DETERMINING THE ROLE OF PLANT-SOIL-MICROBIAL RELATIONSHIPS IN FOREST ECOSYSTEM FUNCTION AND BIOGEOCHEMISTRY

BY

JOSEPH D. EDWARDS

DISSERTATION

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Doctoral Committee:

Associate Professor Wendy Yang, Chair and Director of Research
Associate Professor Anthony Yannarell
Professor James Dalling
Professor Adam Davis

ABSTRACT

Plant-soil-microbial relationships are an essential component of ecosystem function; however, they remain critically underexplored relative to aboveground aspects of ecosystems. In my dissertation, I seek to better understand how interactions among plants, microbial communities, and their environment drive surrounding soil microbiome composition and biogeochemical processes in forests. This work integrates important global change factors like invasive species and increased anthropogenic nutrient deposition while taking a global perspective, spanning temperate and tropical ecosystems. In my first and second chapters, I show how the invasive species *Alliaria petiolata* (garlic mustard) has a dynamic relationship with soil microbial communities and nitrogen cycling processes over space and time. In my third chapter, I provide evidence for the important role fungal communities play in acquiring nitrogen from soil organic matter in a montane rainforest of Panama, and how this function is diminished under elevated nitrogen deposition. Finally, a focal component of my research is how the diversity of mycorrhizal relationships between plants and fungi influences their impact on ecosystems. I explore this in-depth in my last chapter, presenting a study investigating how ectomycorrhizal tree species and fungal communities differ in their associated effects on important ecosystem traits like soil organic matter dynamics and extracellular enzyme activities. Overall, my dissertation supports the inclusion of microbial communities as critical independent players in ecological phenomena, promoting the use of microbially-explicit paradigms and models in informing conservation decision-making and predicting ecosystem response to global change. My dissertation shows that the nature of plant-soil-microbial relationships is highly context-dependent, varying based on environmental conditions, temporal factors, and species diversity.

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Dedicated to Athena, this one's for you kid.

Dedicated to my Mama, for not eating me when you had the chance.

Dedicated to Mackensie, I know it's been hard, but thanks for everything.

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CHAPTER 1: INTRODUCTION

Forests are sustained through interconnected relationships between plants, microbes, and soils (Binkley and Fisher 2019). However, there remain critical gaps in our understanding of how these relationships manifest and their implications for ecosystems function in a changing world (Van der Putten et al. 2013). Significant context dependency in the outcome of plant-microbe relationships (Hoeksema et al. 2010) signifies a need for elucidating the factors that affect how plant, microbial communities, and their environment interact. Therefore, it is important to inform an improved mechanistic understanding of how plants and soil microbiomes interact with global change factors like invasive species (Reinhart and Callaway 2006), anthropogenic nutrient deposition (Wei et al. 2013), and biodiversity loss (Schnitzer et al. 2011) and how these interactions will affect ecosystem function (van der Putten et al. 2016).

Invasive plant species are often thought to gain an advantage over native species through novel interactions with soil microbial communities in their non-native ecosystem (van der Putten 2010). These interactions can form through direct interactions with symbiotic microbes, like pathogens (Kardol et al. 2007) and mutualists (Pringle et al. 2009a), or through influencing the effect microbes are having on ecosystems overall, such as by promoting microbial decomposers to affect nutrient cycling (Zhang et al. 2019). However, there remain significant constraints to generalizing the impacts of invasive species on ecosystems (Hulme et al. 2013) potentially due to context-dependent interactions with soil microbiomes based on seasonal and geographical differences. In my second and third chapters, I will address these issues by observing the effects of the invasive forest herb *Alliaria petiolata* (garlic mustard, Cavara and Grande) on soil

microbial communities and nutrient cycling processes at different phenological stages over its life history and over various spatial scales.

Garlic mustard is a prominent invasive forest herb in North America (Rodgers et al. 2008a), and it has been proposed that part of garlic mustard's invasion success is due to novel interactions with soil microbiomes (Callaway et al. 2008). Garlic mustard produces secondary metabolites that reduce inoculum potential, activity, and biomass of mycorrhizal fungi (Anderson et al. 1996, Roberts and Anderson 2001). Because garlic mustard is a non-mycorrhizal plant, this assault on the mycorrhizal mutualists of its competitors has been posited to explain garlic mustard's high invasion success (Stinson et al. 2006, Wolfe et al. 2008, Lankau et al. 2009). However, garlic mustard's influence on microbial communities changes with invasion age because garlic mustard populations produce less allelochemicals over time (Lankau et al. 2009), thereby weakening its influence on soil microbes (Lankau 2011). Furthermore, invasive plant effects on soil microbial communities (Arthur et al. 2012) and microbial processes (Portier et al. 2019) can also vary with plant phenology. Much of our understanding of garlic mustard impact of microbial communities is derived from studies on persistent invasions with high plant densities, mostly located in the north-eastern United States (Stinson et al. 2006, Wolfe et al. 2008, Anthony et al. 2017, Anthony et al. 2019, Burke et al. 2019a) and based on observations during garlic mustard's active life period. Investigating the microbial impacts of younger, less dense garlic mustard populations in understudied regions of their invasion range and over different points in the plant's life cycle can provide deeper insight into the microbial mechanisms by which garlic mustard promotes its invasion success. I address this question in my second chapter, using DNA sequencing to understand the differences in soil fungal and bacterial/archaeal microbiome composition among soils with garlic mustard present, absent, and

removed. To better understand phenological and geographic variations in garlic mustard associated microbiome effects, I sampled soils under active and senescent garlic mustard plants and collected multiple samples within and across forests in central Illinois.

Invasive plant species impacts on soil microbial communities can also alter biogeochemical processes in their non-native ecosystems (Ehrenfeld 2003, Van der Putten et al. 2007, Vilà et al. 2011). While the impacts of invasive species on biotic and abiotic properties of ecosystems are well documented (Levine et al. 2003, Traveset and Richardson 2014), the mechanisms underlying these impacts are less clear (Zhang et al. 2019). Garlic mustard traits may contribute to its invasive success beyond disruption of soil microbiomes through nutrient cycling (Rodgers et al. 2008a). While garlic mustard can disrupt soil microbiomes (Anthony et al. 2017, Duchesneau et al. 2021), it can also increase nitrogen (N) availability in invaded forests (Rodgers et al. 2008b, Morris et al. 2012). Invasive plant species modification of soil N dynamics is widespread (Liao et al. 2008, Vilà et al. 2011, Castro-Díez et al. 2014), but the factors contributing to garlic mustard's alteration of soil N availability are unclear. A major constraint in evaluating the mechanisms by which invasive species alter soil nutrient properties is the paucity of studies measuring the gross N transformation process rates responsible for changes in ecosystem N cycling. By assessing the impact of invasive garlic mustard populations on soil N cycling rates, we can better understand how this species is altering native forest ecosystems. In my third chapter, I seek to better understand garlic mustard associated impacts on soil N cycling and the potential biotic and abiotic mechanism contributing to garlic mustard effects. I measured gross N cycling rates in conjunction with multiple soil environmental and microbial variables from soils with garlic mustard present, absent, and removed. I replicated these measurements at three different points over garlic mustard's phenology (rosette, flowering, and senescent)

corresponding with differing potential nutrient use and allelochemical production strategies, across eight different forests in central Illinois.

Plant-microbial relationships play an important role in ecosystem nutrient dynamics (Čapek et al. 2018). Plants acquire nutrients from soil organic matter (SOM) through symbiotic relationships with microbial communities (Miller and Cramer 2005, Kraiser et al. 2011). By supplying soil microbiota, such as mycorrhizal fungi or rhizosphere prokaryotes, with photosynthate carbon (C), plants can direct microbiome assembly to promote favorable nutrient cycling processes (Jones et al. 2004, Paterson et al. 2007), particularly for soil nitrogen (N; Mushinski et al. 2021). However, the relative importance of fungal and bacterial communities in facilitating N acquisition for plants from SOM is unclear (Bahram et al. 2020). Emerging evidence suggests that rather than plant-fungal or plant-bacterial interactions prevailing, plant N acquisition may be supported by a multi-partite system of interactions with surrounding soil microbial communities (Hestrin et al. 2019, Emmett et al. 2021). In this context, fungal communities, which are often strongly influenced by mycorrhizal associations (Frey 2019), mediate relationships between plants and surrounding soil bacterial communities to form a "multi-trophic" system for N acquisition from SOM. These multi-trophic relationships in support of plant N acquisition likely have broad effects for structuring ecosystem function, but the factors affecting their dynamics remain poorly understood. Projected increases in anthropogenic N deposition (Kanakidou et al. 2016) are likely to alter ecosystem nutrient cycling systems, thus understanding how these systems structure plant-microbial interactions is important for predicting the outcomes of increased N deposition. I investigate these issues in my fourth chapter, comparing the relationships among plants, microbial communities, SOM variables, and extracellular enzyme activities (EEAs) in a montane tropical forest with and without N additions.

I use N addition to infer the effect of relieved plant reliance on N acquisition from SOM to understand how this relationship affects plant interactions with soil microbiomes and the synergistic effects of these changes on biogeochemical aspects of ecosystem function.

Biodiversity is a significant component of plant microbial interactions (Perreault and Laforest-Lapointe 2021). While there is growing understanding of the importance of species-specific attributes of these interactions, particularly for mycorrhizae (Churchland and Grayston 2014), how these conditions influence ecosystem function remains clear. Ectomycorrhizal (EM) mutualisms between trees and fungi are important drivers of ecosystem function, such as biogeochemical processes and SOM cycling (Smith and Read 2010), however, there is considerable variation in their associated effects on ecosystems (Fernandez and Kennedy 2016, Lin et al. 2017). A potential source of this variation may be from overgeneralizing the EM mutualism by homogenizing tree species and fungal communities with this characteristic as a unified functional guild, rather than a diverse group of organisms with a shared morphological characteristic. Given the rising prevalence of mycorrhizal type as a tool for delineating ecosystems to predict factors like nutrient cycling and SOM dynamics (Phillips et al. 2013) and their potential responses to global change (Terrer et al. 2016, Sulman et al. 2019), it is important to elucidate the sources of variation in EM-associated ecosystem effects. Ectomycorrhizal tree species exhibit significant differences in important traits like liter quality, nutrient-economic strategy, belowground carbon (C) allocation, and microbiome specificity (Craig et al. 2018, Fernandez et al. 2019, Keller et al. 2021). Further, mechanisms for SOM decay among EM fungi reflect the evolutionary history of these organisms (Pellitier and Zak 2018), as distantly related species promote decomposition and nutrient assimilation through differing metabolic mechanisms (Nicolas et al. 2019). These plant and fungal trait differences among EM species

could be responsible for the variation in mycorrhizal response among different ecosystems. I explore differences in ecosystems effects like SOM properties and EEAs among three different EM tree genera (*Carya*, *Quercus*, and *Tilia*) within a forest stand in my fifth chapter. I connect these ecosystem effects to soil fungal, EM fungal, and bacterial microbiome composition to better understand the relationship between EM diversity and ecosystem function.

In my sixth chapter, I review the findings of my dissertation, drawing upon these various studies to explain broader patterns in the context and function of plant-soil-microbe interactions. My research shows the important role microbial communities play in connecting plants to forest ecosystems and provides further evidence of how external factors associated with global change can disrupt these relationships. Overall, I conclude that soil microbial communities are important players of plant-mediated ecosystem function, but that this role is context dependent such that temporal factors, environmental conditions, and species diversity should be carefully considered in generalizing plant-microbial-ecosystem relationships.

CHAPTER 2: SOIL MICROBIAL COMMUNITIES ARE NOT ALTERED BY GARLIC MUSTARD IN RECENTLY INVADED CENTRAL ILLINOIS FORESTS

Introduction

Garlic mustard (*Alliaria petiolata,* Cavara and Grande) is a prominent invasive forest herb in North America (Rodgers et al. 2008a), and it has been proposed that part of garlic mustard's invasion success is due to novel interactions with soil microbiomes (Callaway et al. 2008). Garlic mustard produces secondary metabolites that reduce inoculum potential, activity, and biomass of mycorrhizal fungi (Anderson et al. 1996, Roberts and Anderson 2001). Because garlic mustard is a non-mycorrhizal plant, this assault on the mycorrhizal mutualists of its competitors has been posited to explain garlic mustard's high invasion success (Stinson et al. 2006, Wolfe et al. 2008, Lankau et al. 2009). However, garlic mustard's influence on microbial communities changes with invasion age because garlic mustard populations produce less allelochemicals over time (Lankau et al. 2009), thereby weakening its influence on soil microbes (Lankau 2011). Furthermore, invasive plant effects on soil microbial communities (Arthur et al. 2012) and microbial processes (Portier et al. 2019) can also vary with plant phenology. Much of our understanding of garlic mustard impact of microbial communities is derived from studies on persistent invasions with high plant densities, mostly located in the north-eastern United States (Stinson et al. 2006, Wolfe et al. 2008, Anthony et al. 2017, Anthony et al. 2019, Burke et al. 2019a) and based on observations during garlic mustard's active life period. Investigating the microbial impacts of younger, less dense garlic mustard populations in understudied regions of their invasion range and over different points in the plant's life cycle can provide deeper insight into the microbial mechanisms by which garlic mustard promotes its invasion success.

Garlic mustard is primarily known for its allelopathic impacts on mycorrhizal fungi (Callaway et al. 2008), but garlic mustard invasions can also impact other members of the soil microbial community. For example, garlic mustard can promote fungal saprobes and pathogens (Anthony et al. 2017, Anthony et al. 2019), or decrease bacterial richness and abundance (Lankau 2011). Promoting pathogens to which garlic mustard may be immune (Klironomos 2002) and altering decomposers to affect soil nutrient cycling (Hawkes et al. 2005, Hawkes et al. 2007) may potentially confer an advantage over native plants (Fraterrigo et al. 2011, Lee et al. 2018). However, garlic mustard effects on broader microbial communities are more poorly characterized than its effect on mycorrhizal communities (Cipollini and Cipollini 2016).

Invasive plant effects on soil microbial communities and their functions can vary with invasion age (Lankau 2011), plant phenology (Portier et al. 2019), and local soil properties (Li et al. 2006). After initial invasion, garlic mustard population densities increase rapidly then become relatively stable (Anderson et al. 1996, Nuzzo 1999). Because prior garlic mustard studies have focused primarily on older, more stable invasive populations, the effect of initial garlic mustard "booms" on soil microbiomes is not well understood. Many studies on microbial aspects of garlic mustard invasion also focus on early growing season, active life cycle plants, as these are most likely to have the greatest allelochemical production (Smith and Reynolds 2015). However, post-senescent or inactive invasive plants may still have significant impacts on microbial communities and processes (Arthur et al. 2012, Portier et al. 2019). Garlic mustard exhibits an early phenology relative to native competitors (Engelhardt and Anderson 2011a), and so its effects on soil microbial communities may depend on its life stages or broader temporal changes associated with surrounding plants and ecosystem conditions (Saikkonen 2007, Bennett et al. 2013). Finally, local and landscape factors like disturbance regime, soil types, and surrounding

plant communities also influence how quickly garlic mustard invasions spread and their population distribution patterns in invaded forests (Haines et al. 2018, Urbanowicz et al. 2019). These factors suggest that garlic mustard's influence on soil microbes could be variable across space (e.g. between plant populations in different forests) and time (e.g. between active vs. senescent garlic mustard plants).

Here, we asked the questions; 1) How does garlic mustard influence soil microbiomes in relatively young, low-density invasions; 2) Does this influence vary greatly within or between forests; and 3) How does this influence change over time between active and senescent garlic mustard plants? Using high-throughput DNA sequencing, we investigated how garlic mustard affects the community composition and species diversity of soil fungal and bacterial/archaeal communities between three garlic mustard treatments (present, absent, and removed) over two time points (May and August) across five hardwood forests in the midwestern United States. Our goal was to assess whether soils with garlic mustard present were more similar to each other than nearby reference soils (i.e., soils with garlic mustard absent or removed) across various spatial scales and as the growing season progressed. We used community composition to assess overall dissimilarity between samples, community diversity and evenness to assess whether garlic mustard was filtering surrounding microbiomes, and relative abundance of fungal functional guilds to assess the degree to which garlic mustard might be promoting its invasion success by suppressing mutualists, promoting pathogens, or altering nutrient cycling via saprotrophs.

Methods

Study sites

We measured garlic mustard impacts on soil microbial communities across five forests in central Illinois: Brownfield Woods, Collins Pond Woods, Hart Woods, Richter Woods, and Trelease Woods. These sites are located within a 30 km north-south range and a 40 km east-west range. Mean annual temperature and mean annual precipitation ranged 10.0-10.5 ºC and 889-990 mm, respectively, across all sites. The dominant soil series at each site were either silt loams or silty clay loams. Geographical coordinates, climate, soil type, and estimated invasion age for each site are reported in Table A.1. Plant community composition is similar across all sites, with a "prairie grove" species mix with *Quercus, Fraxinus, Carya,* & *Acer* species dominating the canopies. The understories of these forests are made up of a diverse mix of tree seedlings, *Asimina triloba,* and other shrubs including *Sanicula, Laportea, Ageratina, Ribes,* and *Trillium* species. All sites are managed by the Illinois Natural History Survey (INHS; https://www.inhs.illinois.edu/research/natural-areas-uiuc). According to documentation from INHS managers, all of these sites began experiencing invasion between 1991 and 2000 (Table A.1; S. Buck, personal communication). Site managers control garlic mustard populations through hand-pulling, herbicide application, and "weed whacking", and as a result, garlic mustard densities at these sites are generally maintained below the high densities often associated with a decline in garlic mustard allelochemical production (Lankau et al. 2009, Lankau 2011). For all sites except for Collins Pond Woods, garlic mustard was restricted to small clusters of plants, with an average coverage of 30% of each m^2 plot. Large portions of Collins Pond Woods were covered by garlic mustard plants, with an average coverage of 60% of each m^2 plot.

Study design

In mid-March of 2017, we established four replicate 10-m^2 experimental blocks in each forest. Each block contained 1-m^2 plots for each of the following three treatments: garlic mustard present (present), garlic mustard absent (absent), and garlic mustard removed via hand-pulling (removed). We established the garlic mustard removed treatment as a control to account for unknown, meter-scale differences in soils within each block that might confound garlic mustard presence/absence in the other two plots, and the removal plots also allowed us to determine if live garlic mustard plants are required to see their effects on soil microbes. We collected soil samples in May and August, corresponding with the rosette and senescent life stages of garlic mustard, respectively. As we were focused on the broader soil microbiome effect of garlic mustard, soil samples were collected from 0-10 cm depth from the soil surface using a 2 cm diameter hand corer. While this depth may dilute the detectable effects of garlic mustard leaf-litter on surface-soil microbial communities, it allowed us to better characterize the effects of garlic mustard's root-induced effects on microbial communities, an important component of garlic mustard's purported allelopathy (Cipollini and Cipollini 2016). There was very little, if any, development of organic horizons in any of our plots, so all samples consisted primarily of mineral soils. Four soil cores were collected from each 1 m^2 treatment plot, composited, and homogenized by hand in the field. On the same day as collection, the soil samples were transported to the University of Illinois at Urbana-Champaign and frozen at -20ºC until they were freeze-dried (within one week of collection), then stored at -20ºC for later analysis.

DNA extraction, sequencing, identification, and assignments

We used high-throughput DNA sequencing to determine the impact of garlic mustard on microbial community structure as well as the relative abundance of key functional groups. DNA was extracted from 500 mg of freeze-dried soil using the FastDNA SPIN Kit for Soils (MP Biomedicals, Santa Ana, USA). The extracts were purified using cetyl trimethyl ammonium bromide (CTAB) as described in Edwards et al. (2018). DNA extracts were submitted to the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign for Fluidigm amplification (Fluidigm, San Francisco, USA) and Illumina sequencing (Illumina, San Diego, USA). We assessed fungal communities via the *ITS2* gene region using ITS3 and ITS4 primers (White et al. 1990) to amplify DNA and sequence amplicons via MiSeq bulk 2 x 250 bp V2. As we were interested in the broad fungal community effect of garlic mustard invasion, we chose to use a universal ITS2 barcode region (Schoch et al. 2012). However, this barcode is known to discriminate against early-diverging fungal lineages like arbuscular mycorrhizal fungi (Stockinger et al. 2010). While this guild was included in our analysis, we note that this approach favors broader characterization of fungal communities over more comprehensive characterization of the arbuscular mycorrhizal fungal community specifically, decreasing our ability to characterize garlic mustards effect on these plant mutualists. We assessed bacterial and archaeal communities via the bacterial and archaeal *16S* rRNA genes. The samples were amplified using V4_515 forward and V4_806 reverse primers (Apprill et al. 2015, Parada et al. 2016) and amplicons were sequences via MiSeq bulk 2 x 250 bp V2. Primer sequences and references can be found in Table A.2. Sequence data is publicly available from the NCBI SRA database under accession number PRJNA690109.

We used the DADA2 pipeline (Callahan et al. 2016) for bioinformatic processing to produce amplicon sequence variants (ASVs) from the sequencing data. Briefly, quality filtering, denoising, mering forward and reverse reads, and removing chimeric sequences was performed using recommended parameters for *16S* and *ITS* genes. For *16S* processing one sample did not pass quality filtering (for a total of 119 samples), and for *ITS* processing two samples did not pass quality filtering (for a total of 118 samples). We did not cluster sequence variants (Glassman and Martiny 2018) prior to using the default DADA2 classifier (Wang et al. 2007) to assign taxonomy based on reference sequences from the UNITE database (Kõljalg et al. 2013) for *ITS* sequences and the SILVA database (Quast et al. 2012) for *16S* sequences. We rarified all samples to a uniform read depth of 4169 reads for *ITS* and 10280 reads for *16S*, then transformed our final count numbers via a Hellinger transformation (Legendre and Gallagher 2001) prior to further statistical analysis. Data used for analysis consisted of 9362 fungal, 14618 bacterial, and 152 archaeal ASVs. Due to the relatively low number of archaeal sequence variants, and because bacterial and archaeal sequences were derived from the same primer sets, we analyzed bacterial and archaeal communities together. We also compared the relative abundance and species richness of saprotrophic, pathogenic, and mycorrhizal functional guilds among garlic mustard treatments and between sampling dates using FUNGuild (Nguyen et al. 2016a) to assign functional guild to taxa. Assignments with "probable" or "highly probable" confidence scores were included and in cases where taxa were assigned to more than one guild, mycorrhizal classification was given higher priority than pathogenic, which was given higher priority than saprotrophic (Smith et al. 2021).

Statistical analysis

All statistical analyses were performed in the R statistical environment (R Development Core Team 2013). Overall patterns of beta diversity and community structure were analyzed with the VEGAN package (Oksanen et al. 2010). To determine differences in community composition based on treatment, sampling date, and the interaction between treatment and sampling date, we performed a permutational analysis of variance (PERMANOVA) with the *adonis* function on Hellinger-transformed rarified ASV count numbers. We added location (forest or block) as a term in the *adonis* model, rather than stratifying on these terms, in order to estimate the spatial contribution to the total variance in microbial communities; comparison of these models with those that stratified on location instead did not produce different results with respect to treatment, sampling date, or their interaction (Table A.3). We also assessed differences in community composition and heterogeneity visually using nonmetric multidimensional scaling (NMDS) ordinations on Bray-Curtis dissimilarity of rarified ASV count numbers. We assessed alpha diversity as species diversity (Shannon's *H*) and evenness (*H*/log(species #)) using the *diversity()* function in VEGAN. Differences among garlic mustard treatments, sampling dates, and their interaction in the species richness and evenness, as well as relative abundance and richness of functional guilds, were determined using a linear-mixed model with the LMER package (De Boeck et al. 2011), mycorrhizal abundance and richness values were log-transformed to meet assumptions of normality. In this model, we used treatment, sampling date, and their interaction as fixed effects and block nested within forest as a random effect. Statistical significance was assessed as $p < 0.05$.

Results

Soil microbial community composition

Sampling date and forest significantly influenced fungal and bacterial/archaeal community composition, though neither garlic mustard treatment nor its interaction with sampling date had a significant effect (Table 1). Across all samples, forest was responsible for 12% of variation in fungal communities, while within forests the variation attributable to block increased to between 21%-27% ($p<0.001$, Table 1, Figure 1C). For bacterial and archaeal communities forest was responsible for 15.6% of variation among all samples and between 28%-46% for block within each forest ($p<0.001$, Table 1, Figure 1F). Sampling date accounted for 2.2% of variation in fungal communities among all forests ($p<0.001$, Table 1, Figure 1B) and between 4.9% and 12.9% ($p<0.05$, Table 1, Figure 2F-J) in all forests except Collins Pond where it did not have a significant effect. For bacterial and archaeal communities, sampling date was responsible for 4.2% of overall variation among all samples ($p<0.001$, Table 1, Figure 1E) and between 8.3%-10.8% ($p<0.006$, Table 1, Figure 3F-J) in all forests except Hart Woods where it did not have a significant effect. Garlic mustard treatment did not significantly affect overall fungal communities across all forests or within any forest (Figure 1A, Figure 2A-E). Similarly, garlic mustard treatment also had no significant effect on bacterial and archaeal communities across all forests or within any forest (Figure 1D, Figure 3A-E).

Diversity and evenness

Garlic mustard presence did not significantly affect microbial diversity or evenness relative to control or removed plots, though fungal diversity increased in garlic mustard present plots from May to August corresponding with plant senescence (Table 2). The interaction

between garlic mustard treatment and time had a significant effect on fungal diversity ($F_{2,112}$=3.30, p=0.041), with diversity increasing in garlic mustard present plots from May to August (Figure 4A). Fungal evenness increased significantly from May to August ($F_{1,112}$=34.82, p<0.001), though garlic mustard treatment had no significant effect (Figure 4B). There were no significant effects or interactions of garlic mustard treatment and time on bacterial and archaeal diversity (Figure 4C), though garlic mustard present plots had marginally lower diversity (p=0.092) than absent or removed. Bacterial and archaeal evenness significantly increased from May to August ($F_{1,113}$=38.73, p<0.001), and this effect was most pronounced in garlic mustard present plots (interaction $F_{2,113}$=5.94, p=0.004, Figure 4C).

