Microbial Community Dynamics and Function During Coarse Woody Debris and Leaf Litter Decomposition

A Thesis

Presented in Partial Fulfillment of the Requirements for the

Degree of Master of Science

with a

Major in Environmental Science

in the

College of Graduate Studies

University of Idaho

by

Katelyn Marie Conery

Approved by:

Major Professor: Michael S. Strickland, Ph.D.

Committee Members: Zachary Kayler, Ph.D.; Randall Brooks, Ph.D.

Department Administrator: Lee Vierling, Ph.D.

December 2021

Abstract

Nitrogen fertilization is a common soil amendment; however little is known about how surface and belowground wood decomposition are affected by N amendments. We analyzed data from a field study in Norrliden, Sweden where the mass loss of trembling aspen (*Populus tremuloides* Michx.) and loblolly pine (*Pinus taeda* L.) wood stakes was recorded for three years. Wood stakes were placed horizontally on the surface of the organic horizon and at the interface between the mineral and organic horizons and inserted vertically into the mineral soil in plots treated with different amounts of ammonium nitrate fertilizer (no nitrogen (control), 60 kg ha^{-1} (N1), 120 kg ha^{-1}(N2)). Fungi colonizing the wood stakes were also analyzed. Mass loss was greater in the mineral soil for aspen and pine stakes compared to surface stakes and N treatment only affected stakes placed on the surface. Nitrogen treatment did not affect fungal richness, but it did affect fungal community composition. Overall, N treatment had minimal effect on coarse woody debris (CWD) decomposition and CWD decomposition appeared to be driven more by microclimatic conditions of the soil as related to soil depth.

Microbial communities are important drivers of decomposition and the ability to link community structure to function will strengthen our understanding of their role in C and nutrient cycling. We used microcosms to study how seven different microbial communities sourced from areas with different vegetative cover decomposed the same litter by recording mass loss, respiration, and total volatile organic compound (VOC) production weekly for 12 weeks. We sampled microbial community composition at four time periods during the 12-week study. Mass loss was more related to fungal community as a whole while respiration and total VOCs were more related to the fungal class Sorardiomycetes. Microbial communities remained compositionally distinct throughout the study and were related to differences in decomposition, respiration, and total VOC production, suggesting that microbial communities are not functionally redundant. Because of the differences observed in total VOCs and community composition, we propose that microbial community structure could be studied via VOC production.

Acknowledgments

I would first like to acknowledge my advisor Dr. Michael Strickland for his support, guidance, and dedication to this research study. I would like to thank my committee members Dr. Zachary Kayler and Dr. Randy Brooks for their encouragement and wisdom along the way. I am very grateful for Dr. Jane Lucas for her time and effort spent helping me both in the laboratory and with data analysis. I am appreciative of the support from Dr. Ernie Osburn and the knowledge he has shared with me. I would like to thank my fellow graduate students in the Strickland Lab for their teamwork and friendship. Lastly, I would like to give appreciation to Dr. Debbie Page-Dumroese, Dr. Kas Dumroese, Dr. Martin Jurgensen, Dr. Daniel Lindner, Dr. Michelle Jusino, and Dr. Mark Banik with the USDA Forest Service for their collaboration and efforts in this research (Chapter 2).

Dedication

This work is dedicated to my family who understood the commitment it took to complete this degree and showed continuous support throughout my entire academic career. Without the support of my parents and their dedication to me, I would not be as fortunate I am today.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction to Decomposition

