figure 3a) did not show any clear groupings in community composition between disturbance histories. The PERMANOVA for bacterial communities showed no effect of thinning (disturbance) and plot on belowground Shannon diversity (Table 4). The betadisper analysis showed a homogenous dispersion in the bacterial data (p-value = 0.246; Table 5).

Bacteria Relative Abundance

The most abundant bacteria phyla in our samples were Actinobacteria (29.32%), Acidobacteria (18.25%) and Proteobacteria (13.31%; see the others in Table 7). The linear mixed model and simple linear regression analysis results showed that thinning treatment and tree diversity does not have a significant effect on the relative abundances of most bacterial phyla (see Tables 8 & 9; Figure S3). Verrucomicrobiota (ß = -0.053, p-value = 0.033), however, was found to have a negative relationship with tree diversity.

Bacterial Functional Traits

We were able to classify 19.55% of the bacterial OTUs using the FAPROTAX database. From those, we chose to use functional classifications directly relevant to the soil carbon and nitrogen cycle (see Table 13 for relative abundances). There is no evidence that thinning treatment and tree diversity has a significant effect on the relative abundances of bacterial functional groups (Tables 14 & 15).

Fungal Biomass

Tree diversity and treatment were not correlated with Arbuscular Mycorrhizal Fungi Biomass, Non-Arbuscular Mycorrhizal Fungi Biomass and total fungal biomass (see Table 10; Figure S5).

Fungal Diversity

Fungal richness was found to be negatively related to tree diversity ($ß = -34.460$, p-value = 0.012, $R^2 = 0.087$; Table 6, Figure S2b), but not treatment. The fungal NMDS (K = 2, Stress = 0.230; Figure 3b) did not show any clear groupings in community composition between disturbance histories. The PERMANOVA for fungal communities showed no evidence of a significant effect by treatment on belowground Shannon diversity (Table 4). The betadisper analysis showed a homogenous dispersion in the fungal data (p-value = 0.557; Table 5).

Fungi Relative Abundance

The most abundant fungal phyla at our sites were Basidiomycota (77.31%) and Mortierellomycota (10.07%; Table 5). The linear mixed model results showed that thinning

treatment does not have a significant effect on the relative abundances of fungal phyla (see Table 12; Figure S6). Tree diversity was found to be negatively related to Chytridiomycota (ß = -0.110, p-value = 0.025, R^2 = 0.087) and Mortierellomycota (ß = -0.271, p-value = 0.025, R^2 = 0.099) and positively related to Basidiomycota (ß = -0.296, p-value = 0.029, R^2 = 0.074).

Fungal Functional Traits

A total of 90.78% of the fungal OTUs were assigned to functional guilds. Among them, 76.51% were classified as either pathotrophs, saprotrophs or symbiotrophs, whereas 14.27% belong to fungi with more than one trophic strategy. For trophic-level classification, there were 71.98% symbiotrophs and 13.01% saprotroph-symbiotrophs. We used linear mixed models to test how tree diversity and treatment affects different fungal functional groups (see Table 13; Figures S7 & S8). Tree diversity is positively correlated with symbiotrophs (ß = 0.455, p-value = 0.003, R^2 = 0.124), but no evidence of significant treatment effect. For guild classification, 0.01% were arbuscular mycorrhizal fungi, 71.57% were ectomycorrhizal fungi, 0.55% were plant pathogens, and 0.45% were wood saprotrophs. Ectomycorrhizal fungi has a positive correlation with tree diversity (ß = 0.461, p-value = 0.003, R^2 = 0.123), but there is no treatment effect.

Aboveground relationship with soil carbon and nitrogen

We found no relationships between treatment and soil carbon and nitrogen concentrations, nitrate and ammonium concentrations, and nitrification and nitrogen mineralization rates (see Table 18; Figures S9-14). Tree diversity is negatively correlated with nitrate concentration (ß = -0.619, p-value = 0.043, R^2 = 0.081), total inorganic nitrogen (ß =

-0.300, p-value = 0.040, R^2 = 0.099), and SOC stock (ß = -3.030, p-value = 0.028, R^2 = 0.069; see Table 19).

Bacteria's relationship with soil carbon and nitrogen

We found no relationships between the relative abundances of bacterial phyla and soil carbon and nitrogen concentrations, nitrate and ammonium concentrations, and nitrification and nitrogen mineralization rates (see Table 20).

Bacteria functionality, however, was found to be related to soil carbon and nitrogen (see Tables 21 & 22). The relative abundances of predicted chemoheterotrophic bacteria and chitinolytic bacteria were positively related to SOC (ß = 15.410, p-value = 0.002 and ß = 70.385, p-value = 0.033, respectively). We found positive relationships between nitrifying bacteria and soil nitrate concentration (ß = 4.055, p-value = 0.065) and between nitrogen-fixing bacteria and total inorganic nitrogen (ß = 10.689, p-value = 0.080).

Fungi's Relationship to soil carbon and nitrogen

The relative abundance of Chytridiomycota was positively related to SOC (ß = 15.494, p-value = 0.048; Table 23) and soil nitrogen mineralization (ß = 0.119, p-value = 0.048). The relative abundance of mortierellomycota was positively correlated with total inorganic nitrogen (ß = 0.579, p-value = 0.002) and soil nitrogen mineralization (ß = 1.735, p-value = 0.004). The relative abundance of Basidiomycota exhibits a negative relationship with both soil nitrogen mineralization (ß = -1.112, p-value = 0.004) and total inorganic nitrogen (ß = -4.063, p-value = $2.388x10^{-4}$).

Symbiotrophs were negatively correlated with total inorganic nitrogen (ß = -3.631, p-value = 9.120E-06; Table 24) and soil nitrogen mineralization (ß = -0.939, p-value = 0.001). Likewise, ectomycorrhizae (ß = -0.915, p-value = 0.001) also exhibited a negative relationship with total inorganic nitrogen (ß = -3.581, p-value = 8.660×10^{-6}) and soil nitrogen mineralization (ß = -0.915, p-value = 0.001).

Discussion

This study aimed to investigate the effects of intraspecific genetic diversity of aspen and stand disturbance via thinning treatment on soil microbial biomass and community composition. We found that bacterial and fungal biomass did not differ between the treatments. Both intraspecific diversity and thinning treatment did not affect bacterial Shannon Diversity, bacterial richness or beta diversity, the relative abundance of major bacterial phyla (with the exception of Verrucomicrobiota) or bacterial functionality. Bacterial evenness, however, was positively related with tree diversity. On the other hand, fungal richness was negatively related to tree diversity. Tree diversity but not thinning treatment also affected the relative abundances of fungal phyla and functionality. Interestingly, we also found that soil carbon and nitrogen were not affected by thinning treatment and aboveground diversity. Instead, certain bacterial and fungal phyla and functional groups were found to be correlated with soil organic carbon and nitrogen. We offer a more detailed exploration and discussion of our findings in subsequent sections.

Soil carbon and nitrogen not affected by tree diversity and thinning treatment

Whereas previous research in our experimental aspen stands documented alterations to aboveground biomass and net primary production due to changes in genotypic diversity in response to stand disturbance and thinning treatments (Cope et al., 2021), we found that soil

carbon and nitrogen stocks did not follow the same trends. Previous biodiversity-manipulation experiments have demonstrated positive relationships between tree diversity and soil carbon and nitrogen accumulation (Chen et al., 2023). The undisturbed aspen populations in our experiment, which were subjected to high intraspecific competition, exhibited wide variability in stand-level genetic diversity and on average were marginally less genetically diverse than the disturbed stands (p-value = 0.078; Figure 1).

Our findings are in line with existing studies on aboveground-belowground diversity relationships that focus on *inter*specific diversity. Despite our study being centered on intraspecific diversity, research has shown that intraspecific variation can influence ecosystem function as much as variation between species (Des Roches et al., 2018). In boreal forests, tree species diversity interacting with tree functional traits and forest biomass with site properties rather than tree species diversity by itself was found to influence soil organic carbon (Augusto and Boča, 2022). Compared to tree species diversity, tree species identity was also found to be a stronger driver of soil properties, including soil C stocks and nutrient status, compared to tree species diversity (Dawud et al., 2016).

Microbial biomass not affected by tree diversity and thinning treatment

Past research highlighted the greater sensitivity of fungi compared to bacteria to disturbance (Dooley and Treseder, 2012). However, despite a 75% reduction in tree density, bacterial and fungal biomass did not differ between the disturbed and undisturbed plots. This finding is supported by a meta-analysis by (Holden and Treseder, 2013), which found that soil fungi are not more sensitive to forest disturbance events than bacteria. Other research shows that effects of disturbance on the soil microbiome vary greatly between disturbance types. For

example, tree cutting and natural gap formation was found to significantly reduce soil microbial biomass in a subtropical forest in India (Arunachalam et al., 1996).

Tree diversity was also not found to drive microbial biomass shifts. This is in line with past studies showing that tree identity and environmental factors exert more influence than tree diversity on microbial biomass in a deciduous forest in Germany (Scheibe et al., 2015) and a pine forest in Spain (Lucas-Borja et al., 2012).

Tree diversity affects fungi and bacteria diversity differently

As discussed above, microbial biomass was less sensitive than microbial diversity to changes in tree diversity. Here, fungi were generally more responsive to changes in tree diversity than bacteria. When assessing alpha diversity, which refers to the richness and evenness of species within a particular area or ecosystem, we find that tree Shannon Diversity affects bacterial evenness but not bacterial Shannon diversity or richness. There is evidence that tree diversity is not always a strong driver of soil microbial diversity, as evidenced in a seven-year-old common garden experiment consisting of temperate and deciduous tree species (Rivest et al., 2019). In a temperate deciduous forest, aboveground diversity only indirectly influenced soil microbial diversity via changes in soil pH and nutrient status or litter quality (Thoms et al., 2010). Likewise, Yamamura et al., 2013 reported that site-related features play a more important role in regulating the structure of bacterial communities within the bulk soil of a tropical tree plantation than aboveground species richness. In our study sites, soil resources or soil resources and tree diversity combined may have a stronger effect on soil microbial diversity than tree species diversity *per se*.