Functional guilds

Garlic mustard treatment had no significant effect on the relative abundance or species richness of fungal saprotrophic, pathogenic, and mycorrhizal functional guilds (Table A.4, Figure A.1). Mycorrhizal relative abundance and richness increased significantly from May to August (relative abundance: $F_{1,112}$=29.762, p < 0.001; richness: $F_{1,112}$=26.202, p < 0.001, Figure A.1CF). Fungal pathogen richness decreased significantly from May to August ($F_{1,112}$=7.425, p=0.007), though this effect was most pronounced in the garlic mustard removed plots (interaction: $F_{2,112}$=4.928, p=0.009, Figure A.1E).

Discussion

We found extensive spatial variation of microbial community composition both within and between forests, but we did not detect consistent or strong microbial patterns related to garlic mustard presence at these sites. Despite long known (Vaughn and Berhow 1999, Roberts and

Anderson 2001) and widely reported effect of garlic mustard on soil microbiomes (Stinson et al. 2006, Callaway et al. 2008, Rodgers et al. 2008a, Wolfe et al. 2008, Pringle et al. 2009b, Anderson et al. 2010, Anthony et al. 2017), garlic mustard appears to have minimal impacts on the soil microbiome of these recently invaded forests of central Illinois. Further, we did not find significant garlic mustard-related decreases in ectomycorrhizal fungal diversity or relative abundance, so our data are not consistent with the mycorrhizal suppression effect that is often reported with garlic mustard invasions (Stinson et al. 2006, Wolfe et al. 2008). Indeed, we did not find any direct impact of garlic mustard on other fungal functional guilds that have been more recently reported (Anthony et al. 2017, Duchesneau et al. 2021). It is possible, however, that there were physiological changes to the colonization, biomass, or germination of these guilds that we would not have been able to measure with our DNA-based methodology. While soil microbial communities at our sites did change significantly between our May and August sampling dates, we were unable to detect significant garlic mustard effects on soil microbial communities during either the active or senescent garlic mustard phenological stages. Overall, our study suggests that caution should be taken in generalizing microbially-mediated invasion mechanisms for garlic mustard across all potential ecoregions, ecosystems, and invasion stages.

Garlic mustard's minimal impact on soil microbial communities in our study could potentially be due to regional or local soil characteristics of our sites. Many of the soils in our study had higher clay contents than the characteristically sandy soil of more northern and eastern North American forests invaded by garlic mustard (Table A.1). Allelochemicals produced by garlic mustard plants could have bound to these clay particles (Blair et al. 2005), potentially ameliorating their inhibitory effects on mycorrhizal fungi (Cantor et al. 2011). While some of the earliest evidence for garlic mustard's mycorrhizal suppression was also found in central Illinois

soils (Anderson et al. 1996, Roberts and Anderson 2001), these studies found garlic mustard decreased mycorrhizal inoculum potential, which could occur without a concomitant change in microbial community composition. It may also be that there were impacts of these garlic mustard populations constrained specifically to arbuscular mycorrhizal fungi that would be difficult to detect using our methodology. However, arbuscular mycorrhizal fungi are also thought to interact with bacterial communities (Artursson et al. 2006), so were these mutualists impacted by garlic mustard here we would have likely seen some corresponding shift in the bacterial communities we measured. Additionally, our homogenization of soils up to 10 cm likely limited our ability to detect changes in surface soil communities, which may respond faster to environmental change and leaf-litter induced allelopathy from garlic mustard than deeper soils. Leaf litter has been shown to produce greater allelopathic effects than live roots in other plants (Zhang and Fu 2010), though the relative impact of garlic mustard leaves versus roots is more poorly understood and deserving of further investigation. The possibility of certain soils, soil depths, or plant structures mediating garlic mustard's allelopathy could have ramifications for future garlic mustard invasion pattens, though further investigation of the relationship between allelochemical concentrations and microbial responses across different soil types, depths, and litter treatments will be required to support this pattern.

The age of garlic mustard populations in our study may also have influenced their impacts on soil microbial communities. Microbial community suppression by garlic mustard peaks when invasive populations are around 40 years old (Lankau 2011), which may represent a potential ecological filter on microbial communities in garlic mustard invaded soils. According to management records from these sites all garlic mustard population were <26 years old and, due to land management, with low garlic mustard plant density characteristic of much younger

invasions. We found weak evidence of decreased soil microbial diversity in garlic mustard present plots, particularly in May when the plants were active, which could possibly be an indicator of ongoing microbiome manipulation by garlic mustard populations. As the garlic mustard populations we observed in this study have not yet reached the age associated with peak allelochemical production (Lankau 2011), the microbial filtering effect of this allelochemical peak may not yet be fully present. Ecological filtering associated with invasion age or density could explain differences in our results, particularly mycorrhizal suppression, from studies conducted on older, higher density garlic mustard populations (Anthony et al. 2019, Burke et al. 2019b). Direct comparisons between study settings are needed to elucidate whether these differences are due to invasion age or regional differences in ecosystem characteristics. Further, we were unable to discern whether our "absent" plots had any history of garlic mustard invasion, which could explain why they did not differ from plots with garlic mustard present. Long-term monitoring of garlic mustard population extent and impacts on soil microbial communities could also aid in verifying these results.

The influence of spatial variation on microbial communities is often considered a key factor driving community composition (Fierer and Lennon 2011). Spatial patterns at meter to decameter scales, like those within a forest, can have similar or even stronger effects on microbial community composition as spatial patterns at regional scales (Ramirez et al. 2014). This pattern was demonstrated in our study as we found block-within-forest to explain more overall variation in microbial community composition than forest. The many factors that promote this variation (e.g. temperature, moisture, pH, soil chemistry, trophic interactions, plant and animal communities, etc.) necessitates the need for contextualizing extrinsic impacts on microbial communities, like those of an invasive plant, with their neighboring microbiomes.

Experimental designs that account for this variation by comparing the impacts of invasive species with closely situated control soils, like the hierarchical block-with-forest design we used here, should be utilized in future investigations to ensure patterns are consistent and generalizable across a large area.

Temporal variation also played a significant role in structuring microbial communities, particularly the increase in mycorrhizal abundance and richness over the growing season. The impacts of time on microbial community composition is less well understood than that of space (Carini et al. 2020). Temporal factors are likely to be more influenced by short-term patterns, like weather or plant phenology, than spatial factors potentially making them more susceptible to future global change (Balser et al. 2010). Further research on short-term temporal patterns, like phenology or warming, in invasive species-microbiome interactions (Anthony et al. 2020) is needed to further understand how microbial communities respond to invasive species and their environment over time. Improving understanding of spatial and temporal variation in microbially mediated invasion dynamics could help predict the spread of future invasions and their responses to global change.

These sites are the beneficiaries of high-quality management by the University of Illinois and Illinois Natural History Survey, and as such garlic mustard population here have been maintained at relatively low cover areas for their history. Garlic mustard cover area is linked to the magnitude of its effects on fungal communities (Anthony et al. 2017) and garlic mustard population declines (cover area reduction) have also been associated with fungal community recovery following garlic mustard invasion (Lankau et al. 2014, Anthony et al. 2019). Natural area managers, by controlling garlic mustard populations, may also be limiting its effect on soil microbiomes. It may be that there is some cover area “tipping point” below which garlic mustard

fails to influence soil microbial communities, suggesting that long-term management of garlic mustard invasions may be a potential avenue to curb its more noxious effects on surrounding plant communities. Alternatively, by keeping garlic mustard cover areas in these forests low, managers may have prevented potential microbial community filtering caused by "exploding" garlic mustard populations, highlighting the need for increase management on the edges of garlic mustards invasive range. Resistance and recovery of soil microbiomes should be an important consideration in making future management decision in recently and historically invaded garlic mustard populations.

Overall, we present evidence that garlic mustard invasions may vary in their ability to manipulate microbiomes to promote their invasion success across ecoregions. This variation may be due to soil condition, invasion age, or some other factor. Invasive garlic mustard populations were still present in these forests despite their apparently limited microbial impacts. This finding suggests that garlic mustard populations are not solely reliant on microbial mechanisms (like "mycorrhizal suppression" to the degree we could measure it) to invade forest ecosystem. The lack of garlic mustard impacts on the soil microbial community in recently invaded central Illinois forests suggests that these well-documented impacts in the northeastern United States and in older invasions cannot necessarily be generalized across all environmental contexts.

Tables and Figures

Forest	Community	Main Fixed Effect	F-value	R^2	p-value
All	**Fungi**	Treatment	0.883	0.014	0.948
		Sampling date	2.855	0.022	**0.001**
		Treatment*Sampling date	0.753	0.012	1.000
		Forest	3.661	0.115	**0.001**
	Bacteria & Archaea	Treatment	0.906	0.013	0.631
		Sampling date	5.801	0.042	**0.001**
		Treatment*Sampling date	0.652	0.009	0.999
		Forest	5.540	0.156	**0.001**
Brownfield	**Fungi**	Treatment	0.925	0.650	0.673
		Sampling date	3.670	0.129	**0.001**
		Treatment*Sampling date	0.893	0.062	0.767
		Block	2.043	0.216	**0.001**
	Bacteria & Archaea	Treatment	1.132	0.072	0.228
		Sampling date	3.197	0.101	**0.001**
		Treatment*Sampling date	0.835	0.055	0.873
		Block	2.863	0.282	**0.001**
Collins Pond	**Fungi**	Treatment	1.022	0.088	0.373
		Sampling date	1.183	0.051	0.124
		Treatment*Sampling date	0.865	0.075	0.919
		Block	2.040	0.265	**0.001**
	Bacteria & Archaea	Treatment	1.063	0.066	0.366
		Sampling date	3.453	0.108	**0.001**
		Treatment*Sampling date	0.960	0.060	0.506
		Block	3.820	0.359	**0.001**
Hart	**Fungi**	Treatment	1.061	0.076	0.274
		Sampling date	1.534	0.055	**0.023**
		Treatment*Sampling date	0.880	0.063	0.813
		Block	2.548	0.272	**0.001**
	Bacteria & Archaea	Treatment	1.337	0.083	0.094
		Sampling date	1.725	0.053	**0.043**
		Treatment*Sampling date	0.786	0.049	0.812
		Block	3.779	0.351	**0.001**
Richter	**Fungi**	Treatment	1.072	0.077	0.249
		Sampling date	1.366	0.049	**0.047**
		Treatment*Sampling date	0.903	0.065	0.80
		Block	2.507	0.270	**0.001**
	Bacteria & Archaea	Treatment	1.064	0.051	0.334
		Sampling date	3.457	0.083	**0.005**
		Treatment*Sampling date	0.870	0.042	0.547
		Block	6.452	0.464	**0.001**
Trelease	**Fungi**	Treatment	1.001	0.074	0.547
		Sampling date	1.941	0.072	**0.001**
		Treatment*Sampling date	0.831	0.061	0.970
		Block	2.140	0.238	**0.001**
	Bacteria & Archaea	Treatment	1.132	0.072	0.206
		Sampling date	3.196	0.101	**0.001**
		Treatment*Sampling date	0.843	0.054	0.828
		Block	3.119	0.297	**0.001**

Table 2.1. PERMANOVA results for fungal and bacterial/archaeal community composition across all forests. Bolded p-values highlight significant effects at $p < 0.05$.

Community	**Main Fixed Effect**	**Diversity**		**Evenness**	
		F-value	p-value	F-value	p-value
Fungi	Treatment	0.244	0.761	0.031	0.969
	Sampling date	0.015	0.721	33.77	**<0.001**
	Treatment*Sampling date	3.298	**0.041**	1.278	0.283
Bacteria & Archaea	Treatment	2.440	0.093	1.920	0.152
	Sampling date	2.888	0.092	38.73	**<0.001**
	Treatment*Sampling date	0.640	0.529	5.940	**0.004**

Table 2.2. ANOVA results for main effects on the species diversity (Shannon's *H*) and evenness (*H*/log(species #)). Block nested within site was included as a random effect in all models. Bolded p-values highlight significant effects at $p < 0.05$.

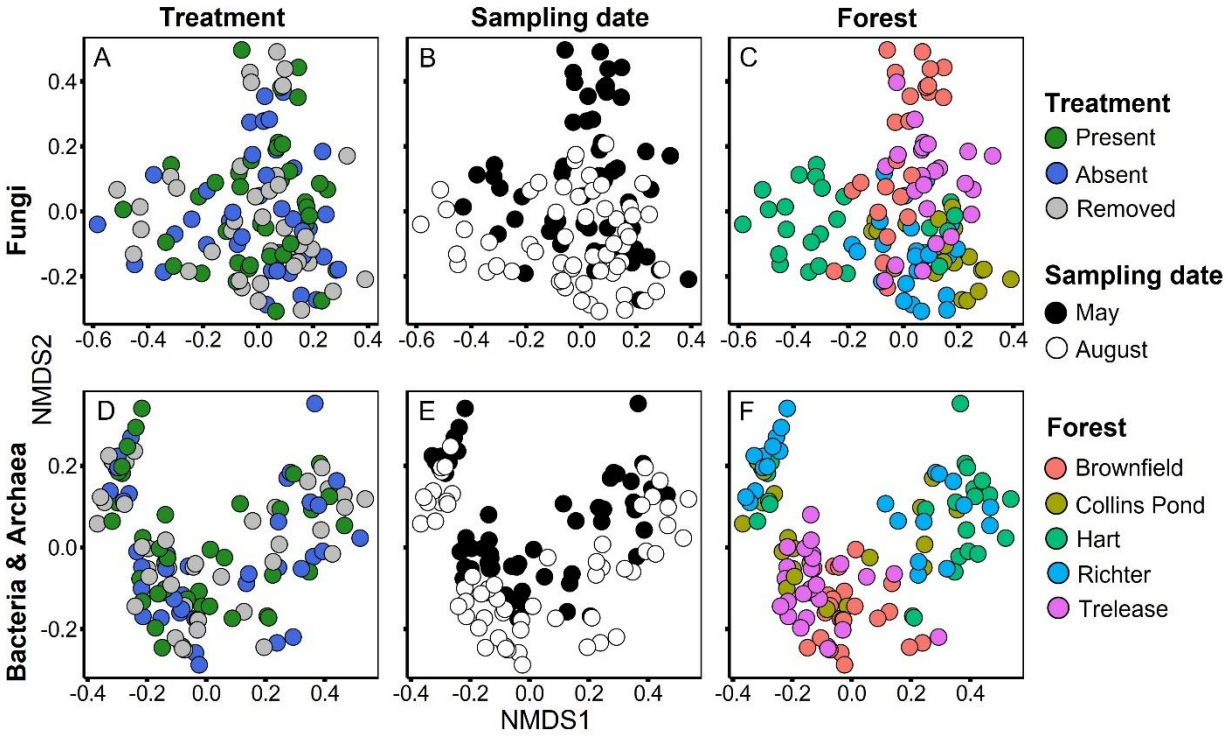

Figure 2.1. Fungal (ABC, Stress = 0.13) and Bacterial/Archaeal (DEF, Stress = 0.05) community composition expressed as nonmetric multidimensional scaling axes depicting Bray-Curtis dissimilarities of rarified count numbers for ASVs within each sample (circle). Panels A & D depict differences between garlic mustard invasion status (treatment), panels B & E depict differences between sampling dates, and panels C & F depict differences between forests. Each panel includes data across all garlic mustard treatments, sampling dates, and forests.

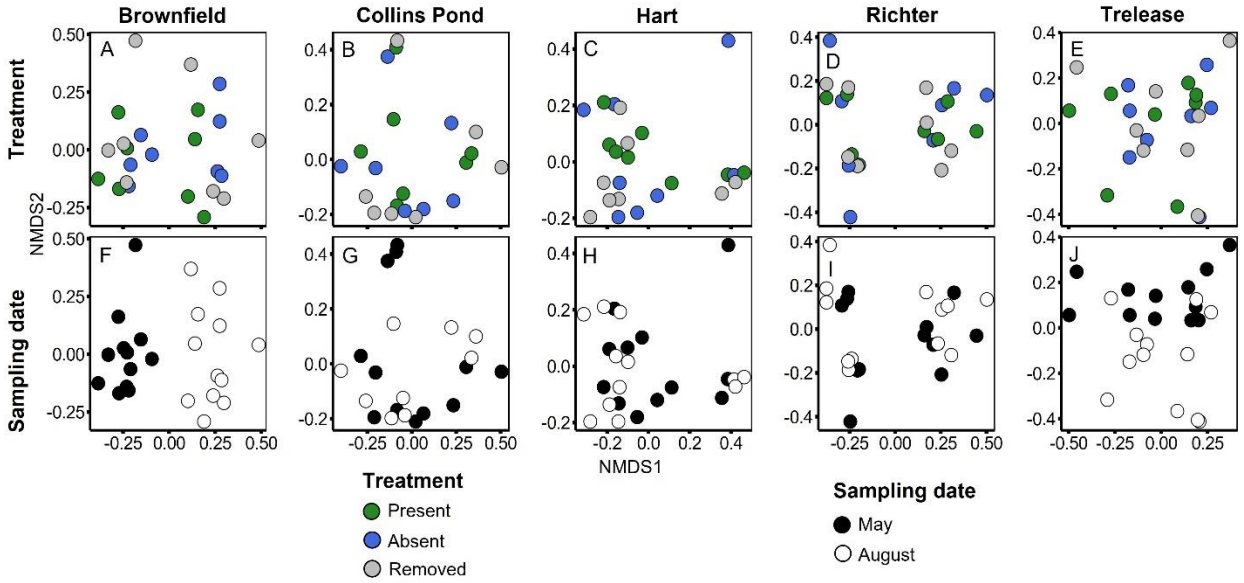

Figure 2.2. Individual forest ordinations of fungal community composition expressed as nonmetric multidimensional scaling axes depicting Bray-Curtis dissimilarities of rarified count numbers for ASVs within each sample (circle). Panels A-E depict differences between garlic mustard invasion status while panels F-J depict differences between sampling dates, with each panel only containing data from the forest listed above each column. Ordination stress ranged from 0.03-0.09.

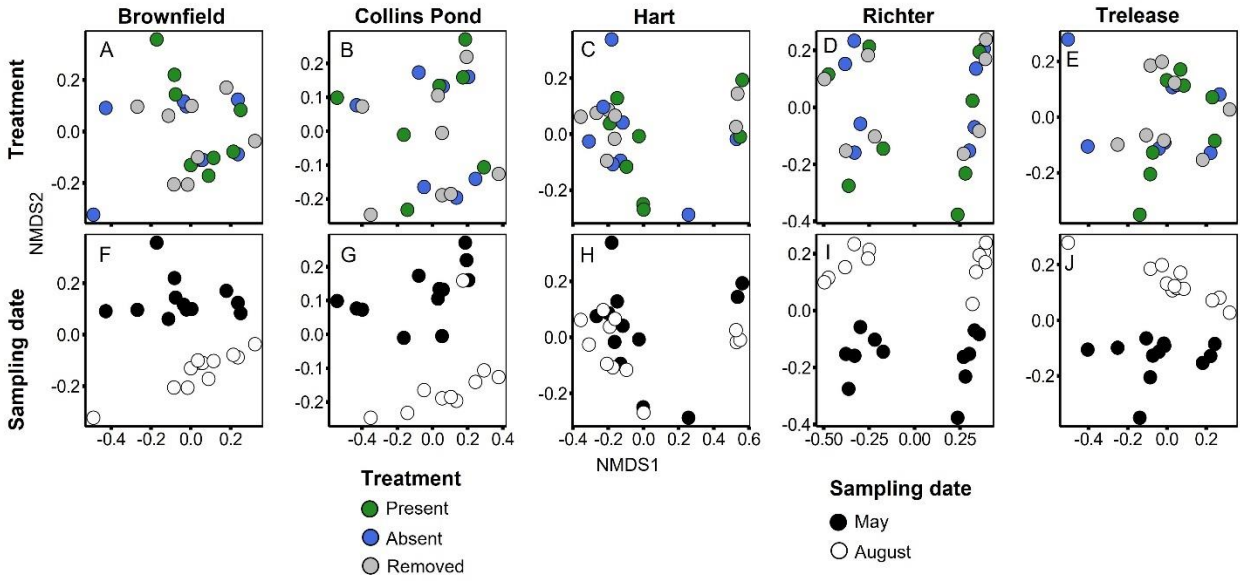

Figure 2.3. Individual forest ordinations of bacterial and archaeal community composition expressed as nonmetric multidimensional scaling axes depicting Bray-Curtis dissimilarities of rarified count numbers for ASVs within each sample (circle). Panels A-E depict differences between garlic mustard invasion status while panels F-J depict differences between sampling dates, with each panel only containing data from the forest listed above each column. Ordination stress ranged from 0.02-0.07.

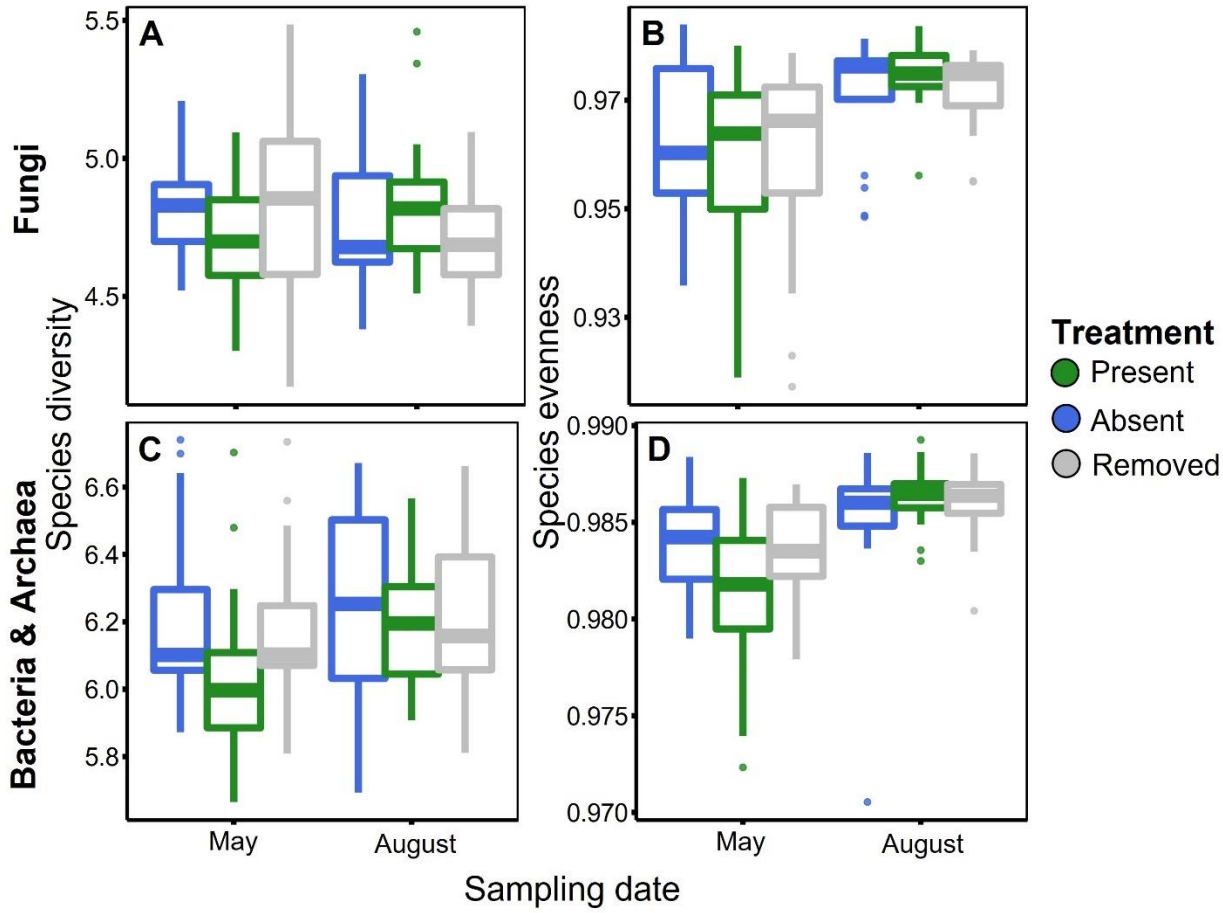

Figure 2.4. Diversity indices for fungal (AB) and bacterial/archaeal (CD) communities. Panels A & C show species diversity, expressed as Shannon's *H*, and panels B & D show species evenness, expressed as *H*/log(species #).

CHAPTER 3: ACCELERATED GROSS NITROGEN CYCLING FOLLOWING GARLIC MUSTARD INVASION IS LINKED WITH ABIOTIC AND BIOTIC CHANGES TO SOILS

Introduction

Invasive plant species often alter soil microbial communities and biogeochemical processes in their non-native ecosystems (Ehrenfeld 2003, Van der Putten et al. 2007, Vilà et al. 2011). While the impacts of invasive species on biotic and abiotic soil properties are well documented (Levine et al. 2003, Traveset and Richardson 2014), the mechanisms underlying these impacts are less clear (Zhang et al. 2019). Garlic mustard (*Alliaria petiolata* (Bieb.) Cavara & Grande), a prominent invasive understory herb in North American forests, may contribute to its invasive success through disruption of soil microbiomes and nutrient cycling (Rodgers et al. 2008a). While garlic mustard can negatively affect mycorrhizal relationships of its native competitors (Stinson et al. 2006, Wolfe et al. 2008) and promote the abundance of saprotrophic and pathogenic microorganisms (Anthony et al. 2017, Duchesneau et al. 2021), it can also increase nitrogen (N) availability in invaded forests (Rodgers et al. 2008b, Morris et al. 2012). Invasive plant species modification of soil N dynamics is widespread (Liao et al. 2008, Vilà et al. 2011, Castro-Díez et al. 2014), but the factors contributing to garlic mustard's alteration of soil N availability are unclear. A major constraint in evaluating the mechanisms by which invasive species alter soil nutrient properties is the paucity of studies measuring the gross N transformation process rates responsible for changes in ecosystem N cycling.