The factors and processes affecting decomposition

Introduction

Soil organic matter (SOM) has several functions including storage and release of nutrients, reducing soil erosion, and improving soil structure (Bot & Benites, 2005). The decomposition of SOM can take several decades to centuries depending on the quality of substrate (i.e., the number of enzymes required to release carbon from a compound) (Bosatta & Ågren, 1999; Wadman & de Haan, 1997). Bacteria and fungi are the primary decomposers of SOM (Van Veen & Kuikman, 1990). The activity of these decomposers is determined partially by availability of nutrients and energy from litter inputs, where litter inputs tend to increase microbial activity and decomposition rate (Thiessen et al., 2013). Soil structure also plays a role in SOM decomposition by affecting microbial accessibility to nutrients, soil temperature, and moisture (Van Veen & Kuikman, 1990). These factors affecting SOM decomposition also affect leaf litter and coarse woody debris (CWD) decomposition. Biotic and abiotic factors interact to control decomposition. Temperature, moisture, wood/litter chemistry, spatial arrangement, and microbial community composition all affect decomposition rate (Aerts, 1997; Bradford et al., 2016; Keiser et al., 2014; Shorohova & Kapitsa, 2014; Strickland et al., 2015; Strickland, Lauber, et al., 2009; Strickland, Osburn, et al., 2009; Zhou et al., 2007). The mechanism of several of these factors and their control on decomposition is difficult to understand because of complex interactions. However, the CWD study described in chapter 2 attempts to disentangle a few of these interactions.

Organic matter is derived from an array of substrates. We studied the major contributing group to OM: plant tissues (Bot & Benites, 2005). Specifically, we studied coarse woody debris and leaf litter to better understand the controls on their decomposition. The study on CWD decomposition (Chapter 2) focused on how wood species, spatial arrangement, and nitrogen additions interact to affect decomposition. Fungal community composition was also studied as a response to these different treatments. This study allowed us to better understand the dominant controls on CWD decomposition and how several factors interact during this process. Most notably, we will be able to gauge how anthropogenic N additions will affect CWD decomposition which affects C storage and the

building of OM. Nitrogen fertilization can affect soil pH by causing soil acidification (Hobbie, 2008). It can also affect cellulose and lignin degrading enzyme activities and microbial community composition (Carreiro et al., 2000; Freedman et al., 2016; He et al., 2021).

Coarse woody debris includes all non-living woody biomass, typically greater than 10cm in diameter (Garrett et al., 2007). Decomposition of CWD has been recognized for its importance is providing a long-term sink of available nutrients (Laiho & Prescott, 2004). This decomposition process is influenced by the properties of the wood and other biotic and abiotic factors, similar to leaf litter decomposition (Freschet et al., 2012). A combination of these factors has been shown to control decay rate; however, temperature has been highlighted as one of the more dominate factors (Finér et al., 2016; Garrett et al., 2007). A study comparing CWD decomposition in a clear-cut and uncut forest found that clear-cut forests had significantly greater mass loss than uncut forests and this was attributed to the higher soil temperatures in the clear-cut forest (Finér et al., 2016). Location of CWD, as represented by wood stakes in several studies, also had significant influence on mass loss. Wood stakes in an uncut forest that were placed on the soil surface, decomposed faster than stakes placed in the mineral soil. The opposite was found in a clear-cut forest (Finér et al., 2016). These patterns were likely controlled by the amount of moisture available at each location and the temperature. Stakes placed on the surface of a clear-cut forest are not protected from moisture loss and have higher soil temperatures as a result of greater exposure compared to uncut forests (Finér et al., 2016). Fungal communities are also important in determining decomposition rates and should be studied during a CWD decomposition experiment. A wood stake decomposition study by Wang et al. (2020) found that wood stakes placed on the forest floor surface had a greater fungal richness than stakes placed in the mineral soil (Wang et al., 2020). They also found that pine stakes had a greater fungal richness than aspen stakes for both stake placement locations. Overall, fungal community richness varied depending on the wood stake species, stake location, and years of decomposition (Wang et al., 2020). CWD decomposition studies are challenging however, because decomposition rate and fungal distribution is likely site-specific (Wang et al., 2020). We will be able to better understand the dominant controls on CWD decomposition with future studies in spite of these potential site-level variations.

The study on leaf litter decomposition (Chapter 3) focused on how different microbial communities decomposed the same litter source. We tracked mass loss, microcosm respiration, and total volatile organic compound (VOC) production weekly for 12 weeks. Microbial community composition was analyzed for a subset of the study period. Microbial communities are often studied via genomics-based methods which are time consuming and costly. This study explored the use of VOCs produced by microbial communities to determine community composition and function. This has the potential to be a much more efficient and cost-effective method to study microbial communities and decomposition, both in situ and ex situ.