In contrast, we find tree diversity affects fungal richness. These results are supported by tree diversity studies conducted in boreal and temperate regions, which found that soil fungal diversity can be affected by tree richness, although these findings are largely context-dependent and guild-specific (Nguyen et al., 2016b; Tedersoo et al., 2016).

Tree diversity and thinning treatment did not have a significant effect on bacterial or fungal beta diversity, which measures species diversity between different areas, indicating that the microbial composition remained relatively consistent across thinning treatments in the experimental aspen stands. Our results are in line with other research findings, which found that it is actually soil properties, particularly pH, that is responsible for up to 82% of variation in soil bacteria beta diversity (Landesman et al., 2014).

Tree diversity, but not thinning treatment, affects bacteria and fungal phyla relative abundances and fungal functional groups

In the experimental aspen stands, thinning treatment did not affect the relative abundances of bacteria and fungal phyla. Our results are in line with a field experiment on native temperate tallgrass species in Virginia. Researchers found that bacterial relative abundance remained stable after a prescribed burn due to rapid recovery (Kang and Mills, 2004). However, longer-term changes in aboveground biomass, plant composition, and litter quality, such as during deforestation and establishment of agriculture or pasturelands, have been found to result in shifts in microbial community composition (Díaz-Vallejo et al., 2021). The thinning treatments in our study altered competitive interactions, stem mortality, and tree growth (Cope et al., 2021), but there were no large disturbances to the soil structure itself, as occurs during managed land use and land cover changes, which may have buffered microorganisms.

In contrast, tree diversity has an effect on the relative abundance of Verrucomicrobiota, a phylum that has been proposed as a potential indicator of land use impact due to its response to forest-to-pasture conversion (Pajares et al., 2016). We found it to have been negatively correlated with tree diversityThe relative abundances of fungal phyla and functional groups also exhibited various different correlations with tree diversity. This suggests a potential shift in fungal composition and functional roles in response to a shifting aboveground diversity. Chytridiomycota and Mortierellomycota were found to have a negative relationship with tree diversity while Basidiomycota, the most abundant fungal phyla in our plots, had a positive relationship. The relative abundances of ectomycorrhizal fungal groups and symbiotrophs were also positively correlated with tree diversity, which highlights the complementary effects of aboveground-belowground interactions. ECM fungi, which are symbionts, play a crucial role in forest dynamics by connecting different trees with an intricate web of hyphae (Khullar and Reddy, 2019), therefore giving plants greater access to nutrients and water by increasing root surface area (Kramer et al., 2012).

Overall, our findings are in line with Lie et al., 2020. Researchers found variable correlations between plant diversity and microbial diversity among taxonomic and functional groups and spatial scales, not all of which are positive. Of the 176 correlations they analyzed for their meta-analysis, 12% reported negative relationships.

Bacterial and fungal phyla and functional characteristics can explain some of the variations in soil carbon and nitrogen

Our research examined the influence of bacterial and fungal phyla and functional groups on soil carbon and nitrogen. We found that different bacterial and fungal phyla exhibited varying

correlations with soil carbon and nitrogen. Actinobacteria, commonly associated with decomposition processes near the soil surface (Štursová et al., 2012), was negatively correlated with SOC. We attribute this to carbon lost from the soil due to microbial respiration during decomposition. Chloroflexi, one of only two bacterial phyla known to contain nitrite-oxidizing bacteria (Sorokin et al., 2012), was negatively related with SOC, bulk soil nitrogen percent, total inorganic nitrogen, and soil nitrogen mineralization rate. Crenarchaeota and Firmicutes were positively correlated with total inorganic nitrogen and soil nitrogen mineralization rate respectively, which implies that the relative abundances of both phyla may have implications in soil nitrogen availability for plant uptake.

For fungi, Chytridiomycota was positively correlated with SOC and bulk soil nitrogen percent. Ascomycota, a phylum consisting of ectomycorrhizal synthesizing fungi (Charya and Garg, 2019) and Mortierellomycota, a phylum consisting of decomposers, were positively correlated with total inorganic nitrogen, highlighting their roles in the nitrogen cycle. However, Basidiomycota, a phylum also closely associated with ectomycorrhizae (Charya and Garg, 2019), was negatively correlated with total inorganic nitrogen and soil nitrogen mineralization rate. We subsequently also found evidence that ectomycorrhizal fungi and symbiotrophs have negative relationships with total inorganic nitrogen and soil nitrogen mineralization rates. As ectomycorrhizal fungi facilitate the uptake of inorganic nitrogen by plants (Smith and Read, 2010), we hypothesize that there might not be enough nitrogen in the soil in our formerly cultivated site, resulting in the trees forming more symbiotic relationships with ECM. Surface mineral soil N in temperate forests typically range between 0.29% to 0.78% N (Perakis and Sinkhorn, 2011), while the soil N in our study site range between 0.21 to 0.24%.

The abundance of nitrifying bacteria and nitrogen-fixing bacteria increased with soil nitrate concentration and total inorganic nitrogen, respectively. Chemoheterotrophic bacteria increased in relation to SOC.

Bacterial groups related to decomposition and carbon cycling variable relationships with SOC. As chemoheterotrophic bacteria obtain energy and carbon synthesized by other organisms, this indicates fluctuations in the soil carbon pool may influence this bacterial group function (Liang et al., 2019). Chitinolytic bacteria decompose chitin from insects and fungi (Cohen-Kupiec and Chet, 1998). However, cellulolytic bacteria, which decompose cellulose in plant litter (Štursová et al., 2012), were negatively related to soil carbon. As cellulose decomposes relatively quickly, we hypothesize that its decomposition results in carbon loss due to microbial respiration.

Functional characterization of microbial communities is important for understanding effects on ecosystem processes. The use of databases to assign functional traits poses several limitations. The FAPROTAX database, which is widely used to assign ecological traits to bacteria, was primarily developed for marine environments, and thus terrestrial bacteria are underrepresented (Louca et al., 2016). In addition, only less than 25% of the database accounts for soil reference genomes, which may explain why only 19.55% of our bacterial community reads were successfully classified (Djemiel et al., 2022). Meanwhile, a limitation of our fungal classifications using the FunGuild database is our decision not to account for the confidence rankings (“highly probable”, “probable,” and “possible”), provided by the database to represent the likeliness that a taxon belongs to a given guild, so as to maximize sample classification. This adds an additional layer of uncertainty. Overall, the use of functional traits has been revolutionary in providing a basic framework for assessing the ecological roles of microbial

communities. Additional work is needed to expand the databases and improve functional prediction to better link microbial communities with their ecological roles in a variety of ecosystems.

Conclusion

Disturbances of intermediate-level intensity have been known to change the genetic compositions of the experimental aspen stands. Our study provides insights into how disturbance-mediated changes to stand-level genetic diversity influence the relative abundances of bacterial and fungal phyla and functional groups and fungal richness. Bacterial and fungal phyla and functional groups appear to exert influence on soil carbon and nitrogen concentrations whilst tree diversity and thinning treatment do not. These findings significantly improve our understanding of the connections between intraspecific biodiversity and ecosystem function and highlight the complex responses of soil microbes to disturbance-mediated changes to aboveground tree diversity. Our research also provides insights on how future disturbance events or silvicultural management practices may affect microbial community composition in natural aspen forests.

Figures

A

B

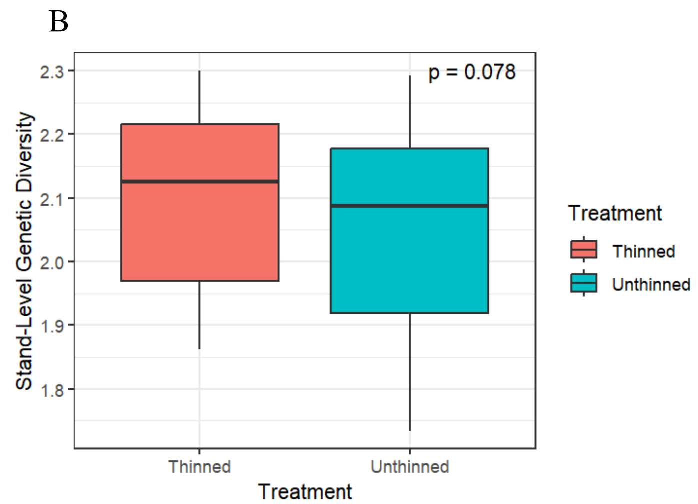


Figure 1. A) Experimental aspen site history and effects of disturbance treatment, B) stand-level genetic diversity (adapted from Shannon's Diversity Index) between disturbance histories since the thinning treatment. P-value in the upper right corner of panel B corresponds to a result from a one-way ANOVA between thinning treatments.