Garlic mustard may promote the availability of soil N in forests it invades by accelerating N cycling process rates through directly altering soil properties. Garlic mustard can increase soil

pH in the relatively acidic soils of forests it invades (Anderson and Kelley 1995). Nitrogen cycling processes responsible for transformations of plant-available soil inorganic N, mineralization and nitrification, are suppressed under more acidic pH (Schimel and Bennett 2004). As garlic mustard is non-mycorrhizal, increasing pH to promote N cycling processes would present a direct benefit to garlic mustard plants by accelerating the production of inorganic N to support nutrient uptake. Garlic mustard can also alter water and nutrient use strategies in competitor native plants (Bialic-Murphy et al. 2021). Competitor species can exhibit decreases water-use efficiency when proximal to garlic mustard plants in order to promote N uptake. This relationship might lead to acceleration of microbial N cycling processes near garlic mustard due to decreased N substrate availability caused by plant uptake in relation to soil moisture.

Another potential mechanism for garlic mustard to alter soil N cycling processes is through manipulating nutrient pools via litter input or nutrient uptake. High-quality leaf and root litter inputs from garlic mustard plants relative to native species (Hale et al. 2011) may decrease carbon (C):N ratios in invaded soils (Burke et al. 2011). Soil organic matter nutrient stoichiometry is closely connected to nutrient cycling rates (Buchkowski et al. 2015) and decomposer community composition (Delgado-Baquerizo et al. 2017). As young garlic mustard plants overwinter in their rosette forms, many die before reaching their reproductive stage (Heckman and Carr 2015) and while native plants are still dormant. High over-winter mortality of first year garlic mustard plants likely represents a strong early-season input of nutrients into soils. Garlic mustard changes to soil nutrient stoichiometry could stimulate microbial decomposers to affect soil nutrient cycling (Ashton et al. 2005, McTee et al. 2017), potentially conferring an advantage over native plants (Fraterrigo et al. 2011, Lee et al. 2018). Garlic

mustard nutrient uptake may also uniquely influence N cycling processes. As garlic mustard plants are already active in early spring, its earlier phenology allows them to uptake nutrients while other plants are still dormant (Anderson et al. 1996, Smith and Reynolds 2015). Increased inorganic nutrient uptake from garlic mustard plants may stimulate microbial activity to promote substrate availability (Raynaud et al. 2006). Garlic mustard also senesces earlier than native plants, potentially contributing to a late growing season flush of lower quality litter (relative to active plants) into soils. Lower-quality post-senescent litter inputs have been found to slow microbial N cycling (Sanaullah et al. 2010), potentially allowing for N accumulation that could promote the success of younger germinating or first-year garlic mustard plants. These phenological patterns in garlic mustard's life history may result in temporal patterns in its impact on soil nutrient cycling.

Garlic mustard could also alter soil N cycling processes through interactions with soil microbial communities, such as by disrupting mycorrhizal relationships (Pringle et al. 2009b). While this allelopathy has the direct benefit of reducing mutualistic relationships among garlic mustard's competitors, it may also provide indirect benefits to garlic mustard by relieving mycorrhizal competition with free-living microbes for inorganic nutrients (Johnson et al. 2013). Garlic mustard's mycorrhizal allelopathy and its changes to soil nutrient stoichiometry could promote the abundance of more saprotrophic microorganisms to accelerate nutrient cycling (Anthony et al. 2017). In addition, garlic mustard may negatively affect bacterial communities (Burke and Chan 2010, Lankau 2011), another potential competitor for inorganic soil N, to promote inorganic N availability. This reduction in microbial N competition would potentially correspond to decreased N in microbial biomass (Treseder 2008). While evidence for garlic mustard affecting microbial communities differently over its life history stages is unclear

(Edwards et al 2022), interactions between garlic mustard phenology and soil microbiomes may impact soil N cycling. Invasive plants can produce different effects on microbial activity and nutrient cycling rates at different phenological stages (Portier et al. 2019). Thus, studying the effect of garlic mustard on soil conditions, nutrient cycling, and microbial communities at different points in its phenology may yield further insight into the mechanisms by which garlic mustard alters soil ecosystems to promote its invasion success. By observing the effects of invasive garlic mustard on nutrient cycling rates concomitantly with soil properties, nutrient stoichiometries, and microbial community composition, we can gain insight into the relative importance of these three potential mechanisms in driving garlic mustard effects on soil N cycling.

Here we investigated how garlic mustard may alter soil N cycling rates in forests it invades (Rodgers et al. 2008b, Morris et al. 2012). We propose three hypothesized mechanisms by which garlic mustard can accelerate N cycling rates: increasing soil pH, decreasing soil C:N ratio, or modifying soil microbial communities. To test these mechanisms, we conducted a study in eight central Illinois forests invaded by garlic mustard, quantifying gross N cycling rates, characterizing microbial community composition, and measuring environmental parameters at three points over the garlic mustard life cycle. Overall, we expected to find greater inorganic N concentrations and accelerated N cycling rates in soils invaded by garlic mustard. Based on our hypothesized mechanisms, we expect these conditions to either correspond with increased soil pH, lower soil C:N ratios during the early garlic mustard life cycle, or differential microbial communities (*e.g.* lower mycorrhizal relative abundance, homogenized prokaryotic communities, or decreased microbial biomass N) corresponding with garlic mustard invasion.

Methods

Study sites

In 2018 we measured garlic mustard impacts on soil N cycling across eight central-Illinois forests spanning 130 km range north-south and 140 km range east-west: Allerton Park, Breens Woods, Bunny Forest, Funk Woods, Richter Woods, Trelease Woods, Warbler Ridge, and the Vermillion River Observatory. Mean annual temperature and mean annual precipitation ranged 10.0-11.1 ºC and 914-977 mm, respectively, across all forests. Plant community composition is relatively similar across these forests, consisting of a "prairie grove" species mix with *Quercus, Fraxinus, Carya,* & *Acer* species dominating the canopies. The understories of these forests are made up of a diverse mix of tree seedlings, *Asimina triloba,* and other shrubs including *Sanicula, Laportea, Ageratina, Ribes,* and *Trillium* species. The dominant soil types at each forest were either silt loams or silty clay loams. Geographical coordinates, climate, and soil series for each site are reported in Table B.1.

Experimental design

This study was designed with forest as the experimental unit of replication to better represent the general effect of garlic mustard on forest soils over a greater geographic area. In March 2018, we established 1-m^2 plots for each of the following three treatments within one 10-m^2 area of each forest: garlic mustard present (present), garlic mustard absent (absent), and garlic mustard removed via hand-pulling (removed). We used the garlic mustard removed treatment as a control to account for meter-scale differences in soils within each block that might promote the growth of garlic mustard and to understand if live garlic mustard plants are required to see their effects. We collected soil samples at three points over the growing season corresponding to

different phenological stages in the garlic mustard life cycle. Samples were collected in mid-May to represent the "rosette" stage, mid-June to represent the "flowering" stage, and mid-August to represent the "senescent" stage of the garlic mustard life cycle. This approach allowed us to observe the distinct effects of early season vs late season litter inputs on soil nitrogen cycling. Additionally, plant allelochemical production has been found to decrease as individuals expend more resources on reproduction (Cipollini and Cipollini 2016), thus sampling across vegetative, reproductive, and senescent life stages will represent a range of plant allelochemical production intensities. Comparisons of the garlic mustard present versus absent treatments controls for the effect of seasonal changes in environmental conditions (*i.e.* temperature, moisture, and light availability) that could potentially be confounded with the effect of garlic mustard phenology on soil N cycling and microbial community composition (Smith and Reynolds 2015).

Soil samples were collected from 0-10 cm depth from the soil surface using a 5 cm diameter hand corer. There was very little to no organic horizon present in any of the study plots, so all samples consisted primarily of mineral soils. Four soil cores were collected from each 1 m^2 treatment plot, composited, gently homogenized by hand in the field, and stored in gas-permeable bags at ambient temperature. On the same day as collection, the soil samples were transported to the University of Illinois at Urbana-Champaign (UIUC) for stable isotope pool dilution assays of gross N cycling rates the next day. A subsample of each soil was frozen at -20ºC on the same day as collection. Subsamples were freeze-dried within three weeks of collection for later DNA and elemental analysis.

Soil chemical properties

Soils were extracted in 2M KCl to determine soil inorganic N concentrations. Soil KCl extractions were analyzed via colorimetric methods using a SmartChem 200 discrete analyzer (Unity Scientific, Milford, MA, USA). Gravimetric soil moisture was assessed by oven-drying another subsample of each soil at 105ºC for 24 hours, pH was determined using a 1:2 ratio of soil : 1 M KCl. We removed rocks, fine-roots, and coarse litter from freeze-dried subsamples of each soil by hand, sieved soils at 2mm, then pulverized soils via bead-beating. Soil samples were analyzed for C and N concentrations using a Vario Micro Cube elemental analyzer (Elementar, Hanau, Germany) interfaced with an IsoPrime 100 isotope ratio mass spectrometer (Cheadle Hulme, UK).

Gross nitrogen cycling rate measurements

We used the ^{15}N pool dilution and tracer techniques to measure gross rates of soil N transformations and to source partition net N_2O fluxes between nitrification and denitrification (Hart et al. 1994, Yang et al. 2017, Krichels et al. 2019). Using $^{15}NH_4^+$ addition, we quantified gross N mineralization, NH_4^+ assimilation, and nitrification-derived N_2O fluxes. Using $^{15}NO_3^-$ addition, we quantified gross nitrification rates, microbial NO_3^- assimilation, and denitrification-derived net N_2O fluxes. The day after samples were collected, soils were subsampled for three different ^{15}N treatments: control with no ^{15}N label addition, $^{15}NH_4^+$ addition, and $^{15}NO_3^-$ addition. Samples from each location were collected within one month prior to each experiment and used in determining concentrations of $^{15}NH_4^+$ and $^{15}NO_3^-$ solutions used for enrichment such that ^{15}N enrichment would represent ~10% of the overall respective N pool. After adding 2 mL ^{15}N label solution to a plastic bag containing 150 g soil, ^{15}N label was gently incorporated by massaging

the bag by hand to evenly distribute the ^{15}N solution. After 15 minutes, soil subsamples were extracted in 2M KCl to determine the initial ^{15}N pool size. The remaining soil was transferred to a pint mason jar and sealed with an airtight lid fitted with a butyl rubber septa for gas sampling. Several ambient air samples were collected to characterize the initial concentration and N isotopic composition of N_2O in the jar headspaces. After 4 hours of incubation, two gas samples were collected from each mason jar to determine the final headspace N_2O concentration using gas chromatography via Shimadzu GC-2014 gas chromatograph (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with an electron capture detector and the final ^{15}N-N_2O isotopic composition using an IsoPrime 100 isotope ratio mass spectrometer interfaced to an IsoPrime trace gas preconcentration unit (Cheadle Hulme, UK) and a GX-271 autosampler (Gilson, Inc., Middleton, WI). After collecting gas samples, a subsample of soil from each mason jar was extracted in 2M KCl for inorganic N concentrations, as well as 0.5M K_2SO_4 with and without a direct chloroform addition (Brookes et al. 1985) to estimate total dissolved N (TN) and microbial biomass N (MBN) based on an extraction efficiency of 0.52 (Brookes et al. 1985). Soil K_2SO_4 extracts were digested using persulfate oxidation, then analyzed colorimetrically for NO_3^- concentrations as previously described for KCl extracts. Both KCl and digested K_2SO_4 soil extracts were prepared for ^{15}N isotopic analysis using acid-trap diffusion (Brooks et al. 1989), and filter disks were analyzed for ^{15}N isotopic composition as previously described for solid soil samples. Microbial assimilation of NH_4^+ and NO_3^- were only measured in the early season, rosette stage as this was the time when plant-microbial competition for N was expected to be highest (Jaeger III et al. 1999). Gross N mineralization and nitrification rates were calculated according to Hart et al. (1994). Microbial NH_4^+ and NO_3^- rates were calculated according to

Yang et al. (2015). Nitrification- and denitrification-derived net N_2O fluxes were calculated according to Krichels et al. (2019).

We also measured soil N loss via NH_4^+ and NO_3^- leaching using 5 cm diameter resin lysimeters installed at 50 cm soil depth in July 2018 and retrieved in March 2019. To make the lysimeters, PVC cylinders were filled with 15g AmberLite mixed bed ion exchange resin (MB 20; Sigma-Aldrich) between two layers of nylon mesh, with acid-washed sand packed on both openings of the lysimeter. Two lysimeters were installed in each experimental plot as technical replicates. Following lysimeter retrieval, the resin was extracted using a 2M NaCl solution; extract NH_4^+ concentrations were measured colorimetrically via the salicylate method, and extract NO_3^- concentrations were measured via the Vanadium (III) method on a SpectraMax M2 plate reader (Molecular Devices, San Jose, CA).

DNA extraction, sequencing, identification, and assignments

We used high-throughput DNA sequencing to determine the impact of garlic mustard on microbial community structure as well as the relative abundance of key functional groups and individual taxa. DNA was extracted from 500 mg of freeze-dried soil using the FastDNA SPIN Kit for Soils (MP Biomedicals, Santa Ana, USA). The extracts were purified using cetyl trimethyl ammonium bromide (CTAB). DNA extracts were submitted to the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign for Fluidigm amplification (Fluidigm, San Francisco, USA) and Illumina sequencing (Illumina, San Diego, USA). We assessed fungal communities via the *ITS2* gene region using ITS3 and ITS4 (Ihrmark et al. 2012) primers to amplify DNA and sequence amplicons via MiSeq bulk 2 x 250 bp V2. As we were interested in the broad fungal community effect of garlic mustard invasion, we chose to

use a universal ITS2 barcode region (Schoch et al. 2012). However, this barcode is known to discriminate against early-diverging fungal lineages, such as arbuscular mycorrhizal fungi (Stockinger et al. 2010), which we therefore excluded from our analysis. We assessed bacterial and archaeal communities via the bacterial and archaeal *16S* rRNA genes. Samples were amplified using Arch519 forward and BAC785 reverse primers and amplicons were sequenced via NovaSeq 2 x 250 bp. Primer sequences and references can be found in Table B.2. Sequence data is publicly available from the NCBI SRA database under accession number PRJNA690109.

We used the DADA2 pipeline (Callahan et al. 2016) for bioinformatic processing to produce amplicon sequence variants (ASVs) from the sequencing data. Briefly, quality filtering, denoising, merging forward and reverse reads, and removing chimeric sequences was performed using recommended parameters for *16S* and *ITS* genes. One sample did not pass quality filtering, thus a total of 71 samples were included for microbial analysis. We did not cluster sequence variants (Glassman and Martiny 2018) prior to using the default DADA2 classifier (Wang et al. 2007) to assign taxonomy based on reference sequences from the UNITE database (Kõljalg et al. 2013) for *ITS* sequences and the SILVA database (Quast et al. 2012) for *16S* sequences. Final count numbers were transformed via a Hellinger transformation (Legendre and Gallagher 2001) prior to further statistical analysis. Data used for analysis consisted of 3498 fungal, 61704 bacterial, and 516 archaeal ASVs. Due to the relatively low number of archaeal sequence variants, and because bacterial and archaeal sequences were derived from the same primer sets, we analyzed bacterial and archaeal communities together.

Statistical analysis

All statistical analyses were performed in the R statistical environment (R Core

Development Team 2013). Differences in soil properties and N cycling rates between garlic mustard treatments and over time were assessed using a two-way mixed effect ANOVA model with garlic mustard treatment, sampling time, and their interaction as fixed effects and forest as a random effect. To meet model assumptions of homogeneity of variance and normally distributed residuals, soil NH_4^+ concentrations, gross N mineralization rates, and nitrification-/denitrification-derived net N_2O fluxes were log-transformed. Linear models were used to assess the correlation between N cycling rates and soil properties. Post-hoc pairwise differences between treatments and between time points were determined using a Tukey test. Overall patterns of microbial beta diversity and community structure were analyzed with the VEGAN package (Oksanen et al. 2010). To determine differences in community composition based on garlic mustard treatment, sampling date, and their interaction, we performed a permutational analysis of variance (PERMANOVA) with the *adonis* function on Hellinger-transformed ASV count numbers stratified by forest to account for the large effect of physical location on microbial community composition. We also assessed differences in community composition and heterogeneity using a constrained principal coordinates analysis (PCoA), conditioned on forest with Bray-Curtis dissimilarity of relativized ASV count numbers. Differences in species diversity (Shannon's *H*), evenness (*H*/log(species #)), and in the relative abundance of functional groups saprotrophic, pathogenic, and ectomycorrhizal (ECM) fungal functional groups were determined using the same model described for soil properties. Fungal functional groups were assigned using the FUNGuild database (Nguyen et al. 2016b) on ASVs designated with a "probable" or "highly probable" confidence score for saprotrophic, pathogenic, or ECM classifications. To assess correlation of microbial community composition with environmental and nutrient cycling variables, we performed a redundancy analysis with the *envfit()* function in

VEGAN on PCoA ordinations of fungal and bacterial/ archaeal communities. Statistical significance was assessed as $p < 0.05$.

Results

Soil properties

Garlic mustard treatment and sampling date affected soil properties differently. Soil pH was significantly lower in garlic mustard absent plots ($p < 0.001$, Figure B.1, Table 1) relative to garlic mustard present and removed, and did not differ among sampling dates. Soil moisture declined as the growing season progressed ($p < 0.001$, Figure B.1, Table 1), and was lower in garlic mustard present plots than absent or removed ($p = 0.01$). Soil C:N ratios did not significantly differ among garlic mustard treatments, sampling dates, or their interaction.

Soil NH_4^+ and MBN changed over the growing season while soil NO_3^- did not, yet none were significantly affected by garlic mustard treatment. Soil NH_4^+ concentrations were greatest in the early growing season, significantly declining from the rosette (May) into the flowering stages (June; $p < 0.001$) and remaining consistent between the flowering (June) and senescent (August) stages (Table 1). Microbial biomass N was significantly reduced during flowering relative to the rosette and senescent stages ($p = 0.01$, Table 1). Soil NO_3^- concentrations did not differ significantly among garlic mustard treatments of sampling dates.

Gross nitrogen cycling rates

Overall, garlic mustard accelerated gross N cycling rates, though there were differences among phenological stages. Gross N mineralization rates were lower in garlic mustard absent plots than present or removed during the late season, senescent stage ($p = 0.02$; Figure 1a), but

the highest overall rates across all garlic mustard treatments were in the early season, rosette stage ($p = 0.004$, Table 1). Gross nitrification rates were lower in garlic mustard absent plots than those with garlic mustard present or removed ($p = 0.02$) and did not differ over time (Figure 1b). Similarly, nitrification-derived net N_2O fluxes were lowest overall in garlic mustard absent plots ($p = 0.04$; Figure 2c), and were highest among all treatments during the early season, rosette stage. Both total and denitrification-derived net N_2O fluxes were greater during the early season, rosette stage than later phenological stages ($p = 0.002$), but were not significantly different between garlic mustard treatments (Figure 1c). Microbial assimilation of NH_4^+ and NO_3^- did not differ among garlic mustard treatments.

These garlic mustard and time point differences in N cycling rates were correlated with differences in environmental factors potentially affected by garlic mustard legacy or growing season conditions. Garlic mustard effects on soil pH may have promoted its effect on gross N mineralization and nitrification rates because as soil pH increased, so did both gross N mineralization ($R^2 = 0.16$, $p < 0.001$, Figure 2a) and gross nitrification rates ($R^2 = 0.17$, $p < 0.001$, Figure 2b). Denitrification-derived net N_2O fluxes exponentially increased as soil moisture increased ($R^2 = 0.14$, $p < 0.001$, Figure 2c), corresponding with seasonal declines in soil moisture. Soil inorganic N loss through leaching did not differ significantly among garlic mustard treatments.

Microbial communities

Fungal and bacterial/archaeal communities shifted under garlic mustard presence and also over time. Fungal community composition differed significantly among garlic mustard treatments ($p = 0.002$, Table 2, Figure 2a). While overall communities differed between

sampling dates ($p = 0.001$, Table 2, Figure 2b), the effect of garlic mustard treatment did not change over time. Bacterial/archaeal communities exhibited a similar relationship with differences among garlic mustard treatments ($p = 0.001$) and among sampling dates ($p = 0.001$, Table 2, Figure 2cd), but no interaction between treatment and time. Visual inspection of constrained ordinations for both fungal and prokaryotic communities suggests garlic mustard presence restricted microbial community composition relative to garlic mustard absent or removed treatments (Figure 2). However, there was no significant effect of garlic mustard treatment on fungal or bacterial/archaeal diversity or evenness (Table B.3), though bacterial diversity increased over the course of the growing season ($p = 0.03$, Table B.3). Additionally, garlic mustard treatment did not significantly affect the relative abundance of fungal saprotrophic, pathogenic, or ECM functional guilds, but ECM abundance increased over the growing season ($p = 0.03$, Table 2).

Microbial community composition significantly correlated with environmental and nutrient cycling variables, with key differences between fungal and bacterial/archaeal communities. Both fungal and prokaryotic partial ordinations significantly correlated with soil pH, gross N mineralization rates, and TN ($p < 0.05$, Table 3, Figure 4ab), while only fungal communities correlated with soil NH_4^+ concentrations ($p = 0.001$, Table 3, Figure 4a) and only bacterial/archaeal communities correlated with soil moisture, gross nitrification rates, and MBN ($p < 0.05$, Table 3, Figure 4b).

Discussion

Invasive garlic mustard alters nutrient availability where it invades (Rodgers et al. 2008b, Anthony et al. 2017), but the mechanisms underlying this invasion effect have remained uncertain. We found that garlic mustard accelerated gross N mineralization and nitrification

rates, potentially through increasing soil pH or manipulating microbial communities. Though we did not observe garlic mustard decreasing microbial diversity or allelopathic effects on fungal functional guilds as has been reported in forests with older, denser garlic mustard invasions (Rodgers et al. 2008a, Anthony et al. 2017), we did find these community differences corresponded to changes in soil properties and N cycling rates. Overall, our finding supported the proposed mechanisms that garlic mustard associated differences in soil N cycling corresponded to increases in soil pH and changes to microbial communities, likely with some interaction between these factors. However, we did not find evidence to support garlic mustard manipulating nutrient conditions via litter input or nutrient uptake to affect N cycling rates, based on soil C:N ratios, inorganic N pools, and microbial N assimilation. Our results suggest that invasive garlic mustard could affect both soil biotic and abiotic properties associated with accelerated N cycling. These changes may help promote garlic mustard invasion by creating more favorable soil N dynamics for garlic mustard in its non-native habitats.

The evidence we found of increased soil pH where garlic mustard was present or had been recently removed, and the correlation pH with increased nitrification and denitrification rates supports our hypothesized soil pH mechanism for garlic mustard effects on soil N cycling. However, as pH did not differ between garlic mustard present and removed treatments, this leads to the question as to whether garlic mustard invasion caused shifts in soil pH, or existing microsite differences in pH promoted garlic mustard establishment in certain higher-pH soils. Rodgers et al. (2008) found garlic mustard-associated increases in soil pH were not correlated with higher overall measures of soil fertility, suggesting garlic mustard did not simply prefer higher pH soils, but was causing increases in soil pH. In a reciprocal planting study with soils ranging from low (4.2) to medium (6.8) pH, Anderson and Kelley (1995) found garlic mustard

growth was positively correlated to soil pH, but that soil pH was always higher after garlic mustard plant had been grown in it. Additionally, as soil pH increases, garlic mustard's effect on microbial community homogenization increases (Anthony et al. 2019), likely further promoting its competitive advantage through mediating microbial communities. While there is likely some relationship between garlic mustard establishment and higher relative soil pH, the evidence we provide here is further support that garlic mustard may in fact be directly manipulating soil pH to modify soil microbial communities and nutrient cycling. If garlic mustard is indeed increasing pH, the evidence we present suggests that this effect does not dissipate after garlic mustard is removed. This legacy effect of garlic mustard on soil pH may contribute to the lack of recovery of soil microbiomes that has previously been seen following garlic mustard removal (Lankau et al. 2014, Anthony et al. 2019). This was supported in our study by the strong correlation between pH and partial fungal ($R^2 = 0.23$) and prokaryotic ($R^2 = 0.41$) community ordinations. Manipulative experiments in which garlic mustard treatments are experimentally established, rather than observing existing populations, are needed to definitively establish the linkage between soil pH increases and accelerated soil N cycling.

We found soil microbial community composition to differ in response to invasive garlic mustard populations, potentially also contributing to its associated impact on soil nutrient cycling. Garlic mustard has been hypothesized to modify ecosystem function through its roots by altering microbial communities. Not only is garlic mustard often associated with declines in mycorrhizal abundance (Stinson et al. 2006, Wolfe et al. 2008), but they have also been found to increase the relative abundance of fungal saprotrophs (Anthony et al. 2017, Anthony et al. 2019), potentially increasing rates of decomposition. While we observed ordination shifts in garlic mustard associated microbial communities consistent with previously reported homogenization

effects of garlic mustard (Anthony et al. 2017), we did not find corresponding declines in microbial diversity (Lankau 2011, Edwards et al. 2022). Furthermore, the differences we found in microbial communities from garlic mustard were not caused by changes to the relative abundance of fungal functional guilds, like ECM fungi or saprotrophs. It is possible, however, that there were physiological changes to the colonization, biomass, or germination of these guilds that we would not have been able to measure with our DNA-based approach. It may also be that there were impacts of these garlic mustard populations constrained specifically to arbuscular mycorrhizal fungi that would be difficult to detect using our methodology. As arbuscular mycorrhizal fungi are also thought to interact with surrounding fungal and bacterial communities (Artursson et al. 2006, Van Der Heijden and Scheublin 2007), the microbial community shift in response to garlic mustard may have been a product of its impact on arbuscular mycorrhizal fungi. Microbial community partial ordinations corresponded with several important N cycling variables, particularly gross N mineralization rates for both fungi and prokaryotes, and gross nitrification rates for prokaryotes. As mineralization is facilitated by multiple microbial domains (Fraterrigo et al. 2006), while nitrification is thought to be almost exclusively driven by bacteria and archaea (Isobe et al. 2011), this pattern may indicate garlic mustard specifically modifying these domains differently to promote certain N cycling processes. The relationship between fungal communities and soil NH_4^+ concentrations may help to explain the lack of independent garlic mustard effects on soil N pools, as garlic mustard has been shown to alter resource economic strategies for competitors without differences in overall soil nutrient pools (Bialic-Murphy et al. 2021). The changes to soil pH and microbial community composition associated with N cycling we document here supports a more root-driven model of garlic mustard associated effects on ecosystem processes. That these effects did not dissipate after

garlic mustard removal potentially indicates that garlic mustard legacy may have more severe long-term consequences for recipient ecosystems.