Leaf litter decomposition is important in providing a quick release of nutrients back into the soil. Carbon is another important element released during decomposition. Mostly studied as respiration i.e., CO_2, carbon is also released in the form of volatile organic compounds. Carbon can be used as an energy source by organisms and VOCs are an important, yet overlooked source of C. Active decomposition by microbes can produce a variety of VOCs depending on the litter type present (Gray et al., 2010). What remains unclear however, is if variations in VOC production is observed when varied microbial communities decompose the same litter type. It has been shown that microbial communities decompose litters at different rates based on their past resource history (Strickland, Lauber, et al., 2009), but the VOC profile of these communities as they decompose litter has not been well studied. Understanding whether microbial community composition affects VOCs is important to our understanding of soil ecosystems and the carbon cycle. For example, litter-derived VOCs, specifically acetone and methanol, were shown to increase labile carbon and increase microbial respiration in a microcosm experiment (McBride et al., 2019). Additionally, VOC derived C can contribute significantly to soil microbial biomass and organic matter pools (McBride et al., 2020). Volatile organic compounds have also been shown to impact the N-cycle, potentially via the inhibition of nitrification (Amaral et al., 1998.; Gray et al., 2010; McBride et al., 2020). Volatile organic compounds have other impacts among microbial communities. They are used as infochemical molecules that affect the behavior and gene expression in receiving microorganisms and are used to suppress or eliminate potential enemies (Schmidt et al., 2016). There are many implications of VOC production and the types and quantity of VOCs produced depends on the presence of

microbes, microbial biomass, and litter type (Gray et al., 2010; Leff & Fierer, 2008; Strickland, Lauber, et al., 2009; Strickland, Osburn, et al., 2009). Understanding if and how litter decomposing communities alter the amount and composition of VOCs will have ecosystem scale implications for understanding C and nutrient cycling and also studying microbial community structure and function.

Both studies highlight unknowns about the decomposition process of leaf litter and CWD in the environment. Specifically, we disentangled biotic and abiotic controls on CWD decomposition and attempted to bring about a new method for studying microbial community composition and function. Both studies analyze microbial community composition and its relation to decomposition over time. These two studies further our understanding on the controls of the decomposition process, which is important to understanding soil C and nutrient storage.

Chapter 2: Coarse Woody Debris Decomposition Field Study

The effect of wood stake species, stake placement, and legacy nitrogen fertilization on coarse woody debris decomposition

Introduction

Coarse woody debris (CWD) is an integral component of forest ecosystems. Woody materials are composed primarily of lignin and cellulose, which when broken down into simpler compounds, provide carbon and nutrients for bacteria, fungi, and other organisms. CWD decomposes slowly, making it a long-term nutrient store and energy source within forest ecosystems (Jia-bing et al., 2005; Zhou et al., 2007), and it plays an important role in the carbon (C) and nitrogen (N) cycles. Generally, the C:N of CWD decreases during the later stages of decomposition (Palviainen & Finér, 2015). Although it has been found that CWD only contributes approximately 5% of N released from decomposing material to the N cycle (Laiho & Prescott, 2004), these nutrient release and retention dynamics should not be overlooked (Harmon et al., 2004). The C and N cycles are tightly coupled and with increasing atmospheric CO_2 levels, the N cycle may either reduce or increase the Earth's ability to take up anthropogenic CO_2 (Gruber & Galloway, 2008). Understanding the feedback between the C and N cycles may lead to potential methods to mitigate climate change (Bradford et al., 2016; Keiser et al., 2014; Shorohova & Kapitsa, 2014; Strickland et al., 2015; Strickland, Lauber, et al., 2009; Strickland, Osburn, et al., 2009; Zhou et al., 2007), therefore all amounts of N need to be accounted for in the global N cycle. The release of nutrients from CWD during decomposition could expand our understanding of not only the N cycle, but nutrient cycling in general. While there is growing recognition of the important role CWD plays in ecosystems, our understanding of the factors that influence CWD decomposition is still incomplete (Fissore et al., 2016).