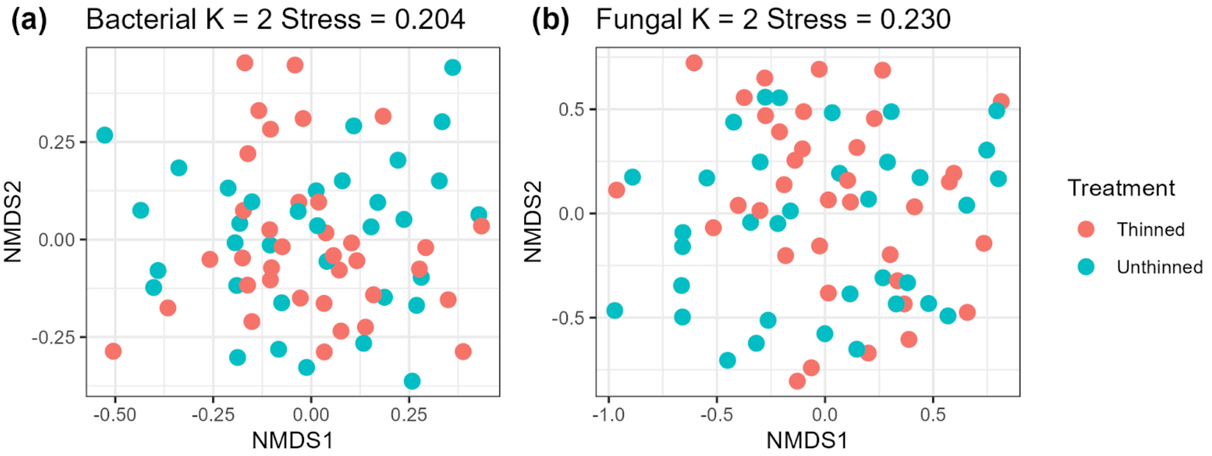


Figure 3. Non-metric scaling analysis for Bacterial and Fungal beta diversity between disturbance histories

Supplementary Figures

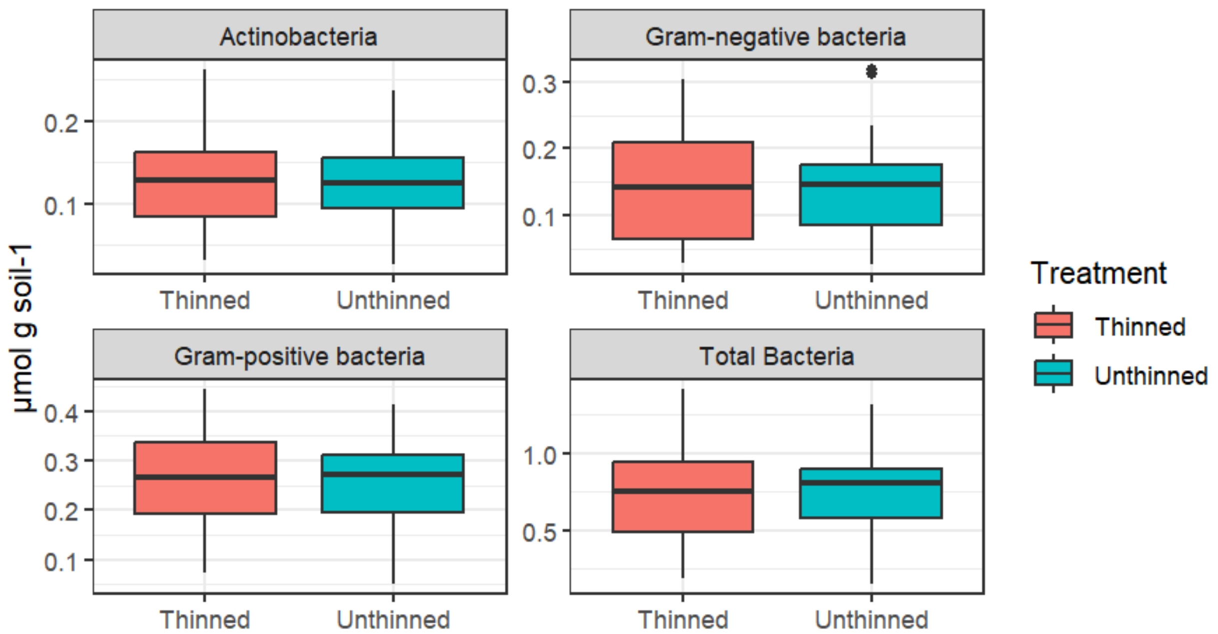
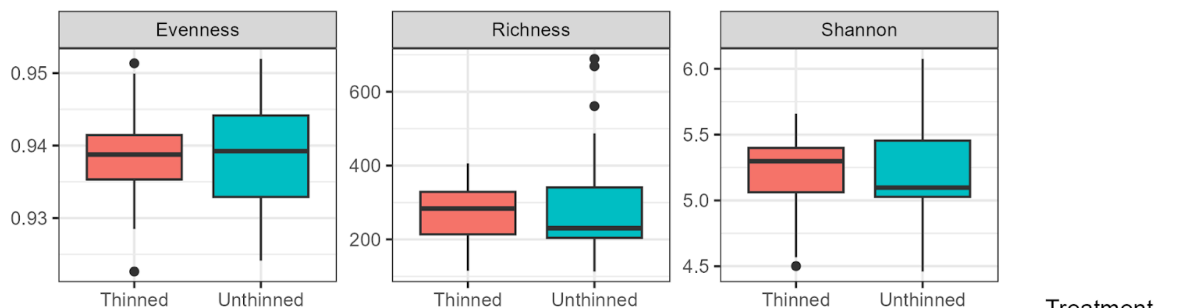