While other invasive species have been found to alter soil nutrient cycling through litter inputs or resource acquisition (Liao et al. 2008, Castro-Díez et al. 2014), we did not find evidence of garlic mustard accelerating soil N cycling through this mechanism. For garlic mustard, the incorporation of nutrient-rich litter from deceased first-year plants early in the growing season or senesced adult plants later in the growing season has been shown to alter nutrient dynamics in soils it invades (Rodgers et al. 2008b, Heckman and Carr 2015). However, bulk soil C:N ratios were not affected by garlic mustard treatment in our study, suggesting that garlic mustard associated differences in N cycling rates were not related to litter inputs based on evidence from soil nutrient stoichiometry. Higher quality garlic mustard litter inputs may have altered patterns in microbial turnover, leading to greater contributions of mineral-associated organic matter (MAOM; Córdova et al. 2018). Altered patterns of MAOM accumulation and composition from garlic mustard litter may have affected N cycling processes by stimulating microbial decomposers, though further research is needed to support this mechanism. Additionally, garlic mustard's emergence early in the growing season has been shown to reduce resource competition with native plants (Engelhardt and Anderson 2011b), but could promote increased competition for N with soil microbes during this period. We did not observe differences in soil inorganic N pools or microbial N assimilation rates, a potential microbial response to decreased N availability, during the early growing season, thus garlic mustard's early-season N acquisition is unlikely to be driving overall patterns in its effect on soil nutrient cycling. Further, as the late-season, senescent stage N mineralization rates were similar between garlic mustard present and removed plots, it does not seem that direct inputs from garlic mustard

plants were necessary to produce this effect. Finally, we did not find a significant correlation between soil C:N ratios and microbial community composition indicating that changes to this soil property were not necessary for garlic mustard to alter soil microbiomes. Based on the lack of impact of garlic mustard invasions on soil nutrient stoichiometry, inorganic N pools, and early season gross microbial N assimilation rates, we do not find support for garlic mustard litter inputs or nutrient acquisition effects on soil N cycling.

In conclusion, garlic mustard may accelerate soil N cycling rates in invaded forests via increases in soil pH and changes to soil microbial communities. Further, some effects associated with garlic mustard are not consistent over its phenology, suggesting garlic mustard could promote different processes at different points in its life history. We also showed that garlic mustard removal is unlikely to remediate its net ecosystem effects, at least in the short term, indicating that garlic mustard invasions may have a lasting legacy on ecosystems. This study provides greater evidence of belowground mediated pathways for invasive plants to alter native ecosystems by synergistically affecting both biotic and abiotic soil properties to support its invasion success. These biotic and abiotic mechanisms highlight the myriad of invasive species traits that can work synergistically to influence recipient ecosystem and potentially promote invasion success.

Tables and Figures

Variable	Residual df	Treatment		Time		Treatment*Time	
		F-value	p-value	F-value	p-value	F-value	p-value
pH	56	**9.22**	**<0.001**	0.61	0.54	0.20	0.94
Moisture	56	**4.81**	**0.01**	**24.10**	**<0.001**	0.71	0.59
C : N	54	1.52	0.23	2.11	0.13	0.43	0.78
NH_4^+	53	0.18	0.83	**28.47**	**<0.001**	0.09	0.98
NO_3^-	56	0.15	0.85	1.69	0.19	0.84	0.50
Microbial biomass nitrogen	56	1.45	0.24	**4.34**	**0.01**	0.14	0.96
Mineralization	52	0.43	0.85	**6.07**	**0.004**	**2.97**	**0.02**
Nitrification	53	**4.02**	**0.02**	0.57	0.56	1.20	0.32
Nitrification-N_2O	50	**3.34**	**0.04**	**26.1**	**<0.001**	1.69	0.16
Denitrification-N_2O	50	0.40	0.66	**7.03**	**0.002**	0.58	0.68
Net N_2O	56	0.69	0.50	**7.21**	**0.001**	0.66	0.61

Table 3.1. F- and p-value results from 2-way mixed effects ANOVA models based on garlic mustard treatment, sampling time, and their interaction as fixed effects, with sampling site (forest) as a random effect. Numerator degrees of freedom for treatment and time was 2 and for their interaction was 4. Bolded values indicate significant relationships of $p < 0.05$.

Community	Fixed effect	df	F-value	R^2	p-value
Fungi	Treatment	2	**1.03**	**0.03**	**0.002**
	Time	2	**1.14**	**0.03**	**0.001**
	Treatment*Time	4	0.70	0.04	1.000
Bacteria/Archaea	Treatment	2	**0.98**	**0.03**	**0.001**
	Time	2	**1.15**	**0.03**	**0.001**
	Treatment*Time	4	0.79	0.04	1.000

Table 3.2. Results from a PERMANOVA test with garlic mustard treatment, time, and their interaction as fixed effects and samples stratified by forest. Bolded values indicate significant relationships of $p < 0.05$.

Variable	Fungi		Bacteria/Archaea	
	R^2	p-value	R^2	p-value
pH	**0.23**	**0.002**	**0.41**	**<0.001**
Moisture	0.06	0.18	**0.11**	**0.04**
NH_4^+	**0.24**	**0.001**	<0.001	0.99
NO_3^-	0.05	0.26	0.07	0.13
Mineralization	**0.22**	**0.002**	**0.19**	**0.007**
Nitrification	0.02	0.54	**0.25**	**0.002**
Denitrification	0.06	0.15	0.002	0.93
Net N_2O flux	0.07	0.12	0.002	0.95
C : N	0.03	0.41	0.09	0.08
TN	**0.11**	**0.04**	**0.28**	**<0.001**
MBN	0.08	0.08	**0.31**	**<0.001**

Table 3.3. Redundancy analysis for correlation of environmental and N-cycling variables with constrained PCoA ordinations for fungal and bacterial / archaeal communities. Bolded values indicate significant relationships of $p < 0.05$.

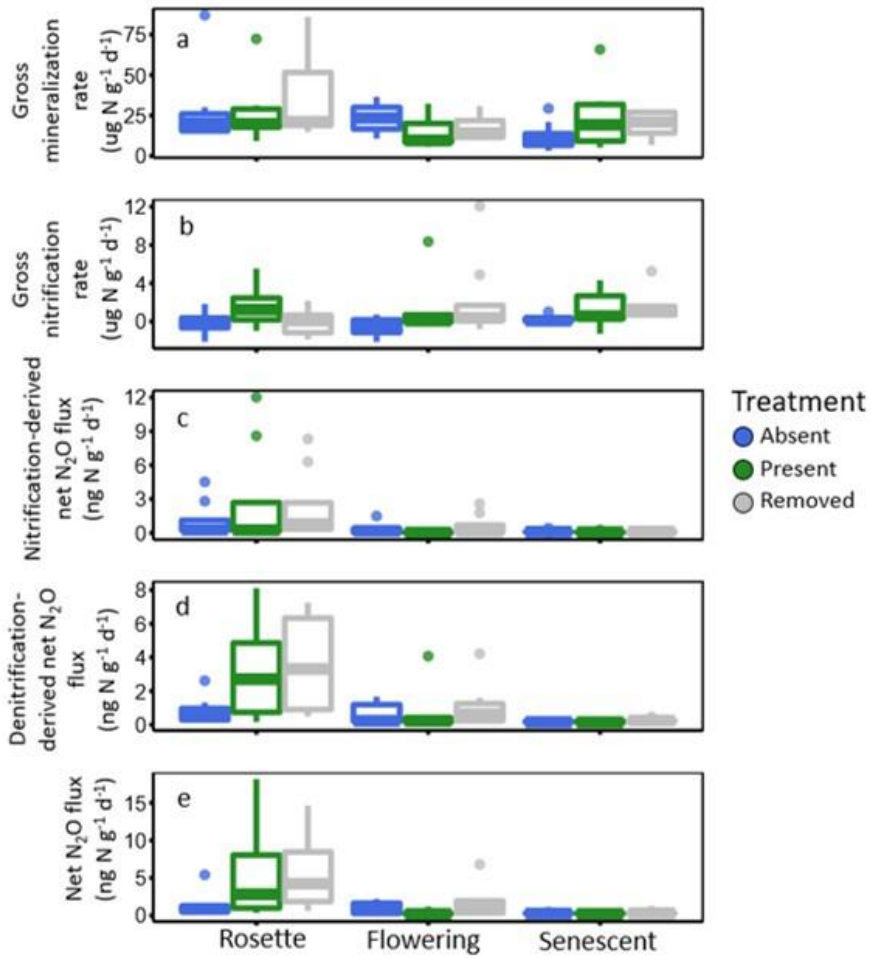

Figure 3.1. N-cycling process rates and net N_2O flux among garlic mustard treatments at different time points.

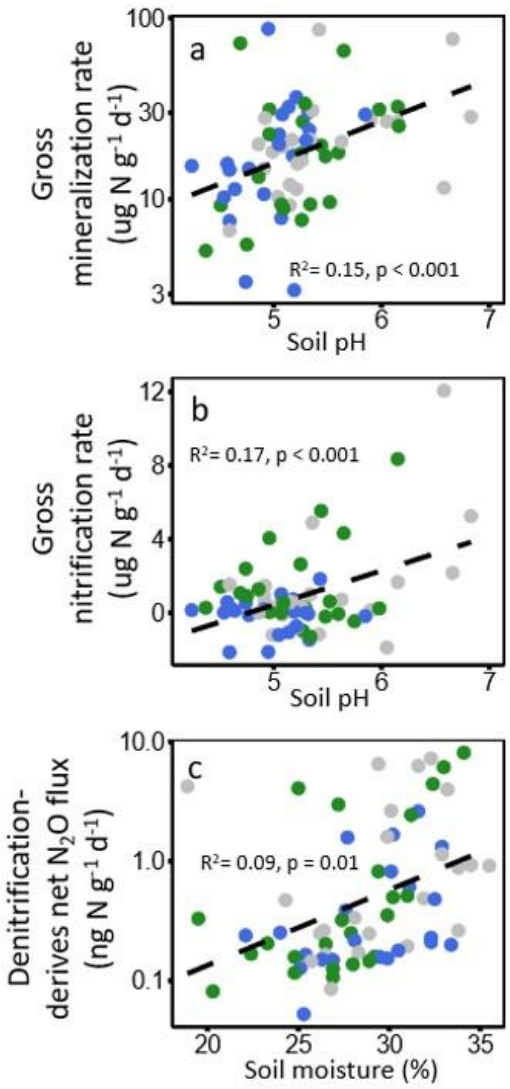

Figure 3.2. Correlations between N-cycling processes and environmental parameters.

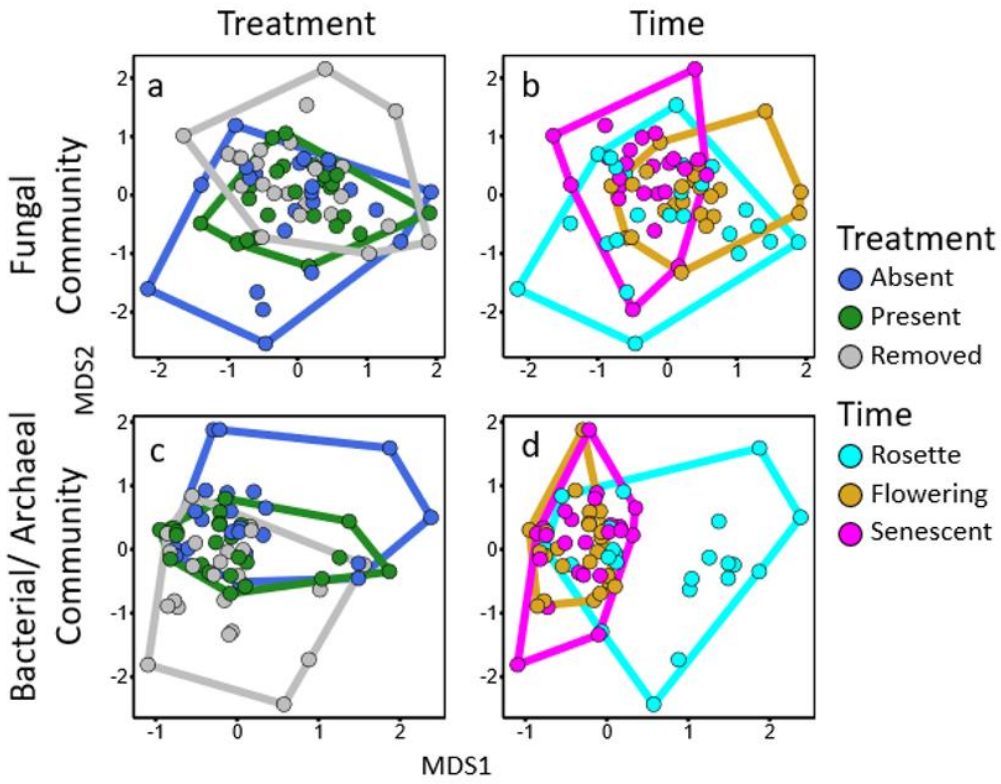

Figure 3.3. Constrained principal correspondence ordinations conditioned on site for Bray-Curtis dissimilarities of relativized ASV count numbers for fungal (ab) and bacterial/ archaeal (cd) communities. Polygons represent convex hulls indicating the extent of each treatment group for garlic mustard treatment (ac) and time point (bd). MDS 1 and 2 for fungal communities explain 11.7% and 9.0% of variation in community dissimilarity, respectively, and MDS 1 and 2 for bacterial / archaeal communities explain 3.5% and 3.0% of variation in community dissimilarity., respectively.

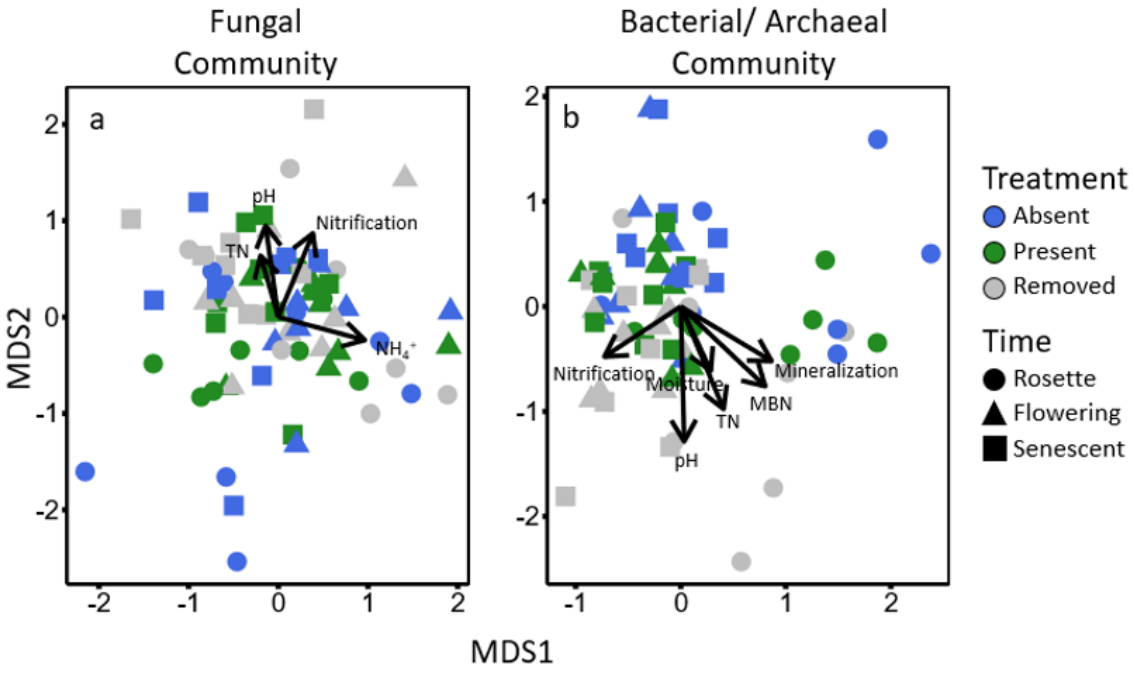

Figure 3.4. Vector scaling analysis of environmental and N-cycling variables against constrained PCoA ordinations for fungal (a) and bacterial / archaea (b) communities. Arrows represent variables with significant correlations to microbial community ordinations, with the length of each arrow roughly proportional to the R^2 of its RDA correlation.

CHAPTER 4: CRITICAL INTERACTIONS AMONG MULTI-TROPHIC RELATIONSHIPS AND PLANT NUTRIENT ACQUISITION FROM SOIL ORGANIC MATTER

Introduction

Forests are sustained through interconnected relationships between plants, microbes, and soils (Binkley and Fisher 2019). Plants can acquire nutrients from soil organic matter (SOM) through symbiotic relationships with microbial communities (Miller and Cramer 2005, Kraiser et al. 2011). By supplying soil microbiota, such as mycorrhizal fungi or rhizosphere prokaryotes, with photosynthate carbon (C), plants can direct microbiome assembly to promote favorable nutrient cycling processes (Jones et al. 2004, Paterson et al. 2007), particularly for soil nitrogen (N; Mushinski et al. 2021). However, the relative importance of fungal and bacterial communities in facilitating N acquisition for plants from SOM is unclear (Bahram et al. 2020). Emerging evidence suggests that rather than plant-fungal or plant-bacterial interactions prevailing, plant N acquisition may be supported by a multi-partite system of interactions with surrounding soil microbial communities (Hestrin et al. 2019, Emmett et al. 2021). In this context, fungal communities, which are often strongly influenced by mycorrhizal associations (Frey 2019), mediate relationships between plants and surrounding soil bacterial communities to form a "multi-trophic" system for N acquisition from SOM. These multi-trophic relationships in support of plant N acquisition likely have broad effects for biogeochemical aspects of ecosystem function (Wagg et al. 2019), but the factors structuring these relationships remain poorly understood.

Trees species form relationships with symbiotic and free-living soil microbiomes (Berendsen et al. 2012) to drive ecosystem processes like C and nutrient cycling (Baldrian 2017). These relationship aid in plants acquiring N from SOM through direct transfers from mycorrhizal fungi or N mineralized by fungi or prokaryotes. While fungal communities are generally considered primary drivers of soil C cycling (Schimel and Schaeffer 2012), bacteria are responsible for the majority of biological N transformation processes (Philippot et al. 2007, Reed et al. 2011). These divergent roles in soil biogeochemical dynamics likely indicate important interactions between soil fungi and bacteria to structure overall ecosystem C and N cycling. Environmental factors such as precipitation and soil pH (Fierer et al. 2003, Hawkes et al. 2011) often drive patterns in microbial community structure and function at broad geographic scales, but local differences are generally more aligned with plant community composition (Bardgett and Wardle 2010). Interactions between plants and microbial communities can affect the composition of SOM (Eskelinen et al. 2009), particularly mineral-associated organic matter (MAOM) and particulate organic matter (POM). Changes in the abundance and composition of MAOM and POM fractions can inform as to the processes and organisms affecting SOM dynamics (Cotrufo et al. 2019, Sokol et al. 2019). Additionally, relieved N limitation can lead to shifts in the extracellular enzyme activities (EEAs) that degrade SOM corresponding with changes in fungal and bacterial community composition (Carrara et al. 2018). While these relationships are likely influenced by other environmental factors, like pH (Averill and Waring 2018), plant nutrient acquisition also likely plays a role in microbially-mediated ecoenzymatic nutrient liberation from SOM (Sinsabaugh et al. 2009, Waring et al. 2014). As plant-microbial interactions are also closely aligned with plant nutritional strategies (Bahram et al. 2020), it is

likely that plant N acquisition from SOM is a strong structural force in shaping ecosystem biogeochemical processes.

Mycorrhizal frameworks can be used to predict the magnitude and direction of plant-microbe-soil relationships, but other interactions between mycorrhizal fungi and surrounding microbiomes are often poorly accounted for in these frameworks (Phillips et al. 2013, Terrer et al. 2016, Crowther et al. 2019). Mycorrhizal fungi are associated with broad patterns in overall fungal community composition, with soils abundant in arbuscular mycorrhizal (AM) fungi having greater fungal diversity and saprotroph richness than those with greater ectomycorrhizal (EM) fungal abundance (Eagar et al. 2021). However, mycorrhizal fungi also demonstrate complex interactions with soil bacteria and other free-living saprotrophs. For example, AM fungi can form cooperative relationships with bacterial communities to aid in SOM decomposition (Nuccio et al. 2013) while EM fungi can competitively suppress bacterial production of SOM-degrading extracellular enzymes (Brooks et al. 2011). Further, the outcomes of these mycorrhizal-bacterial relationships for broader microbiome community assembly seem to differ based on the mycorrhizal species present (Founoune et al. 2002, Emmett et al. 2021). Differing patterns of cooperation or competition between mycorrhizal fungi and free-living fungal and bacterial communities in rhizosphere soils (Kiers and Denison 2008) can influence C and N cycling in surrounding bulk soils (Averill et al. 2014, Moore et al. 2015). Interactions between mycorrhizal fungi and surrounding microbiomes may also explain variation type in SOM dynamics, like MAOM vs POM accumulation, across forests in different locations dominated by the same mycorrhizal (Craig et al. 2018, Keller et al. 2021). Further, as inorganic N availability increases interactive responses in microbial communities and SOM-degrading EEAs manifest differently between ecosystems dominated by different mycorrhizal types (Carrara et al. 2021).

These divergent patterns in microbial-associated ecoenzymatic responses between mycorrhizal regimes suggests multi-trophic interactions may play a role in facilitating N acquisition from SOM. While mycorrhizal fungi are commonly considered important ecosystem architects (Smith and Read 2010), the multi-trophic relationships promoting this function remain underexplored.

Here we sought to elucidate the linkages between multi-trophic relationships and plant N acquisition from SOM. We hypothesized that as the need of plant to acquire N from SOM via microbially-mediated mechanisms decreases, the role of these relationships (particularly plant-fungal) in structuring the overall ecosystem will decline. We leveraged a long-term N fertilization experiment in mixed EM-AM stands in a montane tropical forest in the Fortuna Forest Reserve of western Panama. By evaluating N addition effects on fungal and bacterial community composition, SOM properties, EEAs, and the correlations among these variables, we can understand how these relationships change with decreased plant dependence on SOM-N acquisition. We sampled under three focal tree species that represent variation in mycorrhizal association of trees and the litter chemistry of trees, which can affect microbial community composition and soil C and N cycling. We expected plant species to structure fungal community composition under ambient conditions, but for these relationships to weaken with increased inorganic N availability, as plants invest lower C allocation to their mycorrhizal partners (Figure 1). We also expect fungal community structure to correlate to bacterial community structure, SOM properties, and EEAs under ambient conditions, but for these relationships to weaken with increased inorganic N availability (Figure 1).

Methods

Study site

The study was conducted in an old-growth montane forest in the Quebrada Honda watershed of the Fortuna Forest reserve in the Chiriqui province of western Panama. Background site information (i.e. geographical coordinates, elevation, precipitation, temperature, background N deposition, soil type, etc.) can be found in Table B.1. The site is a highly diverse forest, with over 120 tree species, the most abundant of which are the ECM-associated species, *Oreomunnea mexicana*, and the AM-associated species, *Eschweilera panamensis* and *Micropholis melinoniana* (Prada et al. 2017). Nitrogen isotopic composition of leaf litter and soil organic matter across different watersheds in Fortuna that vary in soil fertility suggest that trees rely more on N transfer from mycorrhizal partners in the low fertility Honda watershed compared to the higher fertility watersheds (Seyfried et al. 2021).

Experimental design

In 2014 we sampled from a long-term N fertilization experiment established in February 2006. The experiment consists of four replicate pairs of 40 m by 40 m plots with N fertilization and control treatments randomly assigned to each member of the pair. Fertilizer is applied at a rate of 125 kg-N ha^{-1} y^{-1} of urea in four equal applications every three months. Short-term changes in aboveground net primary productivity (Adamek et al. 2009), soil trace gas fluxes (Koehler et al. 2009a, Koehler et al. 2009b), and gross soil nitrogen cycling rates (Corre et al. 2010a) in response to fertilization were documented in the first two years after the experiment was initiated. Recently, longer-term changes in the fungal community, soil physicochemical properties, and EEAs were reported (Corrales et al. 2017).

Using tree census data collected in the plots in 2013 (Dalling, unpublished data), we selected tree species with at least three individuals >20 cm diameter at breast height and growth rate > 5mm y^{-1} present in each plot. Two arbuscular mycorrhizal-associated species met those criteria: *E. panamensis* and *M. melinoniana*. The most abundant EM-associated species, *O. mexicana*, met those criteria in all plots except one N fertilized plot; thus, that plot was excluded from the study. These species differ in leaf litter chemistry, with *E. panamensis* having higher quality litter compared to *M. melinoniana* and *O. mexicana* based on nutrient and lignin content (Yang, unpublished data).