The factors controlling CWD decomposition are similar to leaf litter decomposition, with temperature, moisture, spatial arrangement, chemistry, and microbial communities all influencing the overall decomposition rate (Shorohova & Kapitsa, 2014; Zhou et al., 2007). Another factor that influences decomposition is N-availability (Carreiro et al., 2000; Chen et al., 2016; Henriksen & Breland, 1999; Manning et al., 2008). Given its inherent high C:N ratio, most N effects on CWD decomposition are exogenous in nature, occurring via

fertilization and/or deposition. Low N availability in CWD can cause microbes to allocate energy towards N-acquisition enzymes instead of enzymes that degrade lignocellulose, i.e., lignocellulases (Hu et al., 2018; Sinsabaugh et al., 2009). It may be more energy efficient for microbes to gain N via exogenous sources rather than through CWD decomposition. This leads to the assumption that there is an optimal soil N concentration for maximum CWD decomposition. Along with N availability, the chemical composition of CWD also affects decomposition rate. The lignin and cellulose components of CWD are broken down by different organisms and at varying rates due to differences in molecular structure and differences in production of ligninolytic and cellulolytic enzymes by microorganisms (Kaarik, A., 1974). Different types of wood have different lignin contents. Generally, softwoods (gymnosperms/conifers) are composed of a greater percentage of lignin than hardwoods (angiosperms). The molecular structure of lignin is more complex than cellulose, requiring more energy to breakdown. Some fungi only produce ligninolytic enzymes when their growth is N limited (i.e., N-mining), so an increase in available N decreases this enzyme production resulting in a decrease of lignin decomposition (Bebber et al., 2011; Carreiro et al., 2000; Keyser, 1978). This balance between N-availability and CWD decomposition could ultimately be affected by an increase in soil N via fertilization or deposition. In most forests, an increase in N will likely affect CWD decomposition by affecting enzyme production, microbial nutrient acquisition strategies, and changes in microbial community composition, ultimately leading to a decrease in decomposition if N-availability is great (Freedman et al., 2016; He et al., 2021).

As spatial arrangement of CWD on the forest surface is known to affect decomposition rate (Finér et al., 2016), depth within the soil should also be considered (Fissore et al., 2016). Spatial arrangement of CWD can have an effect on microclimatic conditions including temperature and moisture and also impact microbial colonization due to contact with other forest material (Remsburg & Turner, 2006). However, there is a lack of research and understanding as to how soil depth (i.e., within the mineral soil) affects CWD decomposition rates (Finér et al., 2016; Fissore et al., 2016; Wang et al., 2019). There are several factors to consider when studying decomposition at different soil depths. First, CWD found on the surface of the forest floor is exposed to greater moisture and temperature fluctuations throughout the year. While the majority of CWD is found on the soil surface,

CWD can also be incorporated in the soil at depth either via burying or as root material (Moroni et al., 2015). CWD decomposing within the soil will be exposed to more stable temperature and moisture regimes. The soil microbial communities also vary along the soil profile, potentially leading to different decomposer communities at depth (Eilers et al., 2012; Fissore et al., 2016). However, it is relatively unknown whether more stable climate regimes combined with potential differences in the decomposer community will lead to greater or lesser decomposition rates of CWD at depth when compared to debris on the surface.

To better understand how nitrogen additions and soil depth affect CWD decomposition and the fungal decomposer community, we conducted a three-year study in Norrliden, Sweden using two different wood species, trembling aspen (*Populus tremuloides* Michx.) and loblolly pine *(Pinus taeda* L.). The study site was grouped into treatment plots with three levels of N additions, representing no, low, and high rates of N fertilization. Within each treatment plot, wood stakes of each species were placed at three soil depths (i.e., soil surface, interface between organic and mineral horizons, and mineral horizon). Each year, stakes were removed and analyzed for mass loss and fungal community composition. Due to the lower lignin content of aspen, we hypothesized that aspen stakes would decompose more rapidly than pine stakes. We also hypothesized that stakes placed in the mineral soil (greatest depth) should have the greatest mass loss due to there being greater moisture retention and more stable temperatures within the soil compared to stakes placed on the surface of the forest floor. Based on results from previous studies where an increase in available N decreases ligninolytic enzyme production (Bebber et al., 2011; Carreiro et al., 2000; Keyser, 1978), we hypothesized that the control (no N) treatments would be most optimal for decomposition, while the low and high N treatments would decrease decomposition. There is the potential for stake placement and N treatment to have an interacting effect on decomposition, with N having a greater overall control on decomposition (Hu et al., 2018). We also expected to find some fungi that are positively associated with mass loss and potentially correlated to the stake placement. Overall, we expected that this research will improve our understanding of the factors likely to shape CWD decomposition and the fungal communities involved in this critical ecosystem process.