Figure S1. Bacterial biomass between disturbance histories



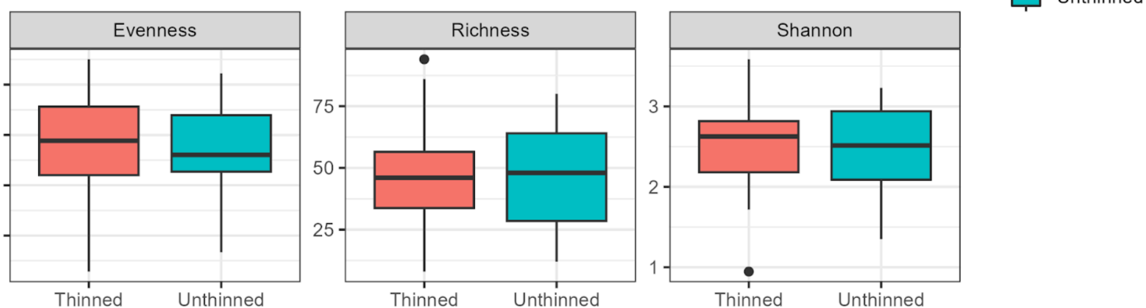


Figure S2. Bacterial and Fungal diversity indexes between disturbance histories

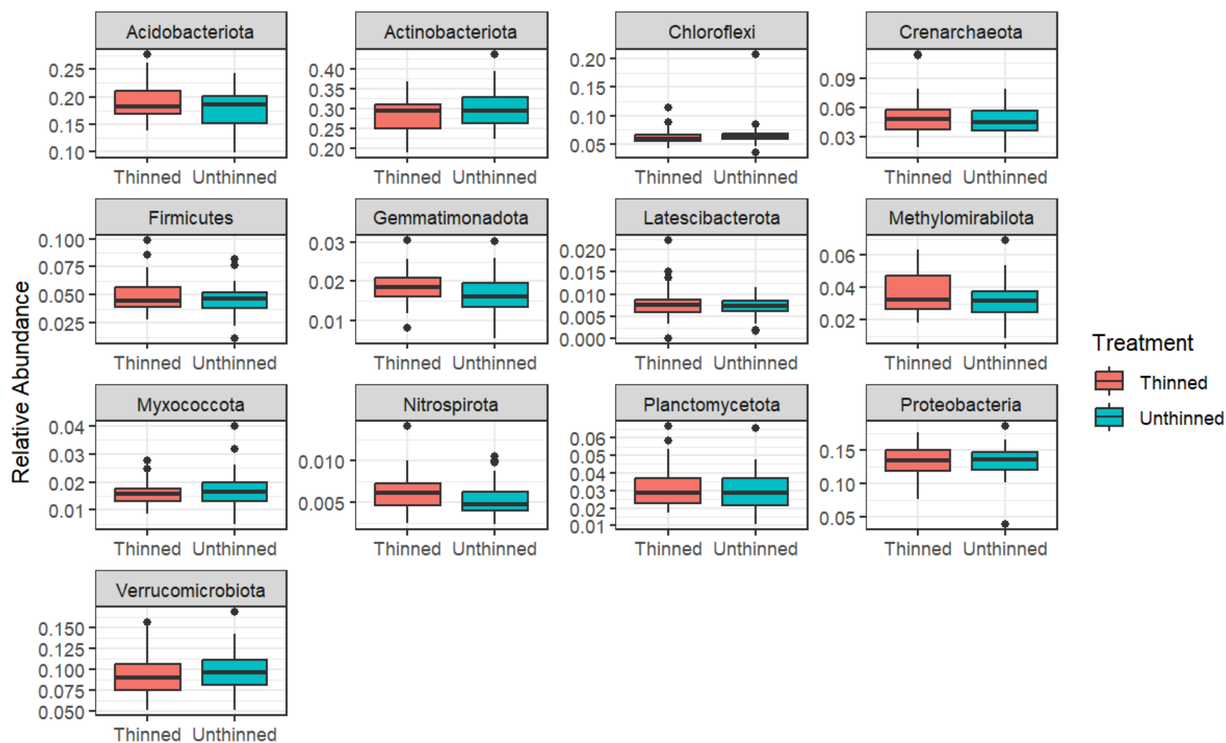


Figure S3. Relative abundances of Bacterial phyla between disturbance histories

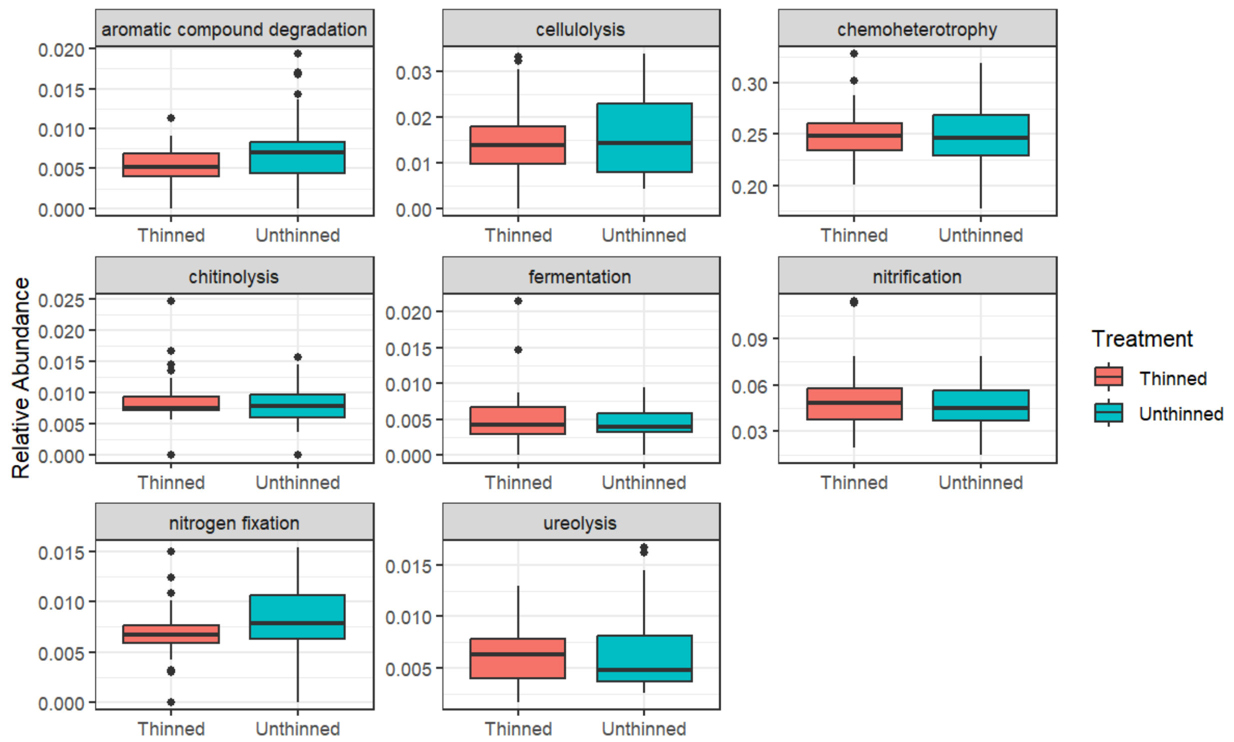


Figure S4. Bacterial functional groups relative abundance between disturbance histories

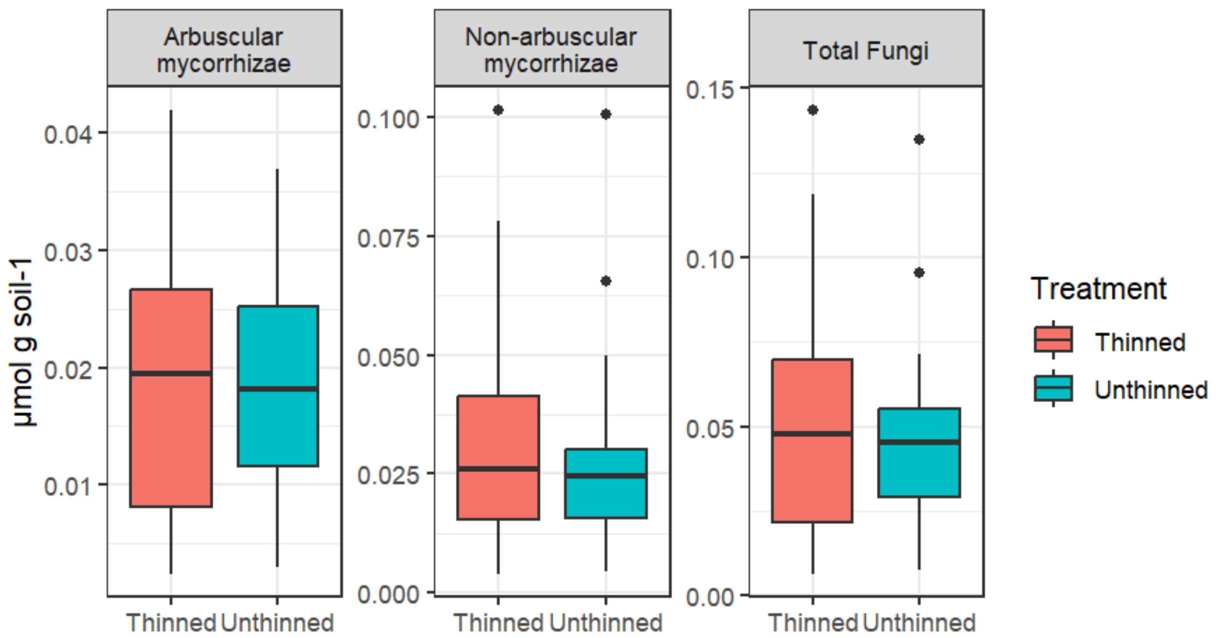


Figure S5. Fungal biomass between disturbance histories

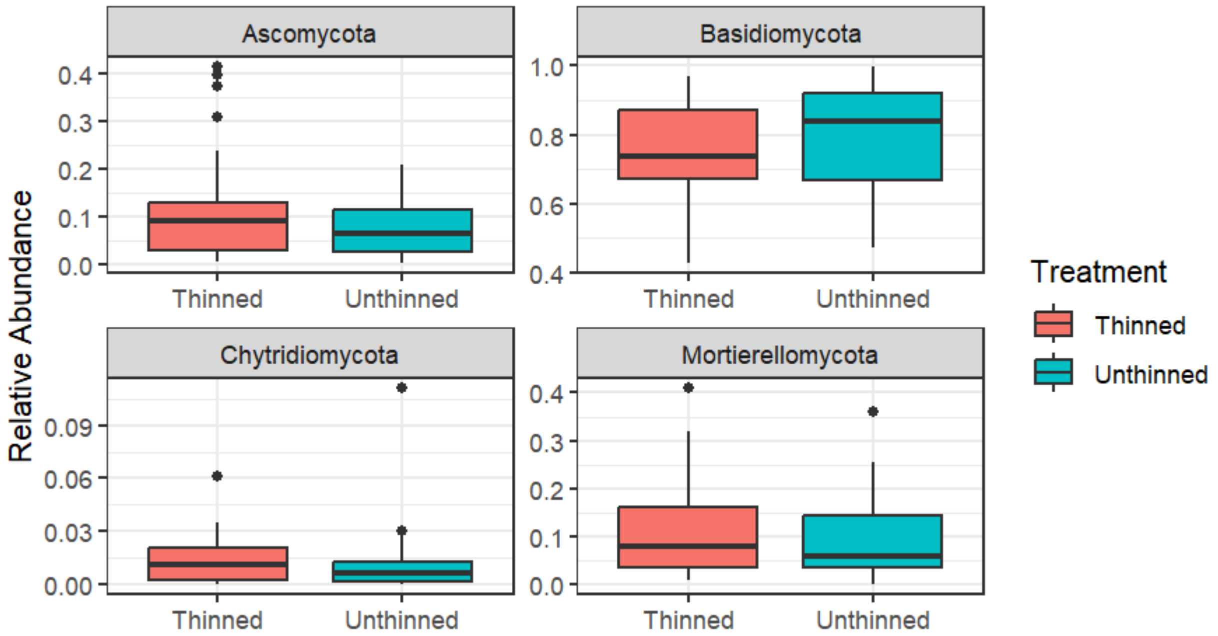


Figure S6. Fungal phyla relative abundances between disturbance histories

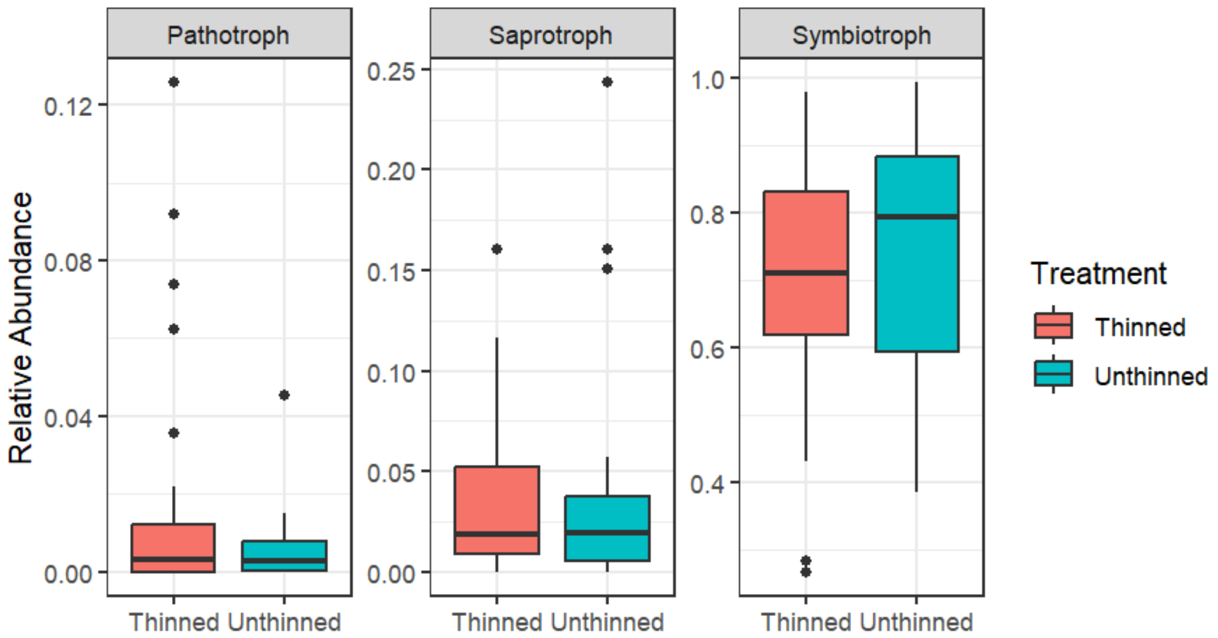


Figure S7. Fungal functional groups relative abundance between disturbance histories