We randomly selected three individuals of each species within each plot as focal trees under which we collected soil samples (i.e., within 1 m from the base of the tree). We used a 2 cm diameter soil probe to collect soil from 0-10 cm depth from the soil surface (i.e., beneath the decomposing litter layer but starting from the organic soil horizon, if present). As the multi-tropic interactions we were primarily interested in here are more pronounced in surface soils (Lucas et al. 1993), we did not discriminate between organic and mineral horizons for this study. We accounted for this by explicitly including soil bulk density and POM into our analysis by using POM percentage of soil mass as an index of O horizon proportion in the surface soil to determine the relative effects of tree species and nutrient treatment on O horizon formation and how these factors influence plant-soil-microbe interactions. Soil taken from three random locations beneath a focal tree was composited in a sealed Ziploc bag for storage and transport. The soil was kept at ambient temperature for transport to the University of Illinois at Urbana-Champaign (UIUC) where the soils were assayed for activity suite of hydrolytic EEAs, C and N elemental and isotopic composition of bulk soil and soil density fractions, and soil pH, as well as processed for molecular analyses described here.

Soil DNA extraction, sequencing, and analysis

After transport to the laboratory, DNA was extracted from 0.5 g of freeze-dried soil using the FastDNA SPIN Kit for Soils (MP Biomedicals, Santa Ana, USA). The extracts were purified using cetyl trimethyl ammonium bromide (CTAB) as described in Edwards et al. (2018). DNA extracts were submitted to the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign for Fluidigm amplification (Fluidigm, San Francisco, USA) and Illumina sequencing (Illumina, San Diego, USA). We assessed fungal communities via the *ITS2* gene region using ITS3 and ITS4 (Ihrmark et al. 2012) primers to amplify DNA and sequence amplicons via MiSeq bulk 2 x 250 bp V2. We assessed bacterial and archaeal communities via the bacterial and archaeal *16S* rRNA genes. The samples were amplified using V4_515 forward and V4_806 reverse primers and amplicons were sequences via MiSeq bulk 2 x 250 bp V2. Primer sequences and references can be found in Table B.2. Sequence data is publicly available from the NCBI SRA database under accession number XXXXXXXX.

To assess the maximum possible variation in microbial communities, we generated amplicon sequence variants (ASVs) from our sequence data using the DADA2 bioinformatics pipeline. Sequences were quality filtered, denoised, forward and reverse reads were merged, and chimeric sequences were removed using recommended parameters for *16S* and *ITS* genes. Taxonomy was assigned via the naïve Bayesian DADA2 classifier, using the SILVA and UNITE reference databases for *16S* and *ITS* taxonomic assignments, respectively. Final count numbers were relativized with a Hellinger transformation prior to statistical analysis. Final datasets consisted of 1915 fungal, 43770 bacterial, and 645 archaeal ASVs, though due to being derived from the same primer sets, bacterial and archaeal communities were analyzed together. We assigned fungi to saprotrophic, pathogenic, and ectomycorrhizal functional guilds using the

FUNGuild database. Assignments with “probable” or “highly probable” confidence scores were included and in cases where taxa were assigned to more than one guild, mycorrhizal classification was given higher priority than pathogenic, which was given higher priority than saprotrophic (Smith et al. 2021).

Statistical analysis

Statistical analysis was performed in the R statistical environment. Patterns in beta diversity, community composition, and relationships between microbial communities and soil variables were analyzed with the VEGAN package. As we were primarily interested in describing the overall relationships between biogeochemical variables (i.e., SOM properties and EEAs) and biotic community structure (i.e., plant species, fungal ASVs, bacterial/archaeal ASVs), we primarily analyzed these variables in aggregate with sample dissimilarities of Z-transformed SOM properties and EEAs. Soil organic matter properties included in our analysis included soil bulk density, pH, isotopic composition and elemental concentrations of C and N, as well as relative abundance of MAOM (hf) and POM (fLF and oLF) fractions of SOM. We also grouped SOM-degrading EEAs: β-Glucosidase (BG); α-Glucosidase (AG); β-1,4-xylosidase (XYL), leucine amino peptidase (LAP), N-acetyl-β-D-glucosaminidase (NAG), and acid phosphatase (AP) together to observe the overall relationship of these enzyme activities with the other variables. Patterns in dissimilarity between focal tree species, N addition treatment, and their interaction on microbial communities, grouped SOM properties, and grouped EEAs were assessed with a PERMANOVA using the *adonis* function in VEGAN. Euclidian distance was used for SOM properties and EEAs while Bray-Curtis distance was used for microbial relative abundances. We then compared the separation of samples for each set of variables using

Principal Correspondence Analysis (PCoA) via the *capscale* function. To assess the correspondence of fungal communities, bacterial/ archaeal communities, soil properties, and EEAs we used a Procrustes analysis with the *protest()* function on each pair-wise combination of PCoA variable ordinations. We assessed the ordination resemblance among the various groups of variables in this study via a Procrustes test to better understand the interconnectedness of soil microbial communities, soil properties, and EEAs. Differences in the relative abundance, species richness, and diversity (Shannon's *H*) of saprotrophic, pathogenic, and EM fungal guilds among focal tree species, N addition treatments, and their interactions were determined using an ANOVA. PERMANOVA and Procrustes tests were performed on all data aggregated and on control and N addition datasets individually to assess differences in patterns of correspondence between experimental treatments. Significance was determined at $p < 0.05$.

Results

Microbial community and ecosystem dissimilarity and correspondence

We observed strong relationships among tree species, soil microbial community composition, and indices of ecosystem function, but these relationships manifested differently between control and N addition plots. Across all samples, we found a significant effect of tree species on dissimilarity patterns of fungal communities ($R^2 = 0.04$, $P = 0.001$), bacterial/archaeal communities ($R^2 = 0.05$, $P = 0.009$). Grouped SOM properties (i.e., bulk density, pH, isotopic composition and elemental concentration of C and N, as well as MAOM and POM percentages of bulk soil mass) also had significant dissimilarity among tree species ($R^2 = 0.10$, $P = 0.009$). Grouped hydrolytic EEAs (i.e., BG, NAG, XYL, AP, and LAP) were also significantly dissimilar among tree species ($R^2 = 0.13$, $P = 0.009$; Table 1, Figure 1). However, when control

and N addition plots were analyzed separately, a divergent pattern emerged. Fungal communities were the only variable to differ significantly among tree species in control plots ($R^2 = 0.08$, $P = 0.001$), whereas they did not differ among tree species in the N addition plots. Conversely, bacterial/archaeal community composition, SOM properties, and EEAs did not differ among tree species in control plots, whereas they differed among tree species in N addition plots (bacteria/archaea: $R^2 = 0.11$, $P = 0.013$; SOM: $R^2 = 0.25$, $P = 0.002$; EEAs: $R^2 = 0.36$, $P = 0.002$).

Soil-microbial relationships most affected by N addition were between fungal communities and EEAs. There was significant Procrustean resemblance among fungal communities, bacterial/archaeal communities, and SOM properties across all samples ($P < 0.01$; Table 3). However, while bacterial/archaeal communities and SOM properties also consistently corresponded to EEAs ($p < 0.009$; Table 3), fungal communities only corresponded to EEAs in control plots ($p = 0.004$; Table 3).

Fungal functional guild abundance

Relative abundance of pathogenic and EM fungal functional guilds significantly differed among tree species and in their interaction with N addition, but there were no significant effects of tree species or N addition on fungal saprotroph relative abundance. Fungal pathogen relative abundance was highest under *E. panamensis* in control plots ($p = 0.03$; Table 3; Figure 3b), but significantly increased under *M. melinoniana* with N addition ($p = 0.03$). *O. mexicana* consistently had the lowers pathogen relative abundance in control and N addition treatments ($p = 0.03$). Ectomycorrhizal relative abundance was significantly greater under *O. mexicana* than either of the AM-associated tree species ($P < 0.001$, Table 3, Figure 3c). We also found a

significant interaction between tree species and N addition treatment on EM fungal relative abundance ($P = 0.006$, Table 1, Figure 1A). Ectomycorrhizal relative abundance nearly doubled under fertilized *O. mexicana* trees compared to unfertilized, while there was a significant decrease in EM fungal abundance under *M. melinoniana* trees.

While EM fungal relative abundance was strongly affected by tree species, soil properties also influenced this fungal guild. When we accounted for soil pH, there was a significant three-way interaction between soil pH, tree species, and N addition treatment on EM fungal relative abundance ($P = 0.008$; Figure 2). In control plots, EM fungal relative abundance was most strongly correlated with soil pH ($R^2 = 0.63$, $P < 0.001$), with EM abundance decreasing as pH decreased across all tree species. In N addition plots, this pattern persisted for both AM-associated tree species, but under the EM-associated *O. mexicana* trees, EM abundance increased with soil pH instead (multiple $R^2 = 0.72$, $P < 0.001$; Figure 4a). We also found a similar relationship between EM fungal abundance and the proportion of soil organic C stored in MAOM, which declined significantly as EM fungal relative abundance increased in control plots ($P < 0.001$). However, like pH, this pattern diverged under *O. mexicana* trees in N addition plots such that the proportion of soil C in MAOM increased with EM fungal relative abundance ($p < 0.05$; figure 4b). Soil pH was strongly correlated with the proportion of soil C stored in MAOM ($R^2 = 0.48$, $P < 0.001$), but this relationship did not differ based on tree species or N addition treatment (Figure 4c).

Discussion

Our study supports the hypothesis that there are important linkages between multi-trophic interactions and plant N acquisition from SOM under more N-limited conditions. By observing

the response of these multi-trophic interactions to increased soil inorganic N availability, we found that fungal-mediated N acquisition from SOM can play a central role in promoting the directional relationship between trees, their fungal mutualists, and the surrounding soil ecosystem. When inorganic N availability increased with experimental N addition, fungal communities associated less strongly with focal tree species and soil biogeochemical processes. Plants may have responded to increased soil inorganic N availability by increasing root growth, rather than investing in mycorrhizal partners to directly acquire soil nutrients. This direct plant-SOM interaction would then have driven broader changes in bacterial communities and their metabolic processes via enzyme activities (Figure 1). Therefore, while it is unclear whether N availability was the primary limiting nutrient in this montane tropical ecosystem (Camenzind et al. 2018), the acquisition of N from SOM was nevertheless an important factor driving ecosystem structure. Overall, multi-trophic interactions play an important role in ecosystem processes facilitating the transfer of N from SOM pools to plants under certain environmental contexts. We found fungal communities can bridge interactions between trees and prokaryotic communities, influencing soil biogeochemical processes and SOM dynamics, but this ecological role is diminished when soil inorganic N availability in increased.

We found the linkage between tree species, microbiome composition, and indices of C and N dynamics were disrupted by increased N availability. While tree species was a significant predictor of fungal community composition under ambient conditions, N addition removed this effect. Conversely, tree species was only a significant predictor of bacterial/archaeal community composition and indices of ecosystem function in N addition plots, describing 11% of variation in bacterial/archaeal communities, 26% grouped SOM properties and 36% in grouped EEAs. This change could be due to decreased C allocation from plants to fungal mutualists under higher

N availability (Högberg et al. 2010, Gorka et al. 2019). As plant exudates have been found to promote mycorrhizal effects on surrounding fungal diversity (Hugoni et al. 2018), reduced C allocation from plants may also reduce mycorrhizal structuring of overall fungal communities (Allison 2012). However, EM fungal relative abundance increased under fertilized *O. mexicana* trees rather than decreased as expected with reduced plant C allocation. Alternatively, increased N availability may alter resource use patterns in fungal communities, decreasing the role of plant litter inputs in shaping fungal community composition (Fitch et al. 2020). Bacterial communities, SOM properties, and EEAs were likely more sensitive to species differences in belowground tree responses to N addition. For example, we saw the average % POM increase ~60% under *O. mexicana* and ~20 % under *E. panamensis*, but decreased 50% under *M. melinoniana*, though due to high variability about the means these differences were not significant (Yang, unpublished data). These differences in relative POM abundance in SOM could represent species-specific responses to increased inorganic N availability in above- or belowground plant litter input, as such patterns are prevalent among tropical tree species (Sun et al. 2018). Further, these plant resource allocation and litter traits are often informative as to the predicted response of rhizosphere and soil microbiomes and biogeochemical processes (Han et al. 2020). Under ambient conditions, tree species appear to directly mediate only fungal communities, but when inorganic N availability increases, fungal mediation is replaced by direct influence on bacterial communities, SOM properties, and EEAs. This pattern was likely indicative of the overall functional path of N acquisition (i.e., from SOM or inorganic N pools) in the ecosystem.

Nutrient addition altered the functional path for N acquisition among tree species, microbial communities, and ecosystem processes. The role of fungi in maintaining plant nutrition and structuring soil ecosystems is a major question in the face of global change (Kraiser et al.

2011, Allen and Allen 2017), particularly in understudied tropical ecosystems (Mohan et al. 2014). Following plant-derived C on a mycorrhizal-mediated path through the ecosystems, it appears that under ambient conditions tree species select for differential fungal communities to acquire N from SOM (Brzostek et al. 2014). Plant mycorrhizal associations have been shown to determine broader microbiome community assembly (Leake et al. 2004) and soil N cycling functionality (Mushinski et al. 2021). When SOM degradation was less necessary for plant N acquisition under experimental inorganic N addition, plant-fungal relationships as well as fungal-ecosystem relationships diminished, suggesting fungal community mediated plant N acquisition from SOM exerted influence on the surrounding ecosystem under more N-limited ambient conditions. Carrara et al. (2018) reported shifts in bacterial communities corresponding to changes in enzyme dynamics under N fertilization were concomitant to declines in AM root colonization, suggesting that AM fungi also lose their ability to mediate soil conditions when N limitation is relieved. Both EM and AM fungal communities shift with increasing concentrations of soil N (Jiang et al. 2018, Argiroff et al. 2021), thus their function in mediating N acquisition from SOM is an important component of both mycorrhizal strategies, though mediated through differing avenues and in different areas of the soil (Carrara et al. 2021).

As plant-microbial relationships become a growing part of predictive models (Sulman et al. 2019), it is increasingly important to realize the context dependence and unique aspects of multi-trophic and interactive plant-fungal-bacterial-soil ecosystem relationships. Mixed evidence for the relationship between microbiome composition and ecosystem function (Cusack et al. 2011, Kivlin and Hawkes 2020) in tropical ecosystems may be due to incomplete assessments or non-hierarchical structures of multi-trophic systems. This inconsistency is particularly keen in montane ecosystems where there is much uncertainty as to the spatial and temporal dynamics

governing nutrient limitation to productivity and ecosystem function (Townsend et al. 2011, Camenzind et al. 2018). While recent empirical and modeling approaches have supported the temperate framework for tropical ecosystems (Lu et al. 2021), there remains much debate as to the generalizability of this pattern particularly in relation to N deposition rates (Lu et al. 2014), affected C pools (Janssens et al. 2010), and microbial community response to nutrient deposition (Cusack et al. 2011). Additionally, while montane ecosystems are more likely to be N-limited due to increased erosion exposing younger soils and greater N loss through run-off (Corre et al. 2010b, Brookshire et al. 2012), P might be co-limiting at certain times or under various environmental conditions (Townsend et al. 2011, Camenzind et al. 2018). As anthropogenic N deposition increases, evidence from temperate forests suggests that the mycorrhizal regimes of these forests are likely to change as well (Averill et al. 2018). The response we observed in SOM dynamics and pathogen abundance under N addition may have been a plant response to the increased relative P limitation (Laliberté et al. 2015). As efforts to model and predict tropical forest responses to global change are severely constrained by uncertainty in the relative importance of N and P limitation for ecosystem NPP (Allen et al. 2020), further investigation into the constraints and structural forces promoted by these limitations is necessary.

Our finding that fungal communities are important drivers on plant N acquisition from SOM adds to a growing understanding of the context-dependency of fungal-mediated ecosystem syndromes (Verbruggen et al. 2013, Seyfried et al. 2021). Historically, fungi were thought to be the drivers of altered enzyme dynamics under increased N deposition (Waldrop et al. 2004), often by slowing overall C cycling and decomposition (Zak et al. 2008). However, as we found here, the role of fungi in multi-trophic relationships is to lessened under increased inorganic N availability, making room for prokaryotes to play a larger role. Although there were no changes

in the overall proportion of C stored in MAOM pools, a product of increased decomposition rates (Cotrufo et al. 2019), under N fertilization when accounting for soil pH, the relationship between fungal communities and soil MAOM C did change. Under more N-limited ambient conditions, the proportion of soil C stored in MAOM decreased linearly as EM fungal abundance decreased, consistent with conventional models of mycorrhizal-mediated soil organic matter dynamics (Craig et al. 2018). This relationship was mirrored with soil pH, where under more N-limited ambient conditions EM relative abundance increased as soils became more acidic, but not under N fertilization. When N limitation was decreased, soil MAOM C and pH remained correlated, but their relationships with EM fungal abundance changed, suggesting an interactive effect of environmental conditions and SOM biogeochemistry with EM fungi. This pattern demonstrates that microbiome composition can be a useful predictor of ecosystem function (such as potential C storage), but that this relationship is not consistent across all environmental contexts. Ectomycorrhizal fungi have a greater competitive advantage over free-living saprotrophs at lower pH, as the bioavailability of many macronutrients significantly declines below pH 5.0 (Truog 1947) and the anabolic capacity of microbial extracellular enzymes is diminished at greater soil acidity (Sinsabaugh et al. 2008, Liang et al. 2017). Increased soil acidity due to N fertilization is predicted to decrease decomposition and promote the accumulation of physically-protected POM (Averill and Waring 2018). However, this does not fully account for plant responses to increased N availability, particularly for root associated inputs. Here, we show that divergent plant-microbial responses to increased inorganic N availability may correlate with unique patters between soil pH and SOM among different species assemblages.

Here, we found tree species to develop stronger relationships with bacterial communities when N limitation was relieved, potentially though altered belowground C allocation by trees.

Trees are often thought to decrease belowground C inputs, like root exudates, in favor of aboveground biomass growth in response to increased N availability (Eastman et al. 2021), yet direct evidence for this phenomenon is limited (Canarini et al. 2019). If N addition decreases belowground C allocation, then we would expect to see a concomitant decline in mycorrhizal abundance; however, EM fungal relative abundance increased under *O. mexicana* trees with N addition. Seemingly counterintuitive increases in ECM abundance following N addition have been reported previously by Karst et al. (2021), who found increased EM fungal biomass without increases in lateral stem growth under fertilization. Indeed, the trees in these experimental plots (both EM and AM) did not have significantly higher growth rates in fertilized plots than unfertilized (Dalling, unpublished data), suggesting that N availability did not primarily limit NPP. We demonstrate an increased importance of prokaryotic communities and plant-prokaryote relationships under increased inorganic N availability. This relationship indicates a need for greater consideration of the context dependency of ecosystem processes structured by multi-trophic relationships in conceptual frameworks for predictive modeling and conservation policy as global N deposition increases (Kanakidou et al. 2016).

Conclusion

Here, we demonstrate that there are important connections between multi-trophic relations and plant N acquisition from SOM. When inorganic N availability increased, the role fungi played in connecting tree species to soil C and N cycling was diminished whereas the role of bacterial and archaeal communities is increased. This suggests a need to move beyond single kingdom paradigms of biogeochemical cycling to better account for multi-trophic relationships in structuring ecosystem function.

Tables and Figures

Treatment	Group	Species r^2 (p)	Trt r^2 (p)	Sp*trt r^2 (p)
All	Fungi	0.043 (0.001)	0.019 (0.047)	NS
	Bacteria/archaea	0.05 (0.009)	0.024 (0.048)	NS
	SOM	0.099 (0.009)	NS	NS
	Enzymes	0.125 (0.009)	NS	0.086 (0.036)
Control only	Fungi	0.078 (0.001)		
	Bacteria/archaea	NS		
	SOM	NS		
	Enzymes	NS		
N only	Fungi	NS		
	Bacteria/archaea	0.112 (0.013)		
	SOM	0.25 (0.002)		
	Enzymes	0.36 (0.002)		

Table 4.1. Results from a PERMANOVA test with tree species, N addition treatment, and their interaction as fixed effects and for tree species with control only and N addition only samples. Bray-Curtis distance was used for fungal and bacterial/archaeal communities and Euclidian distance was used for SOM properties and enzyme activities.

Procrustes (NMDS)	All			Control			N addition		
Group	Fungi	Bacteria/ Archaea	SOM	Fungi	Bacteria/ Archaea	SOM	Fungi	Bacteria/ Archaea	SOM
Bacteria	0.61 (0.001)			0.61 (0.001)			0.67 (0.001)		
SOM	0.42 (0.008)	0.68 (0.001)		0.38 (0.010)	0.77 (0.001)		0.50 (0.003)	0.53 (0.001)	
Enzyme	0.17 (NS)	0.39 (0.001)	0.37 (0.003)	0.39 (0.006)	0.43 (0.004)	0.40 (0.009)	0.27 (NS)	0.54 (0.001)	0.51 (0.002)

Table 4.2. Results from Procrustes test assessing the correspondence between ordinations of different microbial and ecosystem variable group for all samples, as well as control only and N addition only.

Fungal guild	Species (p-value)	Treatment (p-value)	Species*treatment (p-value)
% Saprotroph	0.22	0.16	0.13
% Pathogen	**0.03**	0.09	**0.03**
% Ectomycorrhizal	**<0.001**	0.788	**0.006**

Table 4.3. Results from a linear model on the abundance of different fungal guilds with the tree species, N addition treatment, and the interaction of species and treatment as fixed effects. Bolded values are significant at $p < 0.05$.

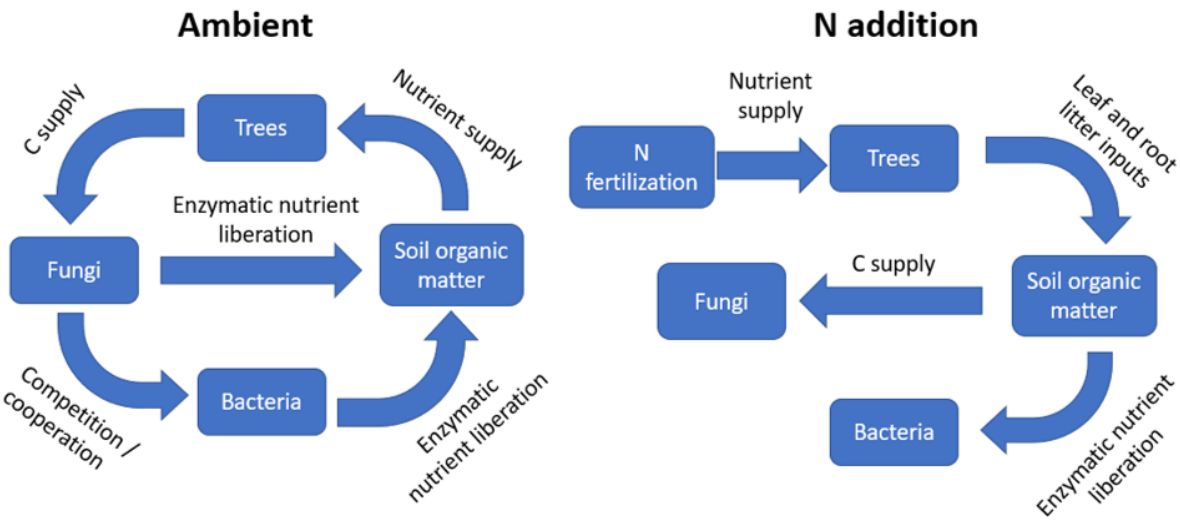

Figure 4.1. Conceptual diagram depicting hypothesized interactions among tree species, microbial communities, SOM, and EEAs under ambient conditions and in response to N addition.

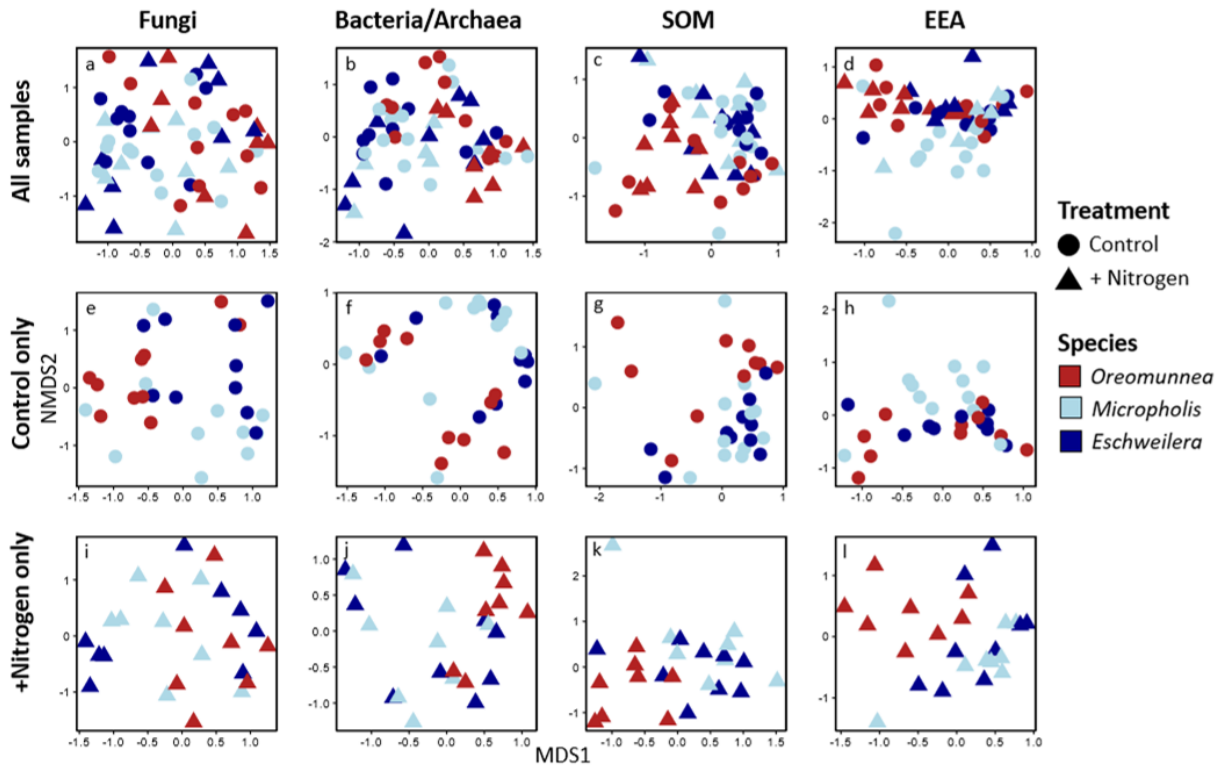

Figure 4.2. PCoA ordinations for fungal community (aei), bacterial/archaeal community (bfj), SOM (cgk), and EEAs (dhl) for all samples (a-d), as well as control only (e-h) and N addition only (i-l). Microbial community ordination were constructed using Bray-Curtis dissimilarity while SOM and EEA ordinations were constructed using Euclidian distance.