Materials and Methods

Study area

We conducted a coarse woody debris decomposition experiment using an ongoing Scots pine (*Pinus sylvestris* L.) forest fertilization study in northern Sweden near Norrliden (64º21'N, 19º46' E). The Norrliden field site has been used since 1972 for fertilization experiments. Plots used correspond to those established for forest fertilization studies. For these studies the 30x30 m fertilized plots had been given ammonium nitrate for 18 consecutive years (1971-1988; Högberg, 1990). Average air temperature is 1.6º C and mean annual precipitation is 595 mm (Holmen et al. 1976). The soil is a podzol developed on glacial till. For more details on study site layout, soils, climate, and fertilizer treatments see Holmen et al. (1976), Högberg et al. (2006), and Tamm et al. (1999).

To assess the effect of N on CWD decomposition, we used three treatment plots, replicated three times each, which varied in N amendments. Treatments included a control (i.e., no nitrogen addition; plot numbers 37, 50, 51), low nitrogen (N1) application with 60 kg ha^{-1} ammonium nitrate applied yearly (plot numbers 38, 53, 57), and high nitrogen (N2) application with 120 kg ha^{-1} ammonium nitrate applied yearly (plot numbers 36, 42, 52). Nitrogen was only applied from 1971 to 1988.

Determination of mass loss associated with wood species, soil placement, and nitrogen fertilizer

In June 2007, to assess the interactive effects of species, placement, and N availability on CWD decomposition, we placed 25 aspen and pine stakes horizontally on the surface of the organic horizons, horizontally at the interface between the mineral and organic horizons, and vertically into the mineral soil of each treated plot and control (3 treatments x 3 replicates x 3 stake locations x 25 stakes x 2 species=1,350 total). Stake construction follows the protocol in Jurgensen et al. (2006) whereby surface and interface stakes (2.5 x 2.5 x 15 cm) and mineral soil stakes (2.5 x 2.5 x 20 cm) were cut from longer, kiln-dried, knot-free sapwood stakes and made from loblolly pine (*Pinus taeda* L.) and trembling aspen (*Populus tremuloides* Michx.). A center section from the larger stake was used as a laboratory control (time=0) to determine mass loss of the stakes after they were sampled. To minimize soil compaction at the wood-soil interface, stakes inserted in the mineral soil were placed into 2.5

cm holes, ~30 cm apart, made by a square 2.5 cm soil coring tool. The top of each mineral soil stake was treated with a wood sealer to reduce moisture loss after installation and was inserted so that the top was even with the mineral soil surface and then covered with the surface organic horizons. Five stakes of each species at each location and treatment were randomly selected for removal annually between 2008 and 2010. Once extracted, all stakes were weighed in the field to determine moisture content and then sent to the School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI, United States for processing.

Before shipping to the United States, stakes were kept cool after extraction and within 24 hours we collected wood shavings from four randomly selected stakes of each species, from each treatment, stake location, and replicate (3 treatments x 3 replicates x 3 stake locations x 4 stakes x 2 species = 216 stakes/sample date) for fungal DNA analysis. Before collecting wood shavings, both ends of each stake were cleaned with a sterile razor blade to remove loose soil. Then the end of each stake was drilled with sterile drill bits and, for each stake, the two shavings were composited into one individual sample. Shavings were placed in 2 ml strip tubes in 96-well format and covered with filter-sterilized cell lysis solution (Lindner & Banik, 2009). Tubes were sealed and frozen before shipping to the Center for Forest Mycology Research (Madison, WI, USA) for fungal community analysis. All fungal samples were frozen to -80ºC until DNA was extracted.

In the laboratory, all stakes were cleaned of adhering soil, dried to 105º C for 48 h and weighed. Decomposition (mass loss) of stakes was measured by subtracting the dry weight of individual surface, interface, and mineral soil field stakes from the dry weight of its corresponding laboratory control (t_0). Mass loss averages for each replicate (n=5) were used as treatment observations in the statistical analyses. Some stakes were unable to be recovered and were recorded as missing.