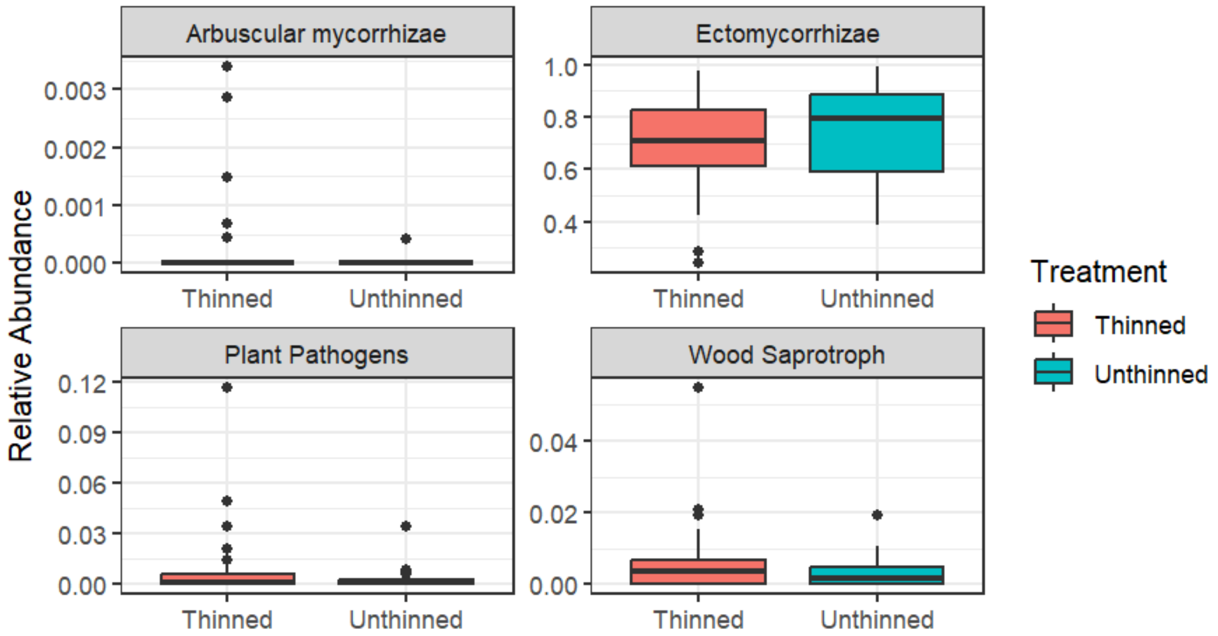


Figure S8. Fungal guild functional groups relative abundance between disturbance histories

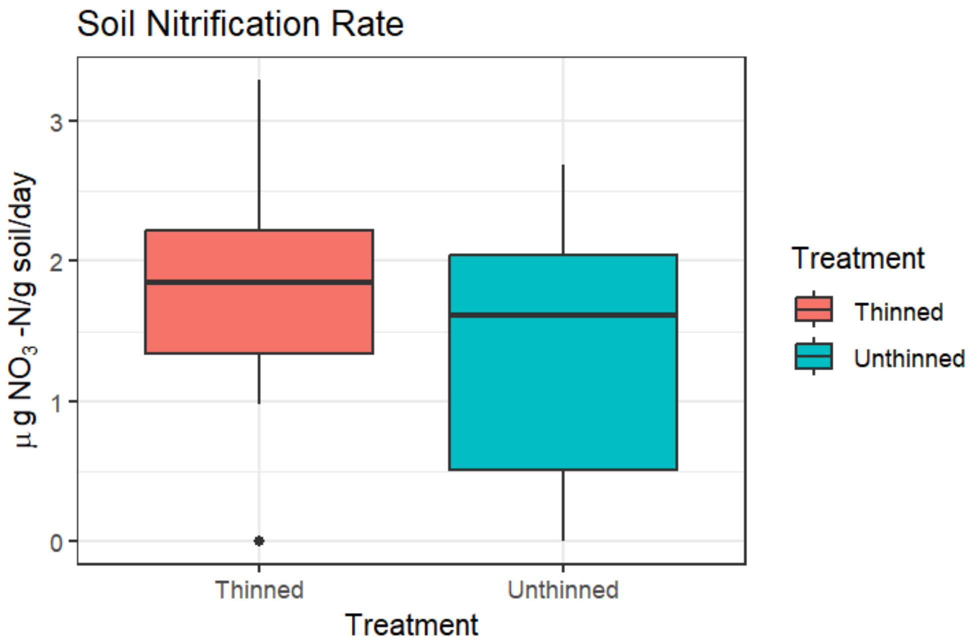


Figure S9. Soil nitrification rate between disturbance histories

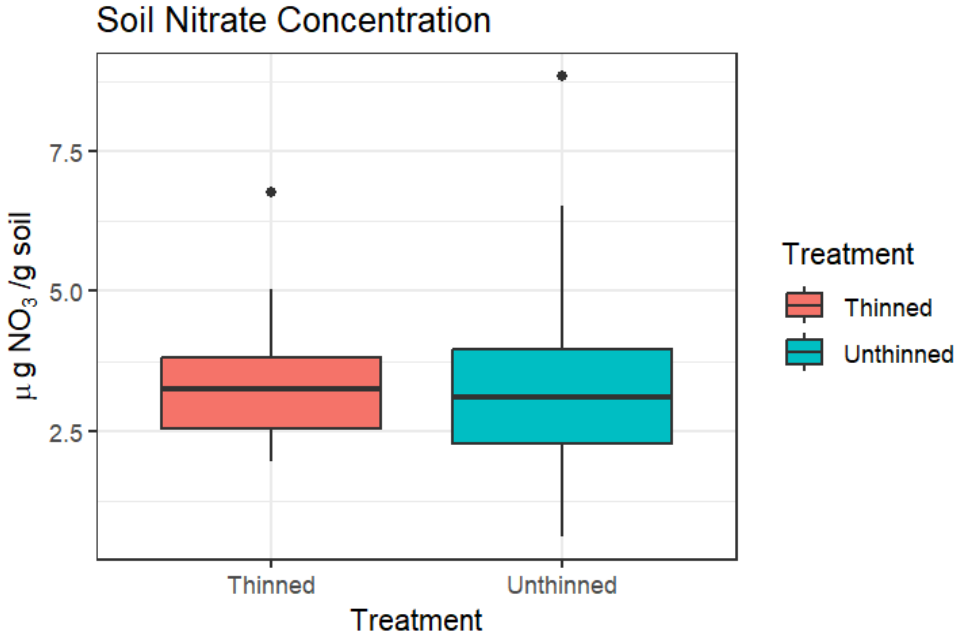


Figure S10. Soil nitrate concentration between disturbance histories

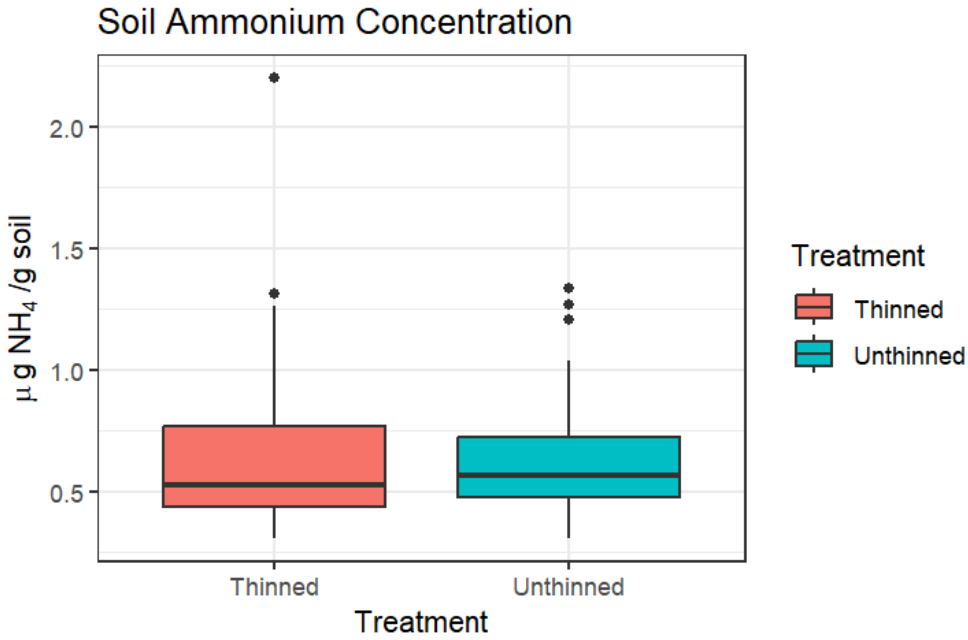


Figure S11. Soil ammonium concentration between disturbance histories

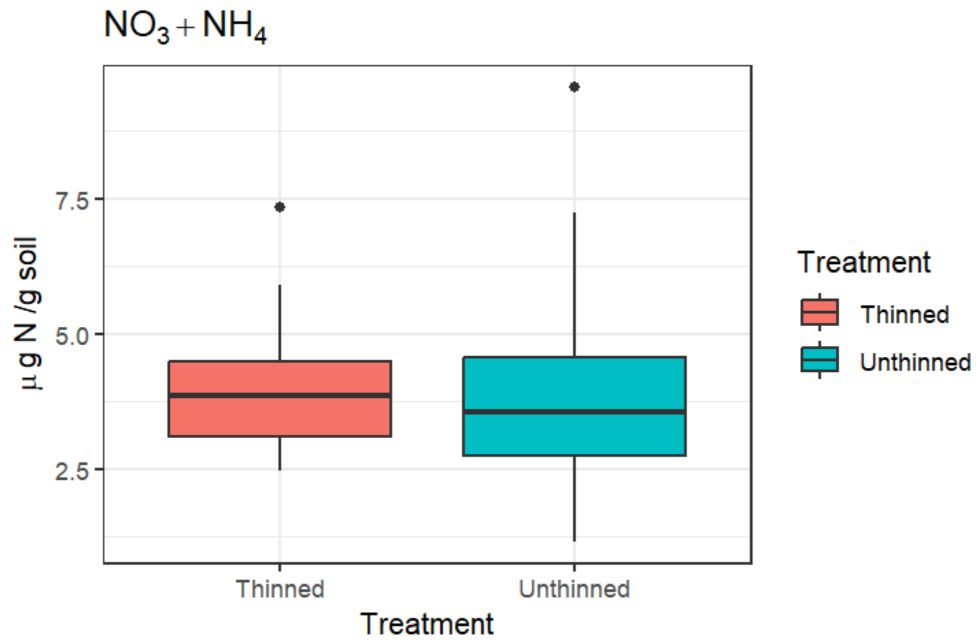


Figure S12. Soil total inorganic nitrogen concentration between disturbance histories