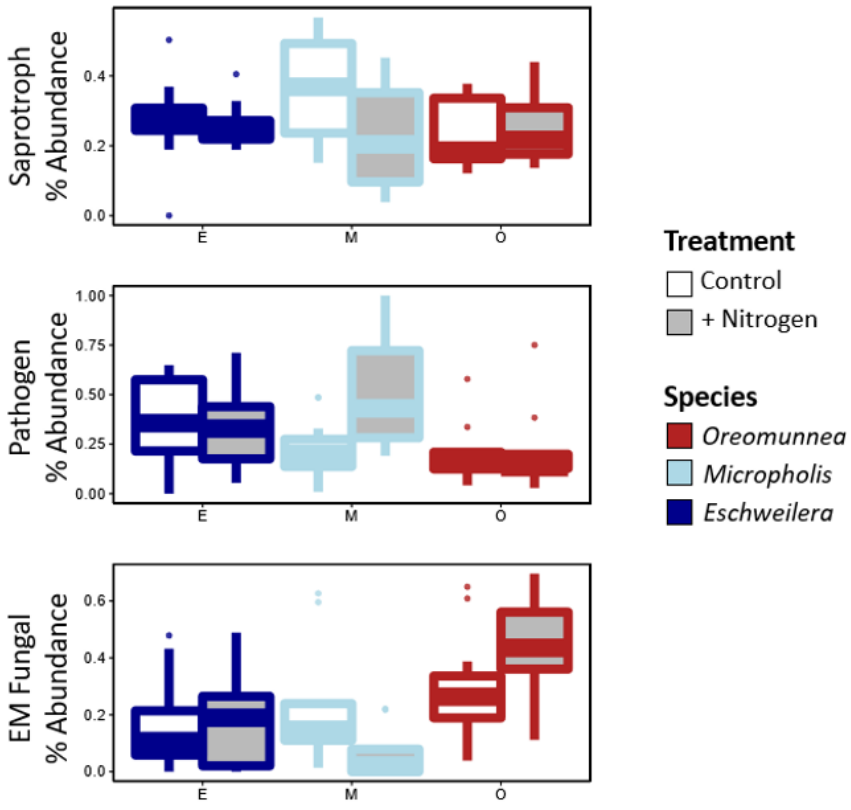

Figure 4.3. Relative abundances of saprotrophic, pathogenic, and EM fungal guilds among different tree species and between nutrient addition treatments.

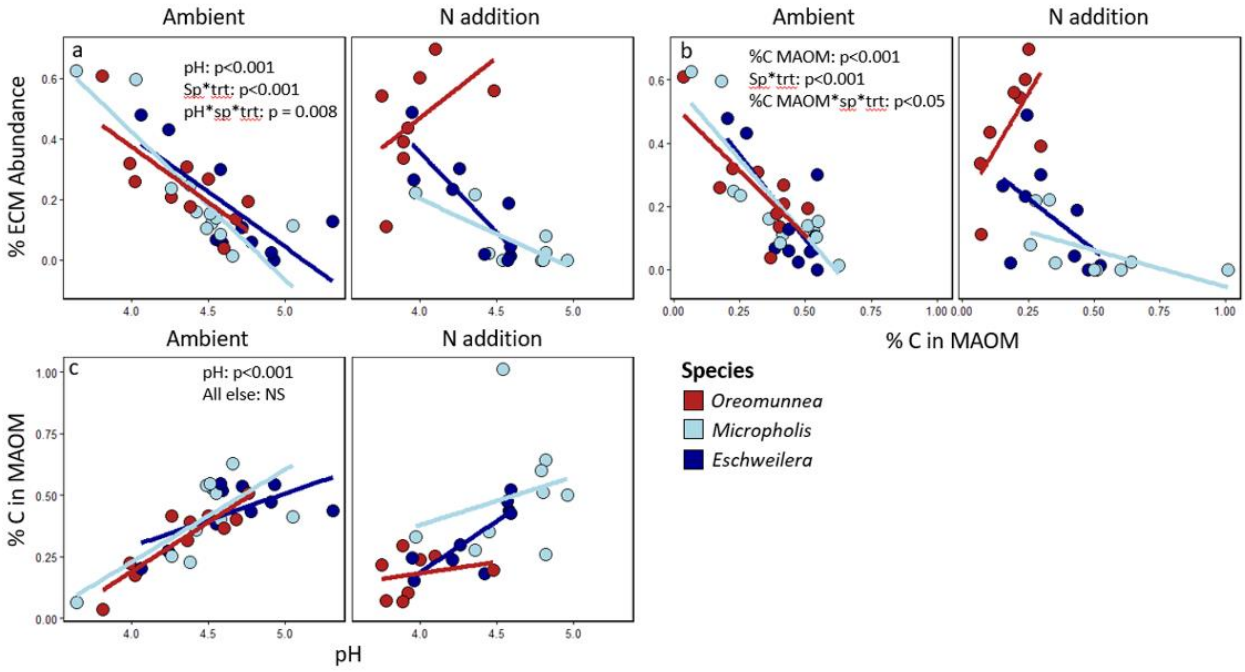

Figure 4.4. Relationships among Ph (ac), % EM fungal abundance(ab), and %C in MAOM (bc) among tree species and between nutrient addition treatments.

CHAPTER 5: DIFFERENCES AMONG ECTOMYCORRHIZAL TREE GENERA RESOURCE USE AND ALLOCATION STRATGIES CORRESPONDS WITH SURROUNDING MICROBIOME COMPOSITION AND ECOSYSTEM PROCESSES

Introduction

Ectomycorrhizal (EM) mutualisms between trees and fungi are important drivers of ecosystem function (Smith and Read 2010). However, there is considerable variation in their effects on ecosystem processes, particularly soil organic matter (SOM) dynamics like carbon (C) and nitrogen (N) cycling (Fernandez and Kennedy 2016, Lin et al. 2017). One potential source of this variation may be from overgeneralizing the ectomycorrhizal mutualism by homogenizing trees and fungi with this characteristic as a unified functional guild, rather than a diverse group of organisms with a shared morphological characteristic. Given the rising prevalence of mycorrhizal type as a tool for delineating ecosystems to predict factors like nutrient cycling and SOM dynamics (Phillips et al. 2013) and their potential responses to global change (Terrer et al. 2016, Sulman et al. 2019), it is important to elucidate the sources of variation in EM-associated ecosystem effects.

Differences among EM tree species in important functional traits could lead to differences in their associated ecosystem effect. Despite EM mutualisms only forming in a small minority of overall plant species (Brundrett 2009), due to this relationship primarily occurring in larger, long-lived woody species, they represent roughly one-third of terrestrial plant biomass (Soudzilovskaia et al. 2019). As there are relatively few plant species that form EM associations, the functional differences between these plant species are often overlooked. However, EM tree species show significant differences in traits that may influence ecosystem function. Deciduous

EM trees can exhibit up to two-fold differences in litter quality (Craig et al. 2018) which could promote which could provide a competitive advantage to root-associated microbes under lower litter quality conditions (Zak et al. 2019). As substrate quality has been found to act as a strong predictor of EM-associated effects on C and nutrient cycling (Fernandez et al. 2019), variation among EM trees could significantly impact their effect on the ecosystem overall. Forests dominated by differing EM tree species assemblages have demonstrated wide ranges of belowground C allocation (Keller et al. 2021) which may be linked to alternative species-specific nutrient economic strategies (Keller and Phillips 2019). Species variation in microbiome assembly can also promote the activity of different extracellular enzymes (Pérez-Izquierdo et al. 2019), which may influence their broader effects on ecosystem. The dynamic interplay among important plant traits like litter quality, nutrient-economic strategy, belowground C allocation, and microbiome assembly could be responsible for variation in EM response between different ecosystems.

Fungal EM traits have evolved many times over the history of fungi (Tedersoo et al. 2012), giving the EMF guild broad phylogenetic and functional diversity. This functional diversity could influence EM-associated effects on soil biogeochemical dynamics, as mechanisms for SOM decay of EM fungi reflect the evolutionary history of these organisms (Pellitier and Zak 2018). Further, distantly related species promote decomposition and nutrient assimilation through differing metabolic mechanisms (Nicolas et al. 2019). Differences in extracellular enzyme activity (EEA) profiles among different EM fungal communities (Rineau and Courty 2011) may contribute to variation in EM fungal capacity to degrade SOM (Averill and Hawkes 2016). Interspecific diversity of plants and fungi helps determine the outcome of these EM mutualisms for ecosystem function (Hazard and Johnson 2018), and often hinges on

the presence of key EM fungal taxa (Hazard et al. 2017). Recent evidence suggests significant variation in EM fungal communities at fine spatial scales corresponds to host and environmental variables that may promote EM associated biogeochemical dynamics (van der Linde et al. 2018). Previous research indicates that changes to EM fungal communities can have a strong impact on their ecosystem function, but more work focusing on active mycorrhizal communities in established ecosystems at spatially explicit scales is needed to further define this relationship.

Here we investigate how much ectomycorrhizal trees and fungal communities differ in their associated effects on ecosystem function. To test the hypothesis that EM functional diversity contributes to differences in their associated ecosystem function, we characterized the effects of EM tree genus and fungal community differences on forest ecosystem properties. We sampled soils and leaf litter from near three different EM tree genera (*Carya, Quercus,* and *Tilia*) in an EM dense forest patch in central-Illinois, USA. From these samples we characterized microbial community composition and ecosystem traits such as SOM properties and EEAs for major hydrolytic and oxidative enzymes associated with SOM degradation. We predict that tree species and fungal community traits will be associated with differing patterns in forest soils, leading to alternative outcomes for EM associated ecosystem function based on tree identity and fungal community composition.

Methods

We conducted our study in an ECM-dominated forest stand in Trelease Woods in Urbana, IL, USA (44.13ºN, -88.14ºW). Census data from 2018 was used to identify a 200m x 175m area dominated by ECM-associated trees of the genera *Carya* (*cordiformis, laciniosa, ovara*), *Quercus* (*macrocarpa, muehlenbergii, rubra*), and *Tilia* (*americana*; Fig.1). These tree

genera differ in traits that are likely to influence EMF community structure and function such as litter production and chemistry (Peterson and Cummins 1974, CÔtÉ and Fyles 1994), root morphology (Beals and Cope 1964, Brundrett et al. 1990), and other functional traits such as phenology or nutrient acquisition (Lechowicz 1984). Leaf litter quality is highest (low C:N, high pH, low lignin) in *Tilia* species (Page and Mitchell 2008) and lowest in *Quercus* species, while *Carya* species are intermediate yet highly variable (CÔtÉ and Fyles 1994, Vesterdal et al. 2008). We identified the 25 largest individual trees of each general within the forest stand for sampling. Samples were collected from within 5 meters of each focal tree in a randomly chosen cardinal direction.

Soil samples were collected in late June of 2020 using a 5 cm diameter soil corer. Soil samples were collected intact to preserve root integrity via a plastic sleeve. Samples were immediately capped on both ends and put on ice to be transported to the University of Illinois at Urbana-Champaign later that day. Following collection, all samples were transported back to the laboratory, hand-picked to remove rocks, coarse litter/roots, and visible fine roots (< 2mm diameter), then sieved through a 2 mm mesh. Soils were then subsampled for the analyses described later and immediately processed (for soil pH, moisture, inorganic N, COC, TN, MBC, and MBN) or frozen at -20^{o} C for later analysis (soil DNA, EEAs, SOM size fractions, and C and N elemental concentrations and isotopic composition). All samples were processed and frozen (if necessary) within 12 hours of collection. We also collected fresh litter using 50 cm by 30 cm litter traps in October – November 2021 because major defoliation from a hailstorm in August 2020 precluded litter collection in the same growing season as soil sampling. Leaf litter was collected daily from litter traps until sufficient to perform chemical analyses, as we did not measure overall litterfall.

Soil and litter chemical properties

To connect tree genera and microbial community difference to ecosystem biogeochemistry, we measured chemical properties of leaf litter, bulk soils, and SOM. Soil and litter samples were dried and pulverized via bead-beating before being analyzed for C and N elemental concentrations and isotopic composition using an Elementar Vario Micro Cube elemental analyzer interfaced with an IsoPrime 100 isotope ratio mass spectrometer (Isoprime Ltd.; Elementar). To partition mineral-associated and particulate organic matter contributions to overall SOM, we used size fractionation following Bradford et al. (2008). Briefly, soils were shaken in 5% NaHMP for ~18 hours, then sieved through 53 μm mesh, and sample that passed through the mesh was considered MAOM, and sample caught by the mesh was considered POM. Size fractions were dried at 65° C, and then subsamples were pulverized and analyzed in the same way as bulk soil and leaf litter. Gravimetric soil moisture was measured by oven-drying another subsample of each soil at 105°C for 24 hours. Soil pH was determined using a 1:1 mass ratio of soil:DI H_2O. To measure soil inorganic N concentrations, soils were extracted in 2M KCl, and soil NO_3^- and NH_4^+ concentrations were determined by analyzing the extracts using colorimetric methods on a SmartChem 200 discrete analyzer (Unity Scientific). To measure dissolved organic C (DOC), total N (TN), microbial biomass C (MBC), and microbial biomass N (MBN), soils were extracted in 0.05M K_2SO_4 with and without a direct chloroform addition (Brookes et al. 1985). Non-purgeable organic C and total N concentrations in the extracts were determined on a Shimadzu TOC-V CSH (Shimadzu Corp., Kyoto, Japan). Microbial extracted organic C and N from chloroform-fumigated samples was converted to MBC and MBN using an assumed extraction efficiency of 0.17 (Gregorich et al. 1990), based on the relatively low concentration of K_2SO_4. Base-cation concentrations and cation exchange capacity (CEC) were

measured by extracting soils in $BaCl_2$ and analyzing extracts for Al, Ca, Fe, K, Mg, Mn, and NA cations on a Perkin-Elmer inductively coupled plasma atomic emission spectrometer (Fisher Thermo Scientific, Waltham, Massachusetts, USA). CEC was calculated based on the sum of total cation cmol + charge per kg.

Extracellular enzyme activities

To assess soil microbial activity, we measured the activity of putative C-degrading, N-degrading, and P-degrading hydrolytic and oxidative enzymes as in Saiya-Cork et al. (2002) and Hobbie et al. (2012). Briefly, ~0.5 g soil subsamples were homogenized in a blender with a maleate buffer (100 mMol, pH 6.5, based on approximate average pH of soils in our study) and dispensed into 96 well plates for hydrolytic enzymes and 1.5 mL posiclick tubes for oxidative enzymes. Samples were incubated at 20^{o} C in the dark with blanks, standards, and controls for ~3.5 hours for hydrolytic and ~20 hours for oxidative assays, respectively. Linearity in the change in fluorescence and absorbance over these time frames was confirmed with prior testing. Hydrolytic enzymes were analyzed fluorometrically using methylumbelliferone (MUB)-labeled substrates (excitation at 365 nm, emission at 450 nm). Oxidative enzymes were assayed using L-3,4-dihydroxyphenylalanine (L-DOPA) and L-DOPA and hydrogen peroxide as substrates, respectively, and measuring absorbance on a microplate spectrophotometer (460 nm). Extracellular enzyme activity was calculated from the linear change in fluorescence (hydrolytic enzymes) and absorbance (oxidative enzymes) after correcting for controls, blanks, and quenching.

Phospholipid fatty acids

To quantitatively assess microbial community abundance, we measured phospholipid fatty acids (PLFA) in our soil samples. Freeze-fried soil samples were shipped to Regen AG Labs (Pleasanton, Nebraska, USA) for PLFA analysis. Lipids were extracted from freeze dried soil and separated into polar and nonpolar fractions using solid-phase chromatography. Polar lipids were converted to fatty acid methyl esters through methanolysis and separated using an HP GC-FID gas chromatograph (HP6890 series; Agilent Technologies Inc., Santa Clara, CA, USA). Peaks were identified using the Sherlock System (v. 6.1; MIDI Inc., Newark, DE, USA). All PLFAs with between 12 and 20 C atoms were included in our analysis, as these are more likely to be from microbial sources in soils (McKinley et al. 2005).

DNA extraction, sequencing, identification, and assignments

To determine differences in microbial community structure among tree genera we used high-throughput DNA sequencing. DNA was extracted from 500 mg of freeze-dried soil using the FastDNA SPIN Kit for Soils (MP Biomedicals, Santa Ana, USA). The extracts were purified using cetyl trimethyl ammonium bromide (CTAB). DNA extracts were submitted to the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign for Fluidigm amplification (Fluidigm, San Francisco, USA) and Illumina sequencing (Illumina, San Diego, USA). We assessed fungal communities via the *ITS2* gene region using ITS3 and ITS4 (Ihrmark et al. 2012) primers to amplify DNA. We assessed bacterial and archaeal communities via the bacterial and archaeal *16S* rRNA genes, which were amplified using Arch519 forward and BAC785 reverse primers. Fungal and bacterial amplicons were sequenced via NovaSeq 2 x 250 bp.

We used the DADA2 pipeline (Callahan et al. 2016) for bioinformatic processing to produce amplicon sequence variants (ASVs) from the sequencing data. Briefly, quality filtering, denoising, merging forward and reverse reads, and removing chimeric sequences was performed using recommended parameters for *16S* and *ITS* genes. We did not cluster sequence variants (Glassman and Martiny 2018) prior to using the default DADA2 classifier (Wang et al. 2007) to assign taxonomy based on reference sequences from the UNITE database (Kõljalg et al. 2013) for *ITS* sequences and the SILVA database (Quast et al. 2012) for *16S* sequences. Final count numbers were transformed via a Hellinger transformation (Legendre and Gallagher 2001) prior to further statistical analysis. Data used for analysis consisted of 6991 fungal, 64266 bacterial, and 549 archaeal ASVs. Due to the relatively low number of archaeal sequence variants, and because bacterial and archaeal sequences were derived from the same primer sets, we analyzed bacterial and archaeal communities together. Ectomycorrhizal fungi were assigned using the FUNGuild database (Nguyen et al. 2016b) on ASVs designated with a "probable" or "highly probable" confidence score for EM classifications.

Statistical analysis

All statistical analyses were performed in the R statistical environment (R Core Development Team 2013). To assess broad differences between tree genera, we separated variables into six groups: overall fungal community, EM fungal community, SOM, EEAs, and PLFAs, see Appendix 1 for more information on these groups. Using the VEGAN package in R (Oksanen et al. 2010) we standardized SOM and EEA variable groups via a Z-transformation and PLFAs with a log-transformation. We used PERMANOVA tests to assess differences based on tree genus and physical proximity (X and Y coordinates) with Bray-Curtis distance for

relativized fungal, EM fungal, and bacterial ASV count numbers, and Euclidian distance for SOM, EEA, and PLFA variable groups. We included X and Y coordinates in this test to account for spatial autocorrelation caused by the non-random spatial distribution of trees in this study. To visually assess differences in variable groups among tree genera, we used constrained principal correspondence analysis (PCoA), conditioned on X and Y coordinates to minimize spatial autocorrelation, with Bray-Curtis distance for microbial ASV count numbers and Euclidian distance for SOM, EEA variable groups. We did not constrain the ordination for PLFAs as we did not find evidence for significant spatial autocorrelation within this group, confirmed by procrustean analysis. To further compare microbial community and soil environmental variables, we used environmental vector scaling with the *envfit()* function in vegan to assess the correlation of SOM and EEA variables with fungal, EM fungal, bacterial, and PLFA ordinations. Correspondence between ordinations was determined using procrustean analysis to infer the strength of relationships among variable groups. Differences among tree genera for individual SOM and EEA variables were tested using a linear mixed-effect model with physical distance between samples as a random effect to account for spatial autocorrelation. Statistical significance was assessed as $p < 0.05$ unless otherwise stated.

Results

Ordinations

Microbial community composition, SOM property, EEA, and PLFA variable groups differed significantly among tree genera (Table 2, Figure 1). Tree genus explained ~ 4% of variation in microbial communities and up to 18% of variation for SOM variables, with genus explaining 7.5% and 6% of variation in EEA and PLFA groups, respectively. Visual inspection

of PCoA ordinations showed greater resemblance within *Tilia*-associated overall fungal communities than *Quercus* or *Carya*. However, *Tilia*-associated bacterial communities were more dispersed and separate from *Carya*- and *Quercus*-associated bacterial communities. *Quercus*-associated EM fungal communities appeared to display more in-group similarity than *Tilia* or *Carya*. Tree genera separated horizontally for SOM variable PCoA loadings between *Quercus* on the lower end and *Tilia* / *Carya* on the higher end. For SOM, one of the strongest patterns that emerged was the enrichment of ^{13}C and ^{15}N in soils under *Tilia*, and to a lesser extent *Carya* trees (Figure 2a, Table 4). This pattern was mirrored for $\delta^{15}N$ in leaf litter, but leaf litter $\delta^{13}C$ was higher for *Quercus* species than the other two genera. Overall patterns of soil and leaf %N, %C, C:N ratio, soil DOC, and MBC were highest under *Quercus* species. EEAs were only significantly lowest under *Tilia* for oxidative enzymes (PO: $p = 0.005$; PE: $p = 0.009$), though rates of BX were greater near *Quercus* species than *Tilia* species ($p = 0.04$, Table C.2). Overall fungal community composition corresponded to all other variable groups except PLFA while EM fungal community composition corresponded to bacterial community composition and SOM. Bacterial/archaeal community composition did not correspond to SOM, but both bacterial/archaeal community composition and SOM corresponded to EEAs. PLFAs corresponded only to EEAs (Table 3).

Comparing microbial communities and ecosystem variables

There were strong patterns of correlation between variable group ordinations with isotopic composition and elemental concentrations of C and N in soils and leaf litter (Table C.3). In overall fungal communities and EM fungal communities, association with *Quercus* had higher C and N concentrations, as well as greater overall DOC and MBC (Figure 2ab). However,

Quercus-associated overall fungal and EM fungal communities corresponded with lower $\delta^{13}C$ and $\delta^{15}N$ for leaf litter and various SOM fractions (Figure 2ab). We also found that *Tilia*-associated bacterial/archaeal communities were correlated with higher $\delta^{13}C$ for bulk soil, POM, and MAOM, but not leaf litter (Figure 2c). Similarly, EEAs were significantly correlated with MAOM $\delta^{13}C$ and $\delta^{15}N$, which were higher near *Tilia* species, as well as abundances of various C and N pools associated with *Quercus* species (Figure 2d). For PLFAs, samples from *Quercus* species were correlated with higher MBC, DOC, %N, and leaf C:N and lower soil $\delta^{13}C$, leaf $\delta^{15}N$, and leaf %N (Figure 2e).

All variable groups aside from EM fungi showed strong ordination resemblance with EEAs (Table 3), yet we found the particular enzymes differed (Table C.4). Overall fungal communities correlated with AP and AG activity (Figure 2f), while bacterial communities correlated with AG and NAG (Figure 2h). Soil organic matter demonstrated a consistent significant correlation with all hydrolytic EEAs (Figure 2i). Oxidative EEAs, as well as CBH and BX were correlated with *Quercus*-associated PLFAs (Figure 2j).

Discussion

While EM tree species and fungal communities are often generalized as a homogenous group in mycorrhizal paradigms, our study shows that EM tree genera can have divergent relationships with soil microbiomes and ecosystem traits. The significant differences we found in microbial communities and soil biogeochemistry among EM tree genera demonstrates the potential importance of considering the interactions between ecophysiology and biodiversity in assessing broad-scale ecosystems patterns. In particular, the trends we observed in C and N isotopic composition of plant matter and SOM pools among EM tree genera, and the relationship

of these isotopic patterns with microbiome composition and soil biogeochemistry, contribute to a greater understanding of the functional diversity among EM mutualisms. Overall, our findings suggest that EM mutualisms can display significant functional diversity, suggesting that EM associated should not be considered a homogenous trait of tree and fungal species in predicting their effects on ecosystems and responses to global change.

EM tree genera differed significantly in SOM properties, potentially corresponding to differing nutrient acquisition and use strategies among these genera. The greatest differences we found among EM tree genera were in SOM variables, with tree genus explaining ~19% of overall variation in SOM dynamics. In line with previous reports of these genera (Craig et al. 2018), leaf litter quality was significantly higher for *Tilia* than *Quercus* or *Carya* species. These differences in foliar nutrient stoichiometries have been previously linked to broader nutrient economic strategies in tree species (Zhang et al. 2018), which may also explain differences we found in leaf isotopic signature among genera. Plants that rely more strongly on mycorrhizal mutualists to acquire N often have lower ^{15}N enrichment in their tissues associated with discrimination against ^{15}N during nutrient transfer from fungi to trees (Hobbie and Högberg 2012). The lower $\delta^{15}N$ we observed in *Quercus* leaves, relative to *Carya* and *Tilia*, may be due to greater reliance of *Quercus* species on mycorrhizal fungi for N acquisition. This is further supported by the greater microbial biomass and more homogenous EM fungal community we found near *Quercus* trees than near *Tilia* trees. Moreover, photosynthesis depleted in ^{13}C relative to atmospheric CO_2 and there is discrimination against ^{13}C when photosynthate is transferred from tree to mycorrhizal fungi, leading to ^{13}C depletion of the fungal biomass (Hobbie et al. 1999). Therefore the C isotopic composition of MAOM, which is largely microbially derived OM, can reflect how much C was transferred from the tree to its mutualist partner. While

Quercus species had the highest leaf $\delta^{13}C$ of the three genera, soil $\delta^{13}C$ (particularly in MAOM) was consistently the lowest for *Quercus*. We argue that this pattern represents allocation of photosynthate C belowground to EM fungi and other mutualist microbes from *Quercus* trees.