Sequencing fungal ITS sequences from wood samples

Samples were stored at the Center for Forest Mycology Research (Madison, WI, USA) at -80° C until processed. Fungal DNA was extracted from drill shavings following the methods outlined in Lindner et al. (2011) and Lindner and Banik (2009). In brief for DNA extraction the 1.5 ml tubes containing samples were thawed to 65° C for 1-2 hours, then

centrifuged at 16.1 rcf for 5 minutes at room temperature with 100 μL of the supernatant transferred to strip tubes. DNA was then extracted as outlined in Lindner and Banik (2009), but modified for use with 200 μL strip tubes as per Lorch et al. (2013). Amplification of the resulting DNA was accomplished using the fungal specific primer pair ITS1F/ITS4 (Lindner & Banik, 2009) and cloning of the resulting amplicons. Eight clones were chosen from each sample for reamplification and sequencing. Fungal identifications are based on the nearest BLAST match in GenBank using similarities of 297% to denote species identification and 90-97% for genus identification. Similarities of less than 90% were tentatively identified to higher level taxa that were the best match (i.e., order or family). In addition, all samples underwent PCR with an *H. irregulare* specific primer (HA2, TACCCCACGGCGTAGACA) paired with ITS1F. This primer was tested against diluted *H. irregulare* positive samples to verify efficacy.

Statistical Analyses

Statistical analyses were conducted in R (R Core Development Team, 2019) and Primer (Anderson et al., 2008). Fungal presence/absence data was recorded in the form of an OUT (operational taxonomic unit) table. The data were not normally distributed, so a logit transformation was tested. A logit transformation did not significantly alter the outputs or distribution of the data, so the original data was used. Wood stake species, stake placement, and N treatment were the experimental factors tested for the response variables stake mass loss and fungal richness. Kruskal-Wallis and Scheirer-Ray-Hare tests ('kruskal.test' and 'scheirerRayHare' functions, 'rcompanion' package) were used for analysis of main effects as they are nonparametric tests (Mangiafico, 2021). Scheirer-Ray-Hare was used to analyze the full models for a significant interaction of factors. If no significant interaction was detected in a model, Kruskal-Wallis was used to test individual main effects against the response variables. Dunn's test ('dunnTest' function) was used for pairwise differences. For statistical significance we assumed an alpha level of 0.05. Since the aspen and pine stakes were significantly different ($P <0.001$(mass loss), $P<0.01$ (richness)), they were analyzed separately. To calculate the percent effect each factor had on the response variables, percent sum of squares was calculated. The 'Rmisc' package was used to calculate means and standard error of data (Hope, 2013).

For fungal communities, we analyzed Jaccard distance matrixes of fungal presence/absence data via a permutational MANOVA (perMANOVA). Pairwise comparisons between treatments were also analyzed via perMANOVA and we tested for homogeneity of dispersions from the centroids via betadisper tests (Anderson et al., 2008). Metric MDS ordination was used to visualize results and employed 999 bootstrap averages of the centroid of to illustrate where 95% of the centroid averages lie within multivariate space. To assess the contribution of specific fungal taxa (i.e., taxa exhibiting the greatest average presence across treatments) to differences between treatments we assessed differences in the presence of taxa across N treatments at each soil depth for aspen and pine via ANOVA.

Results

Mass loss associated with wood species, placement, and nitrogen fertilization

Not surprisingly, mass loss increased with sample year, and overall, aspen stakes exhibited greater mass loss than did pine stakes ($P<0.001$). However, we also found that sample year, N treatment, stake species, and depth of stake placement all interacted to affect mass loss ($P<0.05$). To disentangle this interaction, we examined the response of each stake species for a given placement depth ($P<0.001$ aspen and pine). For aspen stakes, mass loss was greatest for stakes placed at the interface and mineral soil, followed by those placed on the surface (Figure A2.1). Interestingly, the N treatments mainly affected mass loss of aspen stakes placed on the surface (Table A2.1). Specifically, the low N treatment (i.e., N1) was associated with greater mass loss in sample years 2009 and 2010 for aspen stakes placed on the surface. Both the control and high N (i.e., N2) treatments, had similar mass loss and were both lower than N1. Aspen stakes placed at the interface also showed a significant overall N treatment effect, and although significant pairwise treatment differences were not detected, mass loss was greatest for the N1 treatment followed by N2 and control, respectively (Figure 2.1).