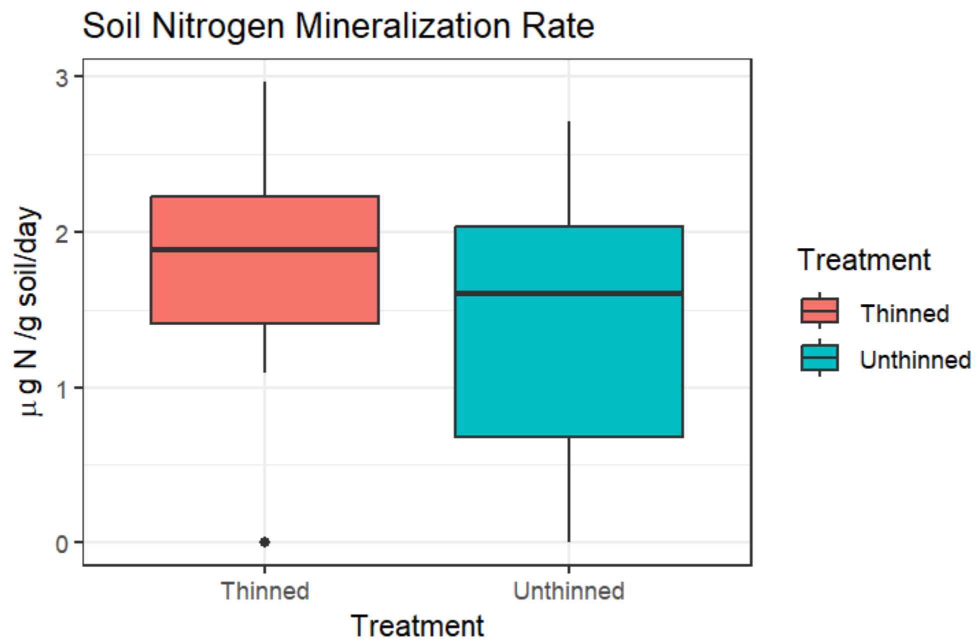


Figure S13. Soil nitrogen mineralization rate between disturbance histories

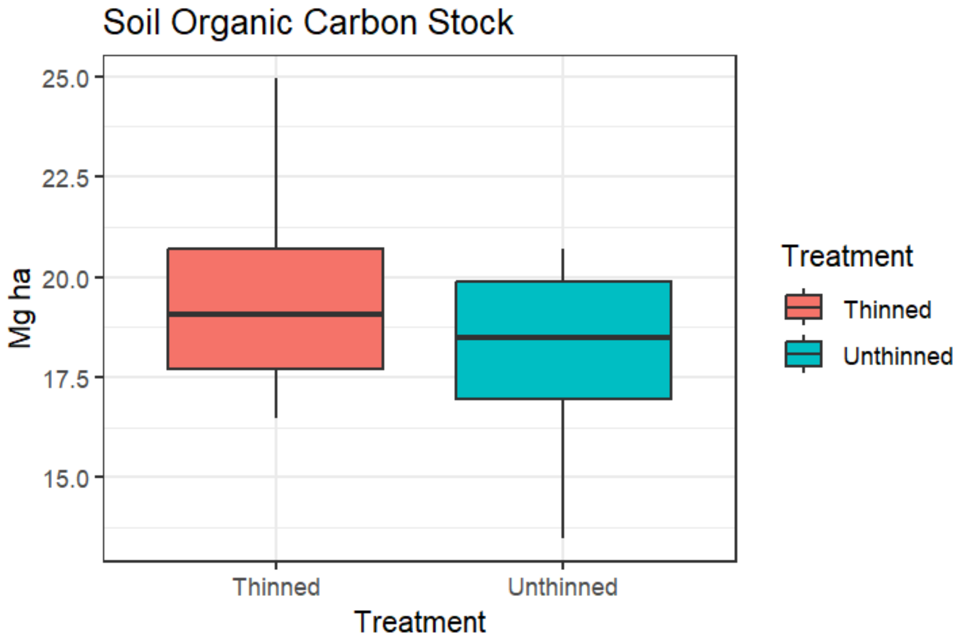


Figure S14. Soil organic carbon stock between disturbance histories

Tables

Table 1. Linear mixed model results for testing if treatment has an effect on Bacterial biomass

		Estimate	SE	t-value	P
Actinobacteria					
	Treatment	-0.004	0.019	-0.239	0.814
Gram-positive bacteria					
	Treatment	-0.005	0.033	-0.144	0.888
Gram-negative bacteria					
	Treatment	-0.010	0.029	-0.347	0.733
Total Bacteria					
	Treatment	-0.001	0.108	-0.008	0.993

Table 2. Simple linear regression results for testing if aboveground Shannon diversity has an effect on Bacterial biomass

		Estimate	SE	t-value	R2	P
Actinobacteria	(Intercept)	-0.027	0.075	-0.366		
	Tree Diversity	0.074	0.036	2.049	0.581	0.444
Gram-positive bacteria	(Intercept)	0.168	0.155	1.083		
	Tree Diversity	0.095	0.074	1.282	0.024	0.204
Gram-negative bacteria	(Intercept)	0.075	0.155	1.083		
	Tree Diversity	0.087	0.074	1.282	0.024	0.204
Total Bacteria	(Intercept)	0.078	0.436	0.095		
	Treatment	0.321	0.209	1.609	0.033	0.130

Table 3. Linear mixed model results for testing differences of Bacterial and Fungal diversity indexes among treatments

			Estimate	SE	t-value	P
Bacteria						
	Richness					
		Treatment	0.004	0.055	0.074	0.942
	Shannon Diversity Index					
		Treatment	0.008	0.110	0.072	0.943
	Shannon Evenness					
		Treatment	-6.019e-05	2.333e-03	-0.026	0.980
Fungi						
	Richness					
		Treatment	0.493	5.895	0.084	0.934
	Shannon Diversity Index					
		Treatment	-0.040	0.145	-0.272	0.789
	Shannon Evenness					
		Treatment	-0.013	0.030	-0.439	0.667

Table 4. PERMANOVA results for Bacterial and Fungal Communities differences among treatments

		df	Sum of Sq	R2	F-value	P
Bacteria						
	Treatment	1	0.114	0.013	1.174	0.238
	Plot	16	3.653	0.415	2.356	**0.001**
	Residual	52	5.039	0.572		
	Total	69	8.806	1		
Fungi						
	Treatment	1	0.403	0.021	1.5135	0.109
	Plot	16	6.325	0.337	1.7417	**0.001**
	Residual	53	12.030	0.641		
	Total	70	18.758	1		

Table 5. Betadisper results from Bacterial and Fungal Communities differences among treatments

		df	Sum of Sq	Mean Sq	F-value	N.Perm	P
Bacteria							
	Treatment	1	0.003	0.003	1.277	999	0.246
	Residuals	68	0.161	0.002			
Fungi							
	Treatment	1	0.002	0.002	0.349	999	0.562
	Residuals	69	0.315	0.005			

Table 6. Simple linear regression results for testing if tree diversity has an effect on Bacterial and Fungal diversity indexes

			Estimate	SE	t-value	R2	P
Bacteria							
	Richness	(Intercept)	2.698	0.244	11.054		
		Tree Diversity	-0.137	0.117	-1.166	0.020	0.248
	Shannon Diversity Index	(Intercept)	5.721	0.503	11.370		
		Tree Diversity	-0.243	0.242	-1.007	0.015	0.318
	Shannon Evenness	(Intercept)	0.920	0.010	98.823		
		Tree Diversity	0.009	0.005	1.907	0.051	**0.061**
Fungi							
	Richness	(Intercept)	118.300	27.950	4.233		
		Tree Diversity	-34.460	13.400	-2.572	0.087	**0.012**
	Shannon Diversity Index	(Intercept)	3.550	0.749	4.743		
		Tree Diversity	-0.503	0.359	-1.402	0.028	0.165
	Shannon Evenness	(Intercept)	0.611	0.139	4.399		
		Tree Diversity	0.028	0.067	0.420	0.003	0.676

Table 7. Bacteria and Fungal relative abundances

	Phylum	Relative abundance (%)
Bacteria	Actinobacteriota	29.321
	Acidobacteriota	18.250
	Proteobacteria	13.311
	Verrucomicrobiota	9.531
	Chloroflexi	6.496
	Crenarchaeota	4.935
	Firmicutes	4.710
	Methylomirobilota	3.429
	Planctonycetota	3.089
	Gemmatimonadota	1.751
	Myxococcota	1.614
	Latescibacterota	0.757
	Nitrospirota	0.577
	Armatimonadota	0.530
	Desulfobacterota	0.345
	Bacteroidota	0.344
	Unknown	0.228
	MBNT15	0.210
	NB1-j	0.198
	Entotheonellaeota	0.153
	RCP2-54	0.091
	Bdellovibrionota	0.063
	Sumerlaeota	0.027
	Patescibacteria	0.023
	Dependentiae	0.007
	Elusimicrobiota	0.005
	FCPU426	0.002
	Cyanobacteria	0.001
	GAL15	0.001
Fungi	Basidiomycota	77.312
	Mortierellomycota	10.069
	Ascomycota	9.371
	Chytridiomycota	1.247
	Fungi_phy_Incertae_sedis	0.966
	Aphelidiomycota	0.315
	Rozellomycota	0.277
	Kickxellomycota	0.136
	Calcarisporiellomycota	0.126
	Blastocladiomycota	0.086
	Mucoromycota	0.040
	Unknown	0.022
	Glomeromycota	0.013
	Basidiobolomycota	0.011
	Zoopagomycota	0.007