The potential association of MAOM ^{13}C composition with relative belowground C rhizodeposition would explain the strong patterns we found correlating MAOM $\delta^{13}C$ with microbiome composition, SOM C and N abundance, and EEAs. Rhizosphere microbiomes, particularly EM fungi, supplied with greater quantities of plant photosynthate would have more capacity liberating nutrients from SOM, particularly given the relatively low litter quality associated with *Quercus* and *Carya* species. Lower reliance on and investments in mutualist fungi from *Tilia* species would also explain their divergent patterns in bacterial community composition and its significant association with greater $\delta^{13}C$. The higher nutritional quality of *Tilia* litter could make it more difficult for *Tilia*-associated EM fungi to competitively exclude other free-living saprotrophs (Fernandez and Kennedy 2016). Greater $\delta^{15}N$ in *Tilia* leaf litter further supports the pattern that *Tilia* are more readily acquiring inorganic N directly from soil, rather than N transferred from EM fungi. Distinct C and N isotopic patterns among EM tree species relating to nutrient economic strategies have previously been observed in seedlings (Keller and Phillips 2019), but how these patterns manifest in older adult trees is still poorly understood. Our study shows that different EM trees can potentially vary in their dependence on fungal partners within the same forest stand, corresponding to broader differences in microbiome assembly, SOM dynamics, and ecosystem processes.

Differences SOM properties and microbiome function we observe among EM trees could help explain variation in broader trends of increasing C concentrations with EM plant biomass (Soudzilovskaia et al. 2019). As soil C is generally more stable when derived from belowground

inputs rather than leaf litter (Sokol and Bradford 2019), differential trends in root and extracellular C allocation among EM tree species may contribute to highly variable soil C stocks in EM-dominated ecosystems. Differences between gymnosperm and angiosperm EM tree species and associated fungal communities lead to alternative patterns in decomposition and C-cycling (Fernandez et al. 2019), though this has previously been primarily associated with substrate quality. Variation in the amount of photosynthate C transferred from trees to rhizosphere communities could represent an additional layer of diversity from EM associations that may drive the functional capacity of these mutualisms. Root-derived soil C from EM-dominated deciduous forests varies significantly at a continental scale (Keller et al. 2021), thus species-specific patterns contributing to this variation could have major implications for using mycorrhizal-associations to predict C storage in forests. Broadly, EM associated plant species are expected to respond to increasing atmospheric CO_2 concentrations with greater investments in plant biomass, rather than soil C (Terrer et al. 2021). However, if EM associate plant species differ in their tissue-soil C allocation strategies, then this would also likely affect how EM dominated ecosystems with differing species assemblages would respond to global change.

Based on the relationships we observed between leaf litter quality with leaf and soil N isotopic patterns, EM tree species might also differ in their nutrient economic strategy and reliance on EM fungi for nutrient acquisition. Relatively low litter quality among EM species has long been considered a key feature in allowing EM fungi to exert influence on surrounding ecosystems (Gadgil and Gadgil 1975, Fernandez and Kennedy 2016), leading to the association of EM dominated ecosystems with slower, more organic nutrient cycling (Phillips et al. 2013). While this pattern manifests globally, it is often weaker for angiosperm species (Lin et al. 2017) than all EM tree species. While this inconsistency in N dynamics may be explained by differing

patterns in acid-base chemistry between gymnosperm and angiosperm EM associated tree species (Lin et al. 2017), we did not find evidence for differences among EM genera in soil pH or CEC. Further, we also found no significant association between acid-base chemistry and patterns of N concentration or N isotopic composition of litter or SOM, suggesting the alternative N dynamics among EM trees we observed may represent an explicit nutrient economic spectrum. Greater reliance on mycorrhizal N acquisition for *Quercus* species is further supported by the relationships between *Quercus*-associated broad fungal and EM fungal communities with lower $\delta^{15}N$ in leaf litter and SOM pools, with ^{15}N depletion signifying increased fugal-plant nutrient transfer. Foliar ^{15}N isotopic abundances are more depleted for EM plants than their nonmycorrhizal counterparts and for plants in soils with lower N-availability (Craine et al. 2009), thus these patterns in our study may reflect alternative nutrient economic conditions among EM tree genera. Reliance of EM fungal acquired organic soil N has been proposed to negatively affect the capacity of EM dominated ecosystems to respond to increasing anthropogenic N deposition (Averill et al. 2018). Our evidence suggests that some EM tree species may be able to respond to these shifting nutrient conditions better than others, potentially complicating predictions of overall EM ecosystem decline in response to global change. There may also be plasticity in EM tree species' reliance on mycorrhizal mutualists based on environmental conditions (Pellitier et al. 2021), indicating a need for further exploration into the dynamic relationship between EM biodiversity and N acquisition.

Conclusion

Overall, we showed how plant C and N use and acquisition strategies can differ among ectomycorrhizal (EM) tree genera. Based on evidence from plant and soil C and N isotopic

composition, EM trees may vary in their reliance on mycorrhizal mutualists for nutrient acquisition, with this relationship extending to other microbial domains, like prokaryotes. These patterns also connect nutrient use and microbial association differences among EM tree genera with broader soil biogeochemical dynamics to suggest that species-specific differences may explain some of the variation in overall EM-associated ecosystem effects. This study demonstrates the importance of biodiversity in understanding plant-microbial interactions and their effects on soil biogeochemical function.

Tables and Figures

Enzyme	Type	Indicator
α-Glucosidase (AG)	Hydrolytic	Degrades starch
β-Glucosidase (BG)	Hydrolytic	Degrades cellulose
Cellobiohydrolase (CBH)	Hydrolytic	Degrades cellulose
β-xylosidase (BX)	Hydrolytic	Degrades hemicellulose
N-acetyl-β-D-glucosaminidase (NAG)	Hydrolytic	Degrades organic N
Acid phosphatase (AP)	Hydrolytic	Degrades organic P
Phenol oxidase (PO)	Oxidative	Degrades lignin (and other SOM)
Peroxidase (PE)	Oxidative	Degrades lignin (and other SOM)

Table 5.1. Names, types, and target of SOM degradation for extracellular enzyme activities.

Group	Effect	Df	F-value	R^2	p-value
Fungal community	Genus	2	**1.503**	**0.040**	**0.001**
	X	1	**1.518**	**0.020**	**0.001**
	Y	1	1.123	0.015	0.066
EM fungal community	Genus	2	**1.624**	**0.042**	**0.001**
	X	1	**1.882**	**0.025**	**0.001**
	Y	1	**1.351**	**0.018**	**0.021**
Bacterial community	Genus	2	**1.494**	**0.040**	**0.001**
	X	1	**1.361**	**0.018**	**0.003**
	Y	1	**1.159**	**0.015**	**0.035**
SOM	Genus	2	**8.661**	**0.188**	**0.001**
	X	1	**3.669**	**0.040**	**0.003**
	Y	1	1.110	0.012	0.314
EEA	Genus	2	**2.964**	**0.075**	**0.011**
	X	1	1.963	0.025	0.103
	Y	1	1.548	0.019	0.188
PLFA	Genus	2	**2.291**	**0.060**	**0.002**
	X	1	1.001	0.013	0.447
	Y	1	0.772	0.010	0.667

Table 5.2. Results from PERMANOVA tests on microbial and ecosystem variable groups based on tree genus, X coordinate, and Y coordinate. Bolded values are significant at $p < 0.05$.

Group	Fungal Community		ECM fungal community		Bacterial community		SOM		EEA	
Stat	Scale	p-value	Scale	p-value	Scale	p-value	Scale	p-value	Scale	p-value
ECM	**0.50**	**0.001**								
Bacterial	**0.51**	**0.001**	**0.25**	**0.016**						
SOM	**0.31**	**0.001**	**0.29**	**0.002**	0.19	0.114				
EEA	**0.29**	**0.003**	0.20	0.1	**0.27**	**0.007**	**0.35**	**0.001**		
PLFA	0.15	0.328	0.14	0.408	0.15	0.402	0.13	0.44	**0.30**	**0.004**

Table 5.3. Results from Procrustes test of pair-wise ordination correspondence between variable groups, “Scale” denotes correlation. Bolded values are significant at $p < 0.05$.

Group	Name	F	p
SOM	**Litter**	**5.51**	**0.006**
	pH	0.18	0.83
	Moisture	**3.29**	**0.04**
	DOC	**20.04**	**< 0.001**
	TN	**7.90**	**< 0.001**
	MBC	**6.97**	**< 0.001**
	MBN	2.33	0.10
	NH_4^+	0.65	0.53
	NO_{3+}^-	2.69	0.08
	% POM	2.84	0.07
	MAOM $\delta^{13}C$	**12.44**	**< 0.001**
	MAOM %C	2.11	0.13
	MAOM $\delta^{15}N$	**4.52**	**0.01**
	MAOM %N	0.18	0.84
	MAOM C:N	0.69	0.51
	POM $\delta^{13}C$	**3.56**	**0.03**
	POM %C	1.71	0.19
	POM $\delta^{15}N$	**4.32**	**0.02**
	POM %N	1.28	0.28
	POM C:N	1.25	0.29
	Bulk $\delta^{13}C$	**11.52**	**< 0.001**
	Bulk %C	**7.33**	**< 0.001**
	Bulk $\delta^{15}N$	**5.35**	**0.007**
	Bulk %N	**5.89**	**0.004**
	Bulk CN	**3.06**	**0.05**
	Leaf $\delta^{13}C$	**18.61**	**< 0.001**
	Leaf %C	**113.01**	**< 0.001**
	Leaf $\delta^{15}N$	**47.08**	**< 0.001**
	Leaf %N	**62.37**	**< 0.001**
	Leaf C:N	**58.17**	**< 0.001**
EEA	AG	1.41	0.25
	BG	1.23	0.30
	CBH	1.60	0.21
	NAG	1.25	0.29
	BX	**3.25**	**0.04**
	AP	0.35	0.70
	PO	**5.00**	**0.009**
	PX	**5.76**	**0.005**

Table 5.4. Results from mixed effects model on SOM and EEAs with tree genus as a fixed effect and spatial position as a random effect. Bolded values are significant at $p < 0.05$.

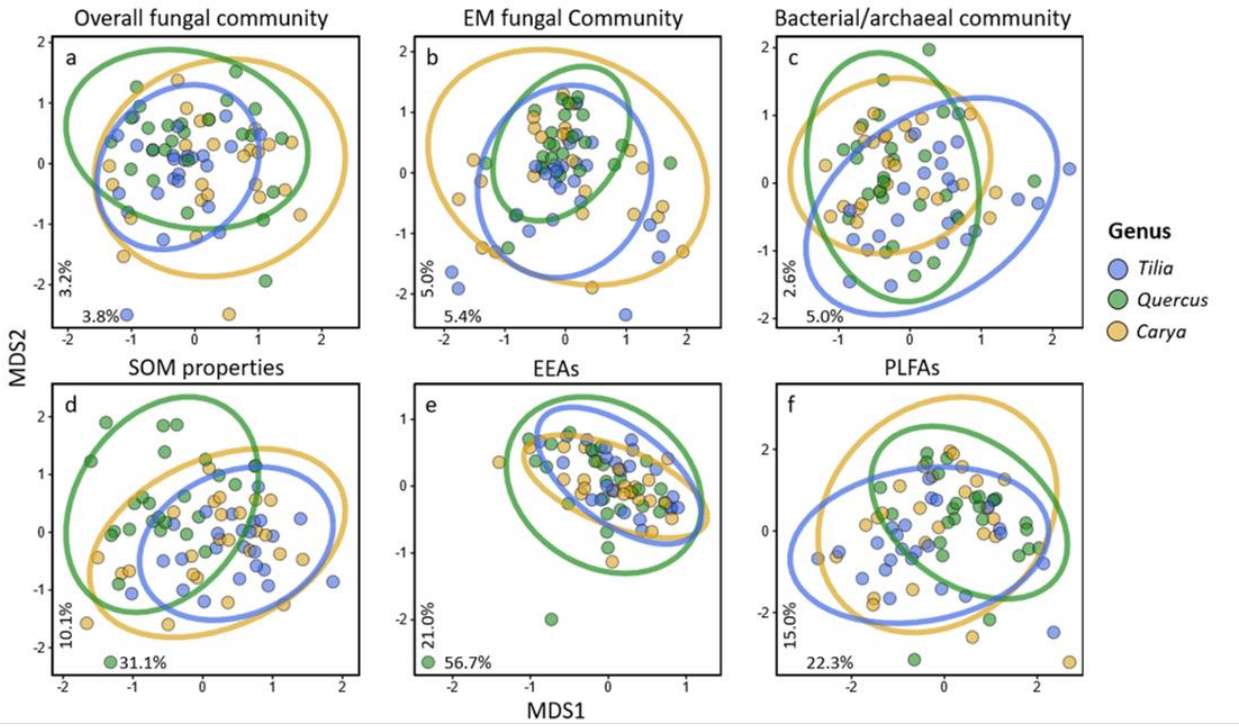

Figure 5.1. PCoA ordinations for microbial community and soil ecosystem variable groups based on Bray-Curtis distance for fungal, EM fungal, and bacterial communities (a-c) and Euclidian distance for COM, EEA, and PLFA variable groups (d-f). Ordinations were conditioned based on X and Y coordinates to account for spatial autocorrelation among samples. Variation in overall dissimilarity explained by MDS1 and MDS2 are shown on their respective axis for each ordination.

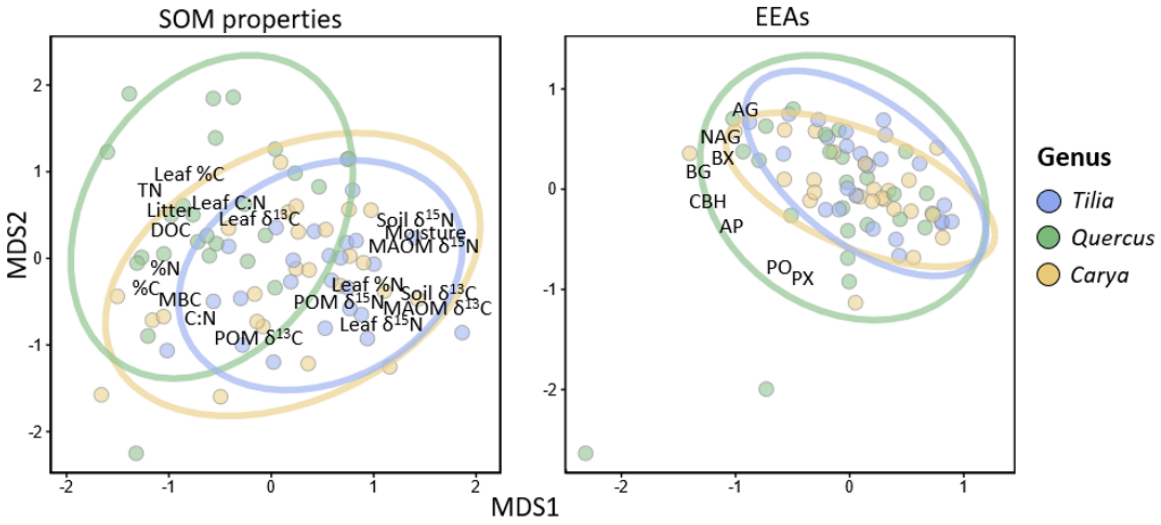

Figure 5.2. SOM and EEA ordinations with variable loadings., for SOM only variables with significant treatments differences among tree genera are displayed.

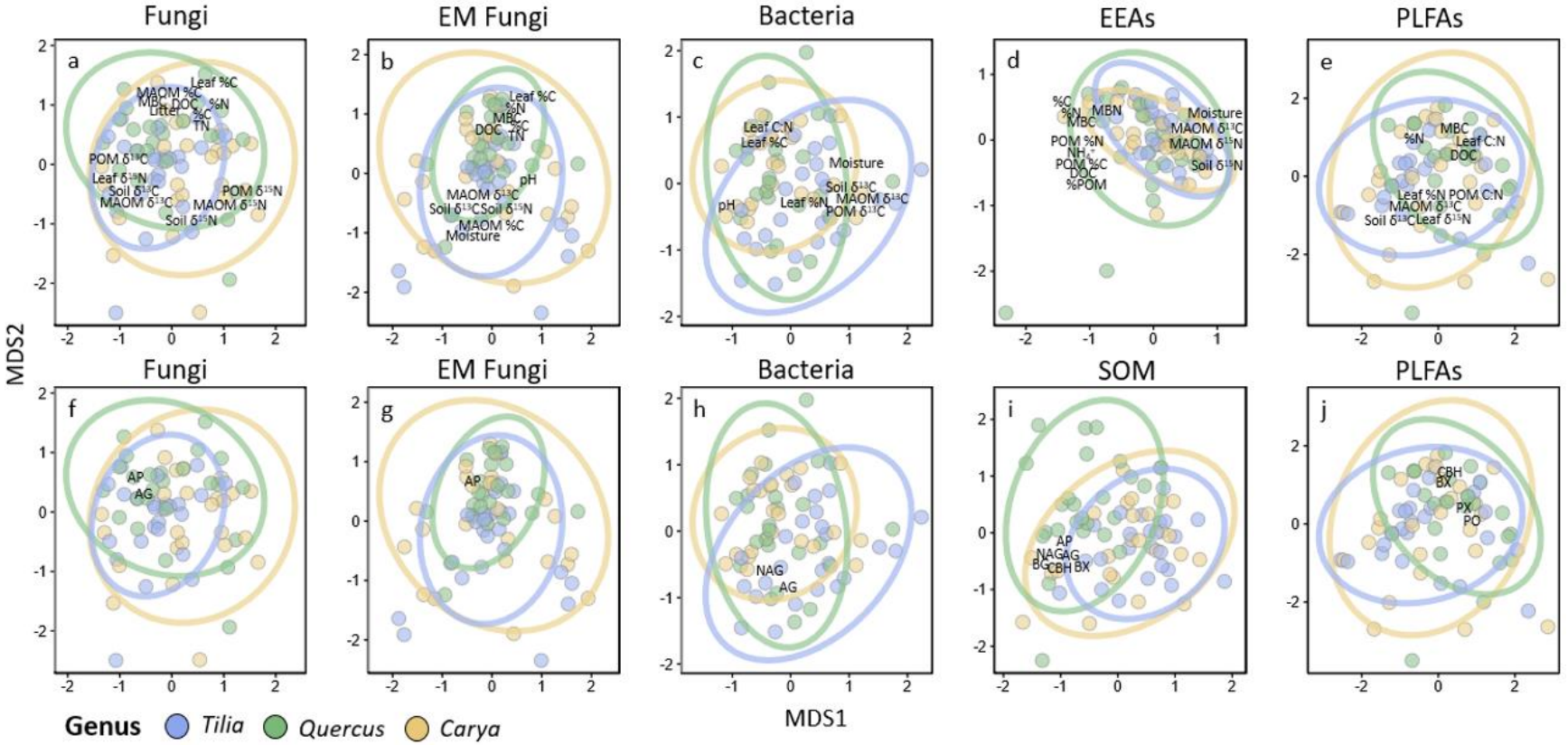

Figure 5.3. Microbial community and soil ecosystem ordinations with vector-scaled correlation loadings for SOM (a-e) and EEA (f-j) variables. Only variables with significant correlations at $p < 0.05$ are displayed.

CHAPTER 6: CONCLUSION

In my dissertation, I investigated how plant-soil-microbial relationships contribute to ecosystem function, as well as factors that may affect the direction and magnitude of these relationships. Though my research, I showed that plant-microbial relationships are not uniformly consistent, described some of the mechanisms by which plant mediated ecosystem outcomes via soil microbial communities, and explored how these relationships can differ based on environmental conditions or species diversity.

In my second chapter, I present evidence that garlic mustard invasions may vary in their ability to manipulate microbiomes to promote their invasion success across ecoregions, speculating that this variation may be due to soil condition, invasion age, or some other factor. The presence of invasive garlic mustard in the forests I sampled despite their apparently limited microbial impacts suggests that garlic mustard populations are not solely reliant on microbial mechanisms (*i.e.* "mycorrhizal suppression" to the degree it was measured) to invade forest ecosystem. The lack of garlic mustard impacts on the soil microbial community in recently invaded central Illinois forests suggests that these well-documented impacts in the northeastern United States and in older invasions cannot necessarily be generalized across all environmental contexts.

In my third chapter, I found garlic mustard may potentially accelerate soil N cycling rates in invaded forests via soil pH increases and changes in soil microbial communities. As some of the effects on soil N cycling associated with garlic mustard are not consistent over its phenology, garlic mustard could promote different processes at different points in its life history. I also showed that garlic mustard removal is unlikely to remediate its net ecosystem effects, at least in

the short term, indicating that garlic mustard invasions may have a lasting legacy on ecosystems. This study provides greater evidence of belowground mediated pathways for invasive plants to alter native ecosystems by synergistically affecting both biotic and abiotic soil properties to support its invasion success.

In my fourth chapter, I show that microbially-mediated plant N acquisition from SOM is linked with multi-trophic interactions in montane tropical forest soils. When inorganic N availability increased, the role fungi played in connecting tree species to soil C and N cycling was diminished whereas the role of bacterial and archaeal communities is increased. This suggests a need to move beyond move beyond the single kingdom paradigm of biogeochemical cycling to better account for multi-trophic relationships in structuring biogeochemical aspects of ecosystem function.

In my fifth chapter, I describe how different plant C and N use and acquisition strategies can differ among ectomycorrhizal (EM) tree genera. I show, based on evidence from plant and soil C and N isotopic composition, that EM trees may vary in their reliance on mycorrhizal mutualists for nutrient acquisition, with this relationship extending to other microbial domains, like prokaryotes. I also connect these patterns in nutrient use and microbial association differences among EM tree genera with broader soil biogeochemical dynamics to suggest that species-specific differences may explain some of the variation in overall EM-associated ecosystem effects. This study demonstrated the importance of biodiversity in understanding plant-microbial interactions and their effects on soil biogeochemical function.

Overall, my dissertation supports the inclusion of microbial communities as critical independent players in ecological phenomena, promoting the use of microbially-explicit paradigms and models in informing conservation decision-making and predicting ecosystem

response to global change. My dissertation shows that the nature of plant-soil-microbial relationships is highly context-dependent, varying based on environmental conditions, temporal factors, and species diversity.

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APPENDIX A: SUPPLEMENTARY TABLES AND FIGURE FOR CHAPTER 2

Site name	Latitude/ Longitude (ºN, ºW)	Mean annual temperature (ºC)	Mean annual precipitation (mm)	Dominant soil series	Year garlic mustard first reported
Brownfield Woods	40.14, -88.16	10.0	965	Drummer silty clay loam	1995
Collins Pond Woods	40.13, -88.03	10.5	990	Raub silt loam and Xenia silt loam	1997
Hart Woods	40.22, -88.35	10.0	889	Ambraw silty clay loam and Martinsville silt loam	1995
Richter Woods	40.09, -87.81	10.0	914	Birkbeck silt loam and Strawn silt loam	2000
Trelease Woods	40.13, -88.14	10.0	965	Flanagan silt loam and Drummer silty clay loam	1991

Table A.1. Study site locations, climate, soil type, and year garlic mustard was first reported. Climate and soil data was obtained from the USDA Web Soil Survey (https://websoilsurvey.sc.egov.usda.gov/) and accessed on [12/06/2020], climate data was averaged between years 2002-2020. Garlic mustard report year was provided by personal communication from S. Buck, the natural areas manager at these sites.

Target gene	PCR primers	Primer sequence (5'→3')	Reference
Fungal ITS forward	ITS3	GCATCGATGAAGAACGCAGC	White et al. (1990
Fungal ITS reverse	ITS4	TCCTCCGCTTATTGATATGC	White et al. (1990)
Bacterial and Archaeal 16S forward	V4_515F	GTGYCAGCMGCCGCGGTAA	Parada et al. (2016)
Bacterial and Archaeal 16S reverse	V4_806R	GGACTACNVGGGTWTCTAAT	Apprill et al. (2015)

Table A.2. Target genes, PCR primer names, primer sequences, and references for genes sequences in fungal and bacterial/ archaeal community analysis.

Forest	Community	Main Fixed Effect	F-value	R^2	p-value
All	**Fungi**	Treatment	0.800	0.014	0.982
		Sampling date	2.622	0.023	**0.001**
		Treatment*Sampling date	0.678	0.011	1.000
	Bacteria & Archaea	Treatment	0.780	0.013	0.668
		Sampling date	5.017	0.416	**0.001**
		Treatment*Sampling date	0.561	0.009	1.000

Table A.3. PERMANOVA test on fungal and bacterial/ archaeal community composition across all forests with forest stratified in the model. Bolded p-values highlight significant effects at $p < 0.05$.