For pine stakes, mass loss was greatest for stakes placed in the mineral soil, followed by interface and surface placement (Figure A2.2). Unlike the aspen stakes, N treatment did not affect mass loss of pine stakes placed at any location (Table A2.1). Mass loss was

impacted to a greater degree by year and stake placement than N treatment for both aspen and pine stakes.

Overall, stake species accounted for 7.31% of the variation in mass loss overall, based on the percent sums of squares. Within stake species, stake placement accounted for 4.22% and 6.07% of the variation in mass loss for aspen and pine, respectively. Nitrogen fertilizer accounted for 2.22% and 2.09% of the variation in mass loss for aspen and pine, respectively.

Fungal richness associated with wood species, placement, and nitrogen fertilization

Fungal richness for both stake species decreased with sample year, and overall, pine stakes exhibited greater fungal richness than did aspen stakes (Figure 2.2). Fungal richness for aspen stakes was relatively similar across placement locations and N treatments (Figure A2.3). The placement locations were not significantly different from each other (P=0.12) and richness was mainly affected by N treatments at the surface placement (Table A2.2). Specifically, differences were observed only within the surface placement in 2009 (Table A2.4) and were driven by N1 treatment having lower fungal richness than both N2 and the control.

Fungal richness was significantly different at the stake placement locations for pine stakes (P<0.05). Specifically, pine stakes placed in the mineral soil had lower fungal richness than the stakes placed on the surface or interface (Figure A2.4). There was no detectable difference in fungal richness between the surface and interface stakes. Nitrogen treatments did affect fungal richness, mainly for stakes placed on the surface in 2009 (Figure 2.2). This difference is driven by the control having greater fungal richness than N1.

Figure 2.1 Means and standard error of percent mass loss of aspen and pine stakes over time, subset by the different stake placements. Asterisks denote a statistical difference (alpha < 0.05) among N treatments for that year. N treatment was only significantly different for stakes placed on the surface. Kruskal-Wallis and Scheirer-Ray-Hare test p-values are listed in each plot.

Overall, stake species accounted for less than 1% of the variation in fungal richness based on the percent sums of squares. Within stake species, stake placement accounted for less than 1% of the variation in fungal richness for both aspen and pine. Nitrogen fertilizer accounted for 1.22% and 2.02% of the variation in fungal richness for aspen and pine, respectively.

We observed an inverse relationship with fungal richness and mass loss where a decrease in fungal richness related to an increase in mass loss (Figure A2.5). As time progressed there was an increase in mass loss and decrease in fungal richness (Figures 2.1 and 2.2), suggesting that there is a relationship between the CWD components remaining at each stage of decomposition and the fungi that can break down these components.

Fungal community composition associated with wood species, placement, and nitrogen fertilization

Overall fungal community composition was affected by all main and interactive effects ($P<0.01$ in all instances). Due to the significant four-way interaction between sample year, stake species, stake placement, and N treatment (pseudo-$F_{8,1146}=1.25$; $P<0.01$), we examined fungal community composition for each stake species across all sampling years for a given stake placement (Figure 2.3). This enabled us to disentangle how communities changed across time and in response to the N treatments.

For the aspen stakes placed on the soil surface, we observed significant main effects of both N treatment (pseudo-$F_{2,189}=3.40$; $P<0.01$) and sampling year (pseudo-$F_{2,189}=3.21$; $P<0.01$), and a significant N treatment × sampling year interaction (pseudo-$F_{4,189}=1.40$; $P<0.01$). This interaction is likely observed because only the control differed from N1 and N2 in 2008, but in subsequent sampling years all three N treatments were significantly different from each other (Figure 2.3). Additionally, across all sample years, the species *Mollisia cinerea* was observed more often in the control N treatment as compared to either N1 or N2 ($P<0.001$; Table A2.5). In fact, *Mollisia cinerea* was never observed in the N1 treatment. *Phanerochaete velutina* was observed more often in N1 compared to either the control or N2 ($P<0.01$; Table A2.5); and *Ascocoryne cylichnium* was observed more often in N2 compared to the control ($P<0.05$; Table A2.5).