Table 8. Linear mixed model results for testing if treatment has an effect on Bacterial phyla relative abundances

		Estimate	SE	t-value	P	Padj
Actinobacteriota						
	Treatment	0.019	0.012	1.546	0.127	0.738
Acidobacteriota						
	Treatment	-0.014	0.018	-0.807	0.431	0.667
Proteobacteria						
	Treatment	8.958e-04	1.006e-02	0.089	0.930	0.738
Verrucomicrobiota						
	Treatment	0.007	0.014	0.498	0.625	0.667
Chloroflexi						
	Treatment	0.011	0.012	0.895	0.392	0.961
Crenarchaeota						
	Treatment	-0.003	0.006	-0.524	0.607	0.739
Firmicutes						
	Treatment	-0.011	0.013	-0.876	0.395	0.738
Methylomirobilota						
	Treatment	-0.005	0.005	-0.995	0.335	0.738
Planctonycetota						
	Treatment	-0.005	0.008	-0.634	0.528	0.961
Gemmatimonadota						
	Treatment	-0.002	0.001	-1.497	0.154	0.739
Myxococcota						
	Treatment	2.924e-04	5.899e-03	0.050	0.961	0.739
Latescibacterota						
	Treatment	-0.001	0.001	-0.767	0.454	0.738
Nitrospirota						
	Treatment	-0.006	0.003	-1.927	**0.058**	0.667

Table 9. Simple linear regression results for testing if aboveground Shannon diversity has an effect on Bacterial phyla relative abundances

		Estimate	SE	t-value	R2	P	Padj
Actinobacteriota	(Intercept)	0.331	0.969	4.788			
	Tree Diversity	-0.018	0.033	-0.544	0.004	0.588	0.850
Acidobacteriota	(Intercept)	0.123	0.051	2.416			
	Tree Diversity	0.029	0.024	1.168	0.020	0.247	0.642
Proteobacteria	(Intercept)	0.128	0.036	3.553			
	Tree Diversity	0.002	0.017	0.132	0.000	0.895	0.989
Verrucomicrobiota	(Intercept)	0.205	0.035	5.857			
	Tree Diversity	-0.053	0.017	-3.138	0.127	**0.003**	**0.033**
Chloroflexi	(Intercept)	0.165	0.051	3.246			
	Tree Diversity	0.044	0.024	1.804	0.046	**0.076**	0.492
Crenarchaeota	(Intercept)	0.174	0.058	2.997			
	Tree Diversity	0.023	0.028	0.806	0.009	0.423	0.825
Firmicutes	(Intercept)	0.285	0.050	5.686			
	Tree Diversity	-0.033	0.024	-1.374	0.027	0.174	0.642
Methylomirobilota	(Intercept)	0.020	0.019	1.068			
	Tree Diversity	0.007	0.009	0.769	0.009	0.444	0.825
Planctonycetota	(Intercept)	0.115	0.048	2.374			
	Tree Diversity	0.028	0.023	1.224	0.022	0.225	0.642
Gemmatimonadota	(Intercept)	0.017	0.007	2.323			
	Tree Diversity	0.000	0.004	0.013	0.000	0.989	0.989
Myxococcota	(Intercept)	0.123	0.032	3.796			
	Tree Diversity	0.001	0.016	0.095	0.000	0.924	0.989
Latescibacterota	(Intercept)	0.101	0.029	3.511			
	Tree Diversity	-0.008	0.014	-0.565	0.005	0.574	0.850
Nitrospirota	(Intercept)	0.079	0.021	3.731			
	Tree Diversity	-0.002	0.010	-0.185	0.001	0.854	0.989

Table 10. Linear mixed model results for testing if treatment has an effect on Fungal biomass

		Estimate	SE	t-value	P
AMF					
	Treatment	0.001	0.015	0.069	0.946
Non-AMF					
	Treatment	-0.043	0.107	-0.407	0.689
Total Fungi					
	Treatment	-0.007	0.023	-0.303	0.766

Table 11. Simple linear regression results for testing if aboveground Shannon diversity has an effect on Fungal biomass

		Estimate	SE	t-value	R2	P
AMF	(Intercept)	0.067	0.060	1.112		
	Tree Diversity	0.031	0.029	1.068	0.016	0.289
Non-AMF	(Intercept)	-2.253	0.444	-5.073		
	Tree Diversity	0.300	0.213	1.409	0.028	0.163
Total Fungi	(Intercept)	0.088	0.095	0.926		
	Tree Diversity	0.058	0.046	1.276	0.023	0.206

Table 12. Linear mixed model results for testing if treatment has an effect on Fungal phyla relative abundances

		Estimate	SE	t-value	P
Basidiomycota					
	Treatment	0.074	0.055	1.341	0.199
Mortierellomycota					
	Treatment	-0.036	0.045	-0.800	0.436
Ascomycota					
	Treatment	-0.048	0.035	-1.376	0.188
Chytridiomycota					
	Treatment	-0.013	0.016	-0.808	0.431

Table 12. Simple linear regression results for testing if aboveground Shannon diversity has an effect on Fungal phyla relative abundances

		Estimate	SE	t-value	R2	P	Padj
Basidiomycota	(Intercept)	0.482	0.263	1.837			
	Tree Diversity	0.296	0.126	2.353	0.074	**0.021**	**0.029**
Mortierellomycota	(Intercept)	0.857	0.206	4.167			
	Tree Diversity	-0.271	0.099	-2.749	0.099	**0.008**	**0.025**
Ascomycota	(Intercept)	0.329	0.207	1.588			
	Tree Diversity	-0.022	0.099	-0.220	0.001	0.827	0.827
Chytridiomycota	(Intercept)	0.321	0.089	3.605			
	Tree Diversity	-0.110	0.043	-2.568	0.087	**0.012**	**0.025**

Table 13. Bacteria and Fungal functional groups' relative abundances

	Phylum	Relative abundance (%)
Bacteria	Chemoheterotrophy	24.97
	Nitrification	4.94
	Cellulolysis	1.52
	Chitinolysis	0.83
	Nitrogen fixation	0.76
	Ureolysis	0.64
	Aromatic compound degradation	0.62
	Fermentation	0.48
Fungi	Symbiotroph	71.98
	Ectomycorrhizae	71.57
	Saptrotroph	3.52
	Pathotroph	1.01
	Plant Pathogens	0.55
	Wood Saprotroph	0.45
	Arbuscular mycorrhizae	0.01

Table 14. Linear mixed model results for testing if treatment has an effect on Bacterial functional groups

		Estimate	SE	t-value	P
Chemoheterotrophy					
	Treatment	7.346e-04	1.041e-02	0.071	0.945
Nitrification					
	Treatment	-0.006	0.014	-0.474	0.642
Cellulolysis					
	Treatment	0.004	0.013	0.309	0.761
Chitinolysis					
	Treatment	-0.001	0.001	-0.994	0.377
Nitrogen fixation					
	Treatment	1.368e-03	1.138e-03	1.202	0.248
Ureolysis					
	Treatment	-0.000	0.005	-0.090	0.930
Aromatic compound degradation					
	Treatment	1.832e-03	1.184e-03	1.547	0.143
Fermentation					
	Treatment	-0.003	0.006	-0.603	0.556

Table 15. Simple linear regression results for testing if aboveground Shannon diversity has an effect on Bacterial functional groups

		Estimate	SE	t-value	R2	P	Padj
Chemoheterotrophy	(Intercept)	0.191	0.042	4.572			
	Tree Diversity	0.028	0.020	1.413	0.029	0.162	0.324
Nitrification	(Intercept)	0.174	0.058	2.997			
	Tree Diversity	0.023	0.028	0.806	0.009	0.423	0.564
Cellulolysis	(Intercept)	0.220	0.049	4.462			
	Tree Diversity	-0.049	0.024	-2.056	0.059	**0.044**	0.295
Chitinolysis	(Intercept)	0.015	0.005	2.683			
	Tree Diversity	-0.003	0.003	-1.149	0.019	0.254	0.407
Nitrogen fixation	(Intercept)	0.015	0.004	3.397			
	Tree Diversity	-0.004	0.002	-1.687	0.040	0.096	0.295
Ureolysis	(Intercept)	0.071	0.030	2.392			
	Tree Diversity	0.003	0.014	0.232	0.001	0.817	0.837
Aromatic compound degradation	(Intercept)	0.005	0.005	0.950			
	Tree Diversity	0.001	0.003	0.206	0.001	0.837	0.837
Fermentation	(Intercept)	0.116	0.031	3.742			
	Tree Diversity	-0.024	0.015	-1.617	0.037	0.111	0.295

Table 16. Linear mixed model results for testing if treatment has an effect on Fungal functional groups

		Estimate	SE	t-value	P
Symbiotroph					
	Treatment	0.057	0.071	0.798	0.437
Ectomycorrhizae					
	Treatment	0.059	0.072	0.820	0.424

Table 17. Simple linear regression results for testing if aboveground Shannon diversity has an effect on Fungal functional groups

		Estimate	SE	t-value	R2	P
Symbiotroph	(Intercept)	0.092	0.304	0.304		
	Tree Diversity	0.455	0.146	3.121	0.124	**0.003**
Ectomycorrhizae	(Intercept)	0.075	0.309	0.244		
	Tree Diversity	0.461	0.148	3.114	0.123	**0.003**