Measure	Main Fixed Effect	Saprotroph		Pathogen		Mycorrhizal	
		F-value	p-value	F-value	p-value	F-value	p-value
Relative abundance	Treatment	0.685	0.506	0.055	0.947	0.700	0.499
	Sampling date	3.032	0.085	0.449	0.504	29.762	**<0.001**
	Treatment*Sampling date	1.259	0.289	1.169	0.315	0.048	0.953
Richness	Treatment	1.186	0.310	0.120	0.887	1.318	0.273
	Sampling date	2.880	0.093	7.425	**0.007**	26.202	**<0.001**
	Treatment*Sampling date	1.230	0.297	4.928	**0.009**	0.190	0.827

Table A.4. Table 2. ANOVA results for main effects on the relative abundance (% read #) and species richness (#) of saprotrophic, pathogenic, and mycorrhizal fungi. Block nested within site was included as a random effect in all models. Bolded p-values highlight significant effects at $p < 0.05$.

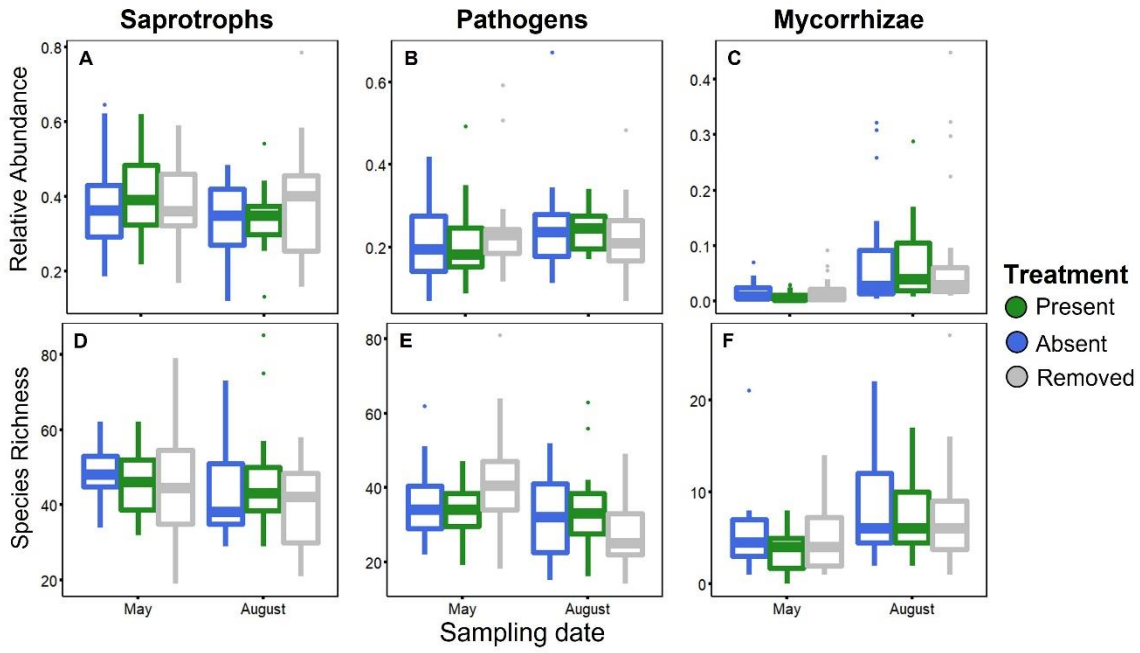

Figure A.1. Relative abundance (A-C) and species richness (D-E) of saprotrophic (AD), pathogenic (BE), and mycorrhizal (CF) functional guilds

APPENDIX B: SUPPLEMENTARY TABLES AND FIGURES FOR CHAPTER 3

Site name	Latitude/ Longitude (ºN, ºW)	Mean annual temperature (ºC)	Mean annual precipitation (mm)	Dominant soil series
Allerton Park	39.98, -88.65	10.5	965	Xenia/ Russell silt loam
Breens Woods	40.64, -88.91	11.1	952	Birkbeck silt loam
Bunny Forest	40.63, -88.83	10.5	914	Mayville silt loam
Funk Woods	40.34, -89.14	11.1	952	Birkbeck silt loam
Richter Woods	40.09, -87.81	10	914	Birkbeck/ Strawn silt loam
Trelease Woods	40.13, -88.14	10	965	Flanagan silt loam/ Drummer silty clay loam
Warbler Ridge	39.44, -88.15	10	965	Senachwine silt loam
Vermillion River Observatory	40.06, -87.56	10.5	977	Senachwine silt loam

Table B.1. Study site locations, climate, and soil type. Climate and soil data was obtained from the USDA Web Soil Survey (https://websoilsurvey.sc.egov.usda.gov/) and accessed on [12/06/2020], climate data was averaged between years 2002-2020.

Target gene	PCR primers	Primer sequence (5'→3')	Reference
Fungal ITS	ITS3	GCATCGATGAAGAACGCAGC	White et al 1990
	ITS4	TCCTCCGCTTATTGATATGC	
Bacterial and Archaeal 16S	Arch519	CAGCMGCCGCGGTAA	Song et al 2013
	V4_805R	GACTACHVGGGTATCTAATCC	Walters et al 2015

Table B.2. Target genes, PCR primer names, primer sequences, and references for genes sequences in fungal and bacterial/ archaeal community analysis.

Variable	Residual df	Treatment		Time		Treatment*Time	
		F-value	p-value	F-value	p-value	F-value	p-value
NH_4^+ assimilation	12	0.74	0.49				
NO_3^- assimilation	12	2.42	0.11				
Immobilization	13	0.37	0.69				
Leaching N-loss	14	2.26	0.14				
Fungal Diversity	55	0.16	0.85	2.39	0.10	0.18	0.94
Fungal Evenness	55	2.16	0.12	2.09	0.13	0.13	0.97
Bacterial/ Archaeal Diversity	55	0.15	0.85	**24.86**	**>0.001**	0.37	0.82
Bacterial/ Archaeal Evenness	55	0.37	0.68	2.33	0.10	0.31	0.86
Saprotroph Relative Abundance	55	0.07	0.92	0.38	0.68	1.31	0.27
Pathogen Relative Abundance	55	0.16	0.85	0.08	0.91	0.51	0.72
ECM Relative Abundance	55	1.27	0.28	**3.5**	**0.03**	0.26	0.90

Table B.3. F- and p-value results from 2-way mixed effects ANOVA models based on garlic mustard treatment, sampling time, and their interaction as fixed effects, with sampling site (forest) as a random effect. Only one time point observation was made for MBN, immobilization rate, and N-loss, so time was not included as a factor in these models. Numerator degrees of freedom for treatment and time was 2 and for their interaction was 4. Bolded values indicate significant relationships of $p < 0.05$.

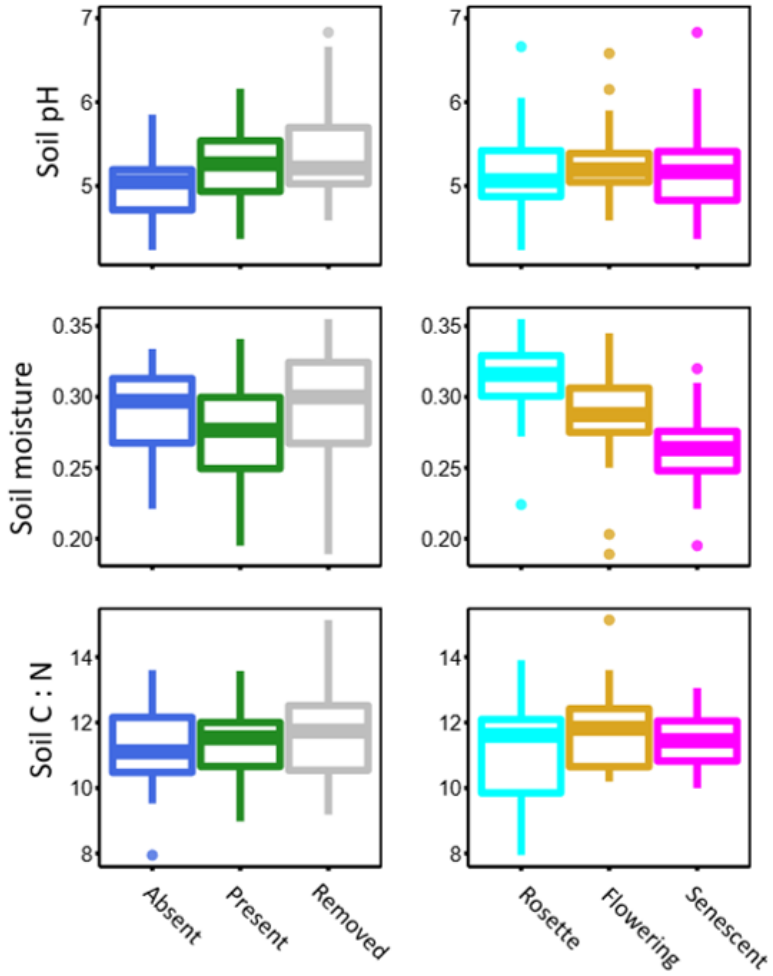

Figure B.1. Soil pH, moisture, and C:N ration among garlic mustard treatment and phenological stage sampling time points.

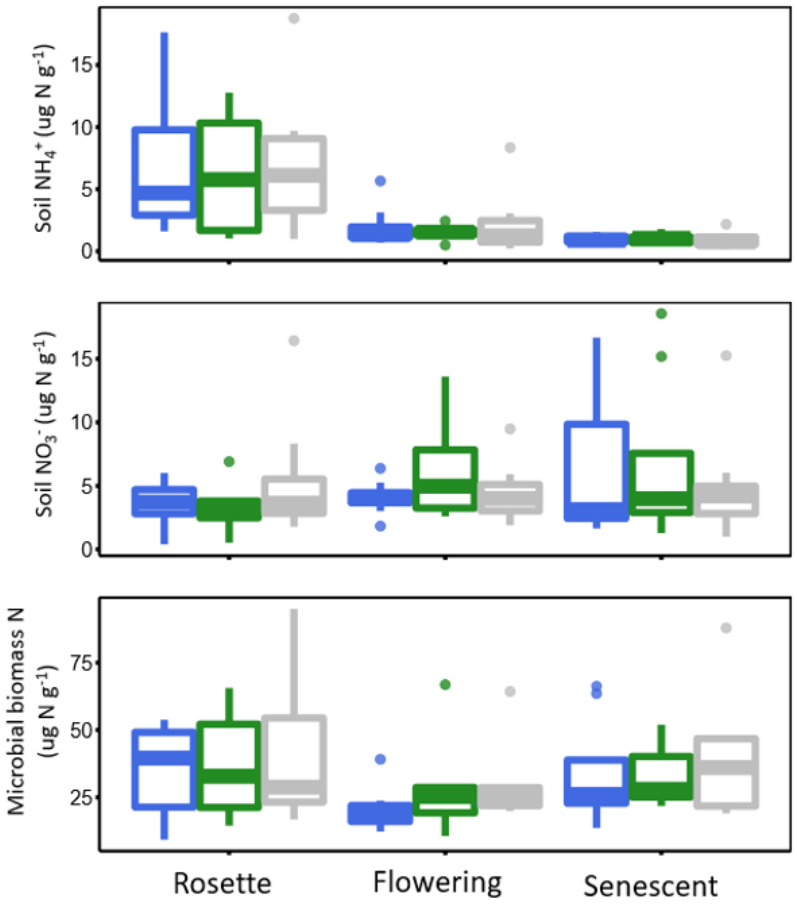

Figure B.2. Soil NH_4^+, NO_3^-, and microbial biomass N concentrations among garlic mustard treatments at different phenological stages.

APPENDIX C: SUPPLEMENTARY TABLES FOR CHAPTER 5

Name	Vector R^2	Vector p	MDS1	MDS2	ANOVA-p
Bulk %N	0.590	0.001	-0.996	-0.090	0.004
CppmN	0.605	0.001	-0.988	-0.155	< 0.001
Bulk %C	0.756	0.001	-0.981	-0.195	< 0.001
DOC	0.554	0.001	-0.974	0.227	< 0.001
CppmC	0.507	0.001	-0.968	-0.253	< 0.001
MBC	0.459	0.001	-0.949	-0.316	< 0.001
Litter	0.369	0.001	-0.893	0.449	0.006
Bulk CN	0.437	0.001	-0.885	-0.466	0.050
TN	0.576	0.001	-0.840	0.543	< 0.001
Leaf C:N	0.258	0.001	-0.825	0.565	< 0.001
Leaf %C	0.460	0.001	-0.768	0.640	< 0.001
Leaf $\delta^{13}C$	0.065	0.108	-0.486	0.874	< 0.001
POM $\delta^{13}C$	0.355	0.001	-0.146	-0.989	0.030
POM $\delta^{15}N$	0.133	0.005	0.725	-0.689	0.020
Leaf $\delta^{15}N$	0.401	0.001	0.778	-0.629	< 0.001
Leaf %N	0.141	0.006	0.868	-0.497	< 0.001
MAOM $\delta^{13}C$	0.403	0.001	0.901	-0.435	< 0.001
Bulk $\delta^{13}C$	0.379	0.001	0.937	-0.350	< 0.001
Bulk $\delta^{15}N$	0.466	0.001	0.987	0.160	0.007
Moisture	0.271	0.001	0.996	0.094	0.040
MAOM $\delta^{15}N$	0.273	0.001	0.997	0.078	0.010

Table C.1. Vector loadings of all SOM variables with significant differences among tree genera.

	Vec-R^2	Vec-p	MDS1	MDS2	ANOVA-p
AG	0.710	0.001	-0.807	0.590	0.250
BG	0.821	0.001	-0.990	0.139	0.300
CBH	0.824	0.001	-1.000	-0.013	0.210
NAG	0.622	0.001	-0.920	0.392	0.290
BX	**0.658**	**0.001**	**-0.974**	**0.225**	**0.040**
AP	0.594	0.001	-0.976	-0.216	0.700
PO	**0.786**	**0.001**	**-0.518**	**-0.855**	**0.009**
PX	**0.759**	**0.001**	**-0.513**	**-0.858**	**0.005**

Table C.2. Vector loadings of all EEAs with p-values denoting significant differences among tree genera.

	Fungi				ECM Fungi			
name	R^2	p	MDS1	MDS2	R^2	p	MDS1	MDS2
Litter	**0.118**	**0.011**	**-0.059**	**0.998**	0.053	0.141	-0.200	0.980
pH	0.050	0.165	0.894	0.448	**0.087**	**0.043**	**0.958**	**-0.287**
Moisture	0.082	0.051	-0.288	-0.958	**0.089**	**0.033**	**-0.448**	**-0.894**
DOC	**0.135**	**0.006**	**0.044**	**0.999**	**0.123**	**0.012**	**0.138**	**0.990**
TN	**0.151**	**0.002**	**0.493**	**0.870**	**0.104**	**0.015**	**0.348**	**0.937**
MBC	**0.143**	**0.003**	**0.117**	**0.993**	**0.150**	**0.003**	**0.011**	**1.000**
MBN	0.024	0.433	-0.968	-0.252	0.036	0.272	-0.970	0.244
NH4+	0.057	0.129	-0.209	0.978	0.065	0.106	-0.025	1.000
NO3+-	0.062	0.096	-0.101	0.995	0.052	0.147	0.217	0.976
% POM	0.013	0.611	0.912	-0.410	0.016	0.564	-0.908	-0.419
MAOM $\delta^{13}C$	**0.142**	**0.004**	**-0.796**	**-0.606**	**0.087**	**0.036**	**-0.698**	**-0.716**
MAOM %C	**0.119**	**0.011**	**-0.191**	**-0.982**	**0.105**	**0.019**	**-0.005**	**-1.000**
MAOM $\delta^{15}N$	**0.161**	**0.001**	**0.669**	**-0.743**	0.039	0.231	-0.262	-0.965
MAOM %N	0.067	0.081	-0.135	-0.991	0.058	0.110	0.024	-1.000
MAOM C:N	0.004	0.873	-0.147	0.989	0.004	0.855	0.037	-0.999
POM $\delta^{13}C$	**0.160**	**0.003**	**-0.995**	**0.103**	0.040	0.209	-0.995	0.098
POM %C	0.051	0.166	0.778	0.629	0.013	0.613	-0.184	0.983
POM $\delta^{15}N$	**0.256**	**0.001**	**0.759**	**-0.651**	0.018	0.502	0.214	-0.977
POM %N	0.053	0.151	0.825	0.565	0.016	0.545	-0.093	0.996
POM C:N	0.013	0.612	-0.411	0.912	0.001	0.975	-0.912	-0.411
Soil $\delta^{13}C$	**0.194**	**0.002**	**-0.786**	**-0.618**	**0.140**	**0.004**	**-0.668**	**-0.744**
Soil %C	**0.204**	**0.001**	**0.275**	**0.961**	**0.143**	**0.004**	**0.247**	**0.969**
Soil $\delta^{15}N$	**0.202**	**0.001**	**0.515**	**-0.857**	**0.087**	**0.035**	**-0.112**	**-0.994**
Soil %N	**0.235**	**0.001**	**0.301**	**0.954**	**0.189**	**0.001**	**0.234**	**0.972**
Soil C:N	0.017	0.558	0.060	0.998	0.003	0.914	0.769	0.639
Leaf $\delta^{15}N$	**0.117**	**0.014**	**-0.401**	**-0.916**	0.059	0.125	-0.153	-0.988
Leaf %N	**0.081**	**0.040**	**-0.997**	**-0.074**	0.035	0.261	0.392	-0.920
Leaf δ13C	0.004	0.889	-0.111	0.994	0.012	0.666	0.229	0.973
Leaf %C	**0.119**	**0.012**	**0.439**	**0.898**	**0.154**	**0.002**	**0.020**	**1.000**
Leaf C:N	**0.089**	**0.028**	**0.933**	**0.359**	0.062	0.116	-0.339	0.941

Table C.3. Vector scaling for SOM variables against fungal, EM fungal, bacterial, EEA, and PLFA ordinations. Bolded values are significant at $p < 0.05$ for given ordination.

	Bacteria				Enzyme			
name	R^2	p	MDS1	MDS2	R^2	p	MDS1	MDS2
Litter	0.010	0.675	-1.000	0.000	0.058	0.129	-0.801	0.599
pH	**0.152**	**0.006**	**-0.951**	**-0.308**	0.007	0.765	0.943	0.334
Moisture	**0.073**	**0.048**	**0.958**	**0.287**	**0.261**	**0.001**	**0.988**	**0.154**
DOC	0.010	0.690	-0.618	0.786	**0.321**	**0.001**	**-0.913**	**-0.407**
TN	0.051	0.163	-0.814	0.581	0.044	0.204	-0.956	-0.293
MBC	0.043	0.200	-0.899	-0.439	**0.384**	**0.001**	**-0.952**	**0.308**
MBN	0.031	0.322	0.517	-0.856	**0.420**	**0.001**	**-0.969**	**0.247**
NH4+	0.002	0.925	-0.008	1.000	**0.372**	**0.001**	**-0.924**	**0.383**
NO3+-	0.002	0.944	-0.476	-0.879	**0.101**	**0.039**	**-0.723**	**0.691**
% POM	0.024	0.395	-0.251	0.968	**0.154**	**0.014**	**-0.996**	**-0.087**
MAOM $\delta^{13}C$	**0.166**	**0.003**	**0.983**	**-0.183**	0.022	0.431	-0.386	0.922
MAOM %C	0.022	0.423	0.276	-0.961	0.041	0.198	-0.830	0.558
MAOM $\delta^{15}N$	0.039	0.233	-0.182	0.983	**0.109**	**0.025**	**-0.923**	**-0.386**
MAOM %N	0.002	0.926	-0.373	-0.928	**0.104**	**0.010**	**0.989**	**0.146**
MAOM C:N	0.031	0.338	-0.920	-0.392	0.022	0.428	0.856	-0.517
POM $\delta^{13}C$	**0.185**	**0.001**	**0.870**	**-0.493**	**0.219**	**0.001**	**0.995**	**-0.103**
POM %C	0.046	0.193	-0.506	0.863	0.029	0.300	0.351	-0.936
POM $\delta^{15}N$	0.022	0.459	-0.830	0.558	0.012	0.642	-0.995	-0.100
POM %N	0.036	0.274	-0.584	0.812	0.051	0.153	-0.485	0.875
POM C:N	0.012	0.643	-0.414	0.910	**0.125**	**0.020**	**-0.992**	**-0.124**
Soil $\delta^{13}C$	**0.204**	**0.001**	**0.987**	**-0.159**	0.030	0.351	0.976	0.216
Soil %C	0.062	0.117	-0.995	-0.100	**0.151**	**0.014**	**-1.000**	**0.000**
Soil $\delta^{15}N$	0.042	0.207	0.021	1.000	0.039	0.245	0.349	-0.937
Soil %N	0.056	0.125	-0.979	-0.204	0.073	0.063	0.987	0.159
Soil C:N	0.026	0.391	-0.959	0.283	**0.223**	**0.001**	**-0.966**	**0.257**
Leaf $\delta^{15}N$	0.059	0.111	0.931	-0.364	**0.250**	**0.001**	**0.969**	**-0.245**
Leaf %N	**0.171**	**0.002**	**0.754**	**-0.657**	**0.267**	**0.001**	**-0.949**	**0.315**
Leaf δ13C	0.004	0.873	0.844	0.536	0.017	0.512	-0.956	-0.293
Leaf %C	**0.114**	**0.012**	**-0.714**	**0.700**	0.037	0.240	1.000	-0.031
Leaf C:N	**0.188**	**0.001**	**-0.697**	**0.717**	0.028	0.359	0.613	0.790

Table C.3. Continued

	PLFA			
name	R^2	p	MDS1	MDS2
Litter	0.010	0.695	-1.000	-0.004
pH	0.078	0.051	0.952	0.306
Moisture	0.027	0.378	-0.860	-0.511
DOC	**0.083**	**0.050**	**0.873**	**0.487**
TN	0.018	0.487	0.690	0.723
MBC	**0.136**	**0.007**	**0.156**	**0.988**
MBN	**0.119**	**0.013**	**0.200**	**0.980**
NH4+	**0.139**	**0.007**	**0.078**	**0.997**
NO3+-	0.052	0.143	-0.320	0.947
% POM	0.023	0.454	-0.521	0.854
MAOM $\delta^{13}C$	0.022	0.458	-0.782	0.623
MAOM %C	0.031	0.331	-0.724	0.689
MAOM $\delta^{15}N$	**0.102**	**0.018**	**0.731**	**0.682**
MAOM %N	**0.127**	**0.011**	**-0.445**	**-0.895**
MAOM C:N	0.025	0.404	0.851	-0.526
POM $\delta^{13}C$	0.053	0.140	-0.616	-0.788
POM %C	0.042	0.220	0.966	-0.257
POM $\delta^{15}N$	0.034	0.287	0.559	-0.829
POM %N	0.074	0.061	-0.732	-0.681
POM C:N	0.027	0.381	0.926	0.379
Soil $\delta^{13}C$	0.019	0.492	-0.942	0.334
Soil %C	0.037	0.267	0.635	0.773
Soil $\delta^{15}N$	**0.112**	**0.015**	**0.388**	**-0.922**
Soil %N	**0.086**	**0.050**	**-0.361**	**-0.933**
Soil C:N	0.046	0.174	-0.026	1.000
Leaf $\delta^{15}N$	0.053	0.141	-0.375	-0.927
Leaf %N	**0.093**	**0.033**	**-0.221**	**0.975**
Leaf δ13C	0.025	0.379	0.827	-0.562
Leaf %C	**0.137**	**0.005**	**-0.362**	**-0.932**
Leaf C:N	**0.142**	**0.004**	**-0.599**	**-0.801**

Table C.3. Continued

	Fungi				ECM Fungi			
name	R^2	p	MDS1	MDS2	R^2	p	MDS1	MDS2
AG	**0.193**	**0.001**	**-0.820**	**0.573**	0.022	0.435	0.010	1.000
BG	0.064	0.105	-0.717	0.697	0.062	0.089	-0.490	0.871
CBH	0.041	0.238	-0.665	0.747	0.045	0.178	-0.513	0.858
NAG	0.068	0.092	-0.961	0.277	0.060	0.117	-0.752	0.660
BX	0.076	0.068	-0.593	0.806	0.071	0.066	-0.529	0.849
AP	**0.134**	**0.010**	**-0.608**	**0.794**	**0.190**	**0.002**	**-0.450**	**0.893**
PO	0.029	0.345	0.836	0.548	0.007	0.756	0.990	0.144
PX	0.033	0.302	0.652	0.758	0.015	0.583	0.954	0.301

	Bacteria				SOM			
name	R^2	p	MDS1	MDS2	R^2	p	MDS1	MDS2
AG	**0.240**	**0.001**	**-0.081**	**-0.997**	**0.178**	**0.001**	**-0.822**	**-0.570**
BG	0.050	0.156	-0.437	-0.899	**0.269**	**0.001**	**-0.941**	**-0.338**
CBH	0.021	0.457	-0.271	-0.963	**0.252**	**0.001**	**-0.910**	**-0.414**
NAG	**0.091**	**0.033**	**-0.487**	**-0.874**	**0.207**	**0.001**	**-0.944**	**-0.329**
BX	0.047	0.164	-0.325	-0.946	**0.271**	**0.001**	**-0.920**	**-0.392**
AP	0.058	0.111	0.766	0.643	**0.267**	**0.001**	**-0.978**	**-0.207**
PO	0.050	0.152	-0.355	0.935	0.036	0.250	-0.999	0.037
PX	0.045	0.181	-0.321	0.947	0.043	0.188	-1.000	-0.004

	PLFA			
name	R^2	p	MDS1	MDS2
AG	0.057	0.125	-0.229	0.974
BG	0.069	0.080	0.100	0.995
CBH	**0.083**	**0.043**	**0.262**	**0.965**
NAG	0.028	0.379	0.320	0.947
BX	**0.133**	**0.011**	**0.054**	**0.999**
AP	0.047	0.170	-0.302	0.953
PO	**0.112**	**0.009**	**0.996**	**0.091**
PX	**0.102**	**0.013**	**0.989**	**0.148**

Table C.4. Vector scaling for EEA variables against fungal, EM fungal, bacterial, SOM, and PLFA ordinations. Bolded values are significant at $p < 0.05$ for given ordination.