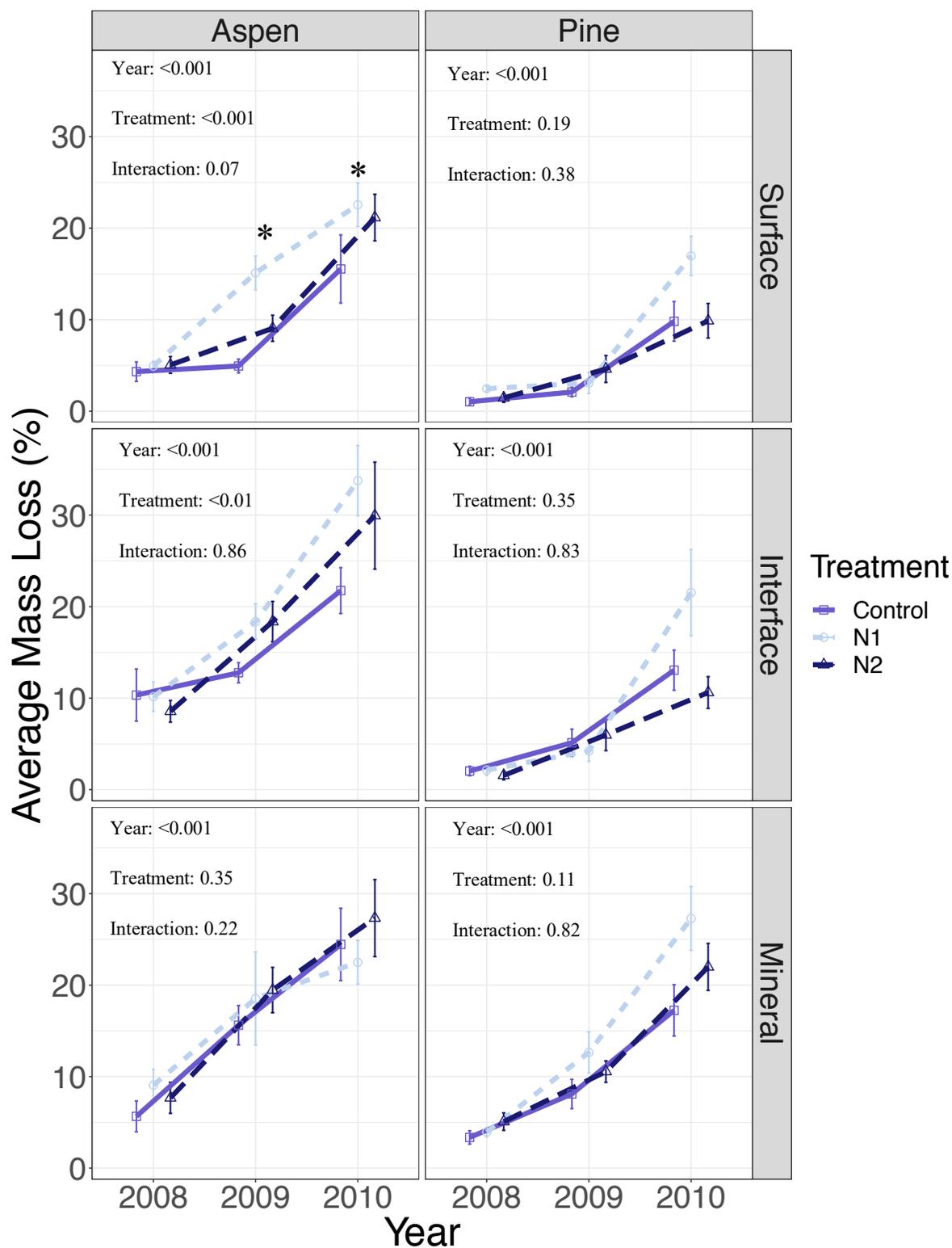