Table 18. Linear mixed model results for testing if treatment affects carbon and nitrogen cycling

			Estimate	SE	t-value	P
Nitrogen						
	NO_3^- rate					
		Treatment	-0.500	0.295	-1.697	0.109
	NO_3^- concentration					
		Treatment	-0.089	0.158	-0.561	0.583
	NH_4^+ concentration					
		Treatment	-0.025	0.101	-0.244	0.810
	NO_3^- + NH_4^+					
		Treatment	-0.052	0.071	-0.741	0.471
	N mineralization rate					
		Treatment	-0.192	0.902	-0.213	0.834
Carbon						
	SOC stock Mg ha					
		Treatment	-1.311	0.9324	-1.406	0.179

Table 19. Simple linear regression results for testing if tree diversity affects carbon and nitrogen cycling

			Estimate	SE	t-value	R2	P	Padj
Nitrogen								
	NO_3^- rate	(Intercept)	2.263	1.270	1.782			
		Tree Diversity	-0.326	0.610	-0.534	0.004	0.595	0.744
	NO_3^- concentration	(Intercept)	3.053	0.525	5.818			
		Tree Diversity	-0.619	0.252	-2.454	0.081	**0.017**	**0.043**
	NH_4^+ concentration	(Intercept)	0.095	0.254	0.373			
		Tree Diversity	-0.154	0.122	-1.261	0.023	0.212	0.353
	NO_3^- + NH_4^+	(Intercept)	1.187	0.229	5.192			
		Tree Diversity	-0.300	0.110	-2.733	0.099	**0.008**	**0.040**
	N mineralization rate	(Intercept)	1.929	1.229	1.570			
		Tree Diversity	-0.148	0.591	-0.250	0.001	0.803	0.803
Carbon		(Intercept)	25.057	2.818	8.893			
	SOC stock Mg ha							
		Tree Diversity	-3.030	1.353	-2.239	0.069	**0.028**	

Table 20. Linear mixed model results for testing if Bacterial phyla has an effect on carbon and nitrogen

		Estimate	SE	t-value	P	Padj
SOC stock Mg ha						
	Acidobacteria	6.018	4.097	1.469	0.147	0.478
	Actinobacteriota	1.874	2.206	0.849	0.400	0.664
	Chloroflexi	-13.304	5.758	-2.311	**0.025**	0.241
	Crenarchaeota	4.687	6.882	0.681	0.499	0.664
	Firmicutes	7.719	8.582	0.899	0.372	0.664
	Gemmatimonadota	-37.951	21.759	-1.744	0.086	0.373
	Latescibacterota	-22.057	36.105	-0.611	0.544	0.664
	Methylomirobilota	-4.696	10.461	-0.449	0.655	0.664
	Myxococcota	-11.232	19.104	-0.588	0.559	0.664
	Nitrospirota	27.294	46.894	0.582	0.563	0.664
	Planctonycetota	-3.769	8.640	-0.436	0.664	0.664
	Proteobacteria	4.895	5.788	0.846	0.401	0.664
	Verrucomicrobiota	-9.573	4.482	-2.136	**0.037**	0.241
Soil N%						
	Acidobacteria	0.065	0.038	1.722	0.090	0.293
	Actinobacteriota	0.017	0.021	0.752	0.455	0.643
	Chloroflexi	-0.119	0.053	-2.237	**0.029**	0.189
	Crenarchaeota	0.073	0.064	1.151	0.255	0.643
	Firmicutes	0.051	0.080	0.629	0.532	0.643
	Gemmatimonadota	-0.403	0.205	-1.967	0.054	0.234
	Latescibacterota	-0.232	0.340	-0.682	0.498	0.643
	Methylomirobilota	-0.064	0.097	-0.658	0.513	0.643
	Myxococcota	-0.083	0.180	-0.463	0.645	0.699
	Nitrospirota	0.317	0.441	0.719	0.475	0.643
	Planctonycetota	-0.008	0.081	-0.101	0.920	0.920
	Proteobacteria	0.033	0.054	0.610	0.544	0.643
	Verrucomicrobiota	-0.104	0.042	-2.497	**0.015**	0.189
$NO_3 + NH_4$						
	Acidobacteria	0.211	0.563	0.375	0.709	0.768
	Actinobacteriota	-0.567	0.311	-1.825	0.074	0.176
	Chloroflexi	-1.698	0.798	-2.127	**0.037**	0.176
	Crenarchaeota	2.089	0.937	2.230	**0.029**	0.176
	Firmicutes	1.635	1.198	1.365	0.177	0.288
	Gemmatimonadota	-0.863	3.198	-0.270	0.788	0.788
	Latescibacterota	8.930	5.029	1.775	0.081	0.176
	Methylomirobilota	-2.247	1.432	-1.569	0.121	0.225
	Myxococcota	4.850	2.727	1.779	0.081	0.176
	Nitrospirota	7.158	6.755	1.060	0.294	0.382
	Planctonycetota	2.299	1.193	1.927	0.059	0.176
	Proteobacteria	1.002	0.779	1.287	0.203	0.293
	Verrucomicrobiota	0.063	0.664	0.395	0.694	0.768
N mineralization rate						
	Acidobacteria	4.082	3.046	1.340	0.185	0.418
	Actinobacteria	-4.012	1.787	-2.250	**0.028**	0.182
	Chloroflexi	-8.238	4.412	-1.867	**0.066**	0.286
	Crenarchaeota	7.105	5.408	1.314	0.193	0.418
	Firmicutes	17.546	6.369	2.755	**0.008**	0.104
	Gemmatimonadota	-12.951	18.249	-0.710	0.480	0.567
	Latescibacterota	29.271	29.312	0.999	0.322	0.567
	Methylomirobilota	-4.815	8.004	-0.602	0.549	0.595
	Myxococcota	13.203	15.881	0.831	0.409	0.567
	Nitrospirota	-27.940	39.081	-0.715	0.477	0.567
	Planctonycetota	6.772	7.183	0.943	0.350	0.567
	Proteobacteria	0.526	4.285	0.123	0.903	0.903
	Verrucomicrobiota	5.248	3.717	1.412	0.163	0.418

Table 21. Linear mixed model results for testing if Bacterial functional groups relative abundance has an effect on carbon cycling

		Estimate	SE	t-value	P	Padj
SOC stock Mg ha						
	Aromatic compound degradation	24.011	33.734	0.712	0.480	0.480
	Cellulolysis	-0.709	0.369	-1.920	**0.060**	0.100
	Chemoheterotrophy	15.410	4.063	3.793	**0.000**	**0.002**
	Chitinolysis	70.385	27.460	2.563	**0.013**	**0.033**
	Fermentation	25.317	34.648	0.731	0.468	0.480

Table 22. Linear mixed model results for testing if Bacterial functional groups relative abundance has an effect on nitrogen cycling

		Estimate	SE	t-value	P
NO_3^- rate					
	Nitrification	7.863	5.554	1.416	0.162
	N fixation				
	Ureolysis				
NO_3^- concentration					
	Nitrification	4.055	2.161	1.876	**0.065**
	N fixation				
	Ureolysis				
NH_4^+ concentration					
	Nitrification				
	N fixation	3.487	7.263	0.480	0.633
	Ureolysis	-4.362	5.846	-0.746	0.458
NO_3^- + NH_4^+					
	Nitrification				
	N fixation	10.689	6.008	1.779	**0.080**
	Ureolysis				

Table 23. Linear mixed model results for testing if Fungal phyla has an effect on carbon and nitrogen

		Estimate	SE	t-value	P	Padj
SOC stock Mg ha						
	Ascomycota	0.316	1.199	0.264	0.793	0.793
	Basidiomycota	-0.917	0.787	-1.166	0.249	0.498
	Chytridiomycota	15.494	5.983	2.590	**0.012**	**0.048**
	Mortierellomycota	1.056	1.253	0.843	0.403	0.537
Soil N%						
	Ascomycota	0.002	0.012	0.139	0.890	0.890
	Basidiomycota	-0.008	0.008	-1.104	0.274	0.511
	Chytridiomycota	0.119	0.059	2.027	**0.048**	0.192
	Mortierellomycota	0.011	0.012	0.880	0.383	0.511
$NO_3^- + NH_4^+$						
	Ascomycota	3.158	1.558	2.027	**0.047**	**0.063**
	Basidiomycota	-4.063	0.946	-4.295	**5.970×10^{-5}**	**2.388×10^{-4}**
	Chytridiomycota	1.320	0.904	1.460	0.150	0.15
	Mortierellomycota	0.579	0.169	3.429	**0.001**	**0.002**
Soil N mineralization						
	Ascomycota	0.667	0.967	0.690	0.493	0.493
	Basidiomycota	-1.112	0.336	-3.312	**0.002**	**0.004**
	Chytridiomycota	5.746	2.849	2.017	**0.048**	**0.064**
	Mortierellomycota	1.735	0.534	3.247	**0.002**	**0.004**

Table 24. Linear mixed model results for testing if Fungal functional groups relative abundance has an effect on carbon and nitrogen cycling

		Estimate	SE	t-value	P
SOC stock Mg ha					
	Symbiotroph	-0.642	0.648	-0.991	0.326
	Ectomycorrhizae	-0.657	0.638	-1.030	0.308
Soil N%					
	Symbiotroph	-0.006	0.006	-0.994	0.324
	Ectomycorrhizae	-0.006	0.006	-1.015	0.314
$NO_3^- + NH_4^+$					
	Symbiotroph	-3.631	0.756	-4.806	**9.120×10^{-6}**
	Ectomycorrhizae	-3.581	0.743	-4.819	**8.660×10^{-5}**
N mineralization rate					
	Symbiotroph	-0.939	0.271	-3.466	**0.001**
	Ectomycorrhizae	-0.915	0.267	-3.423	**0.001**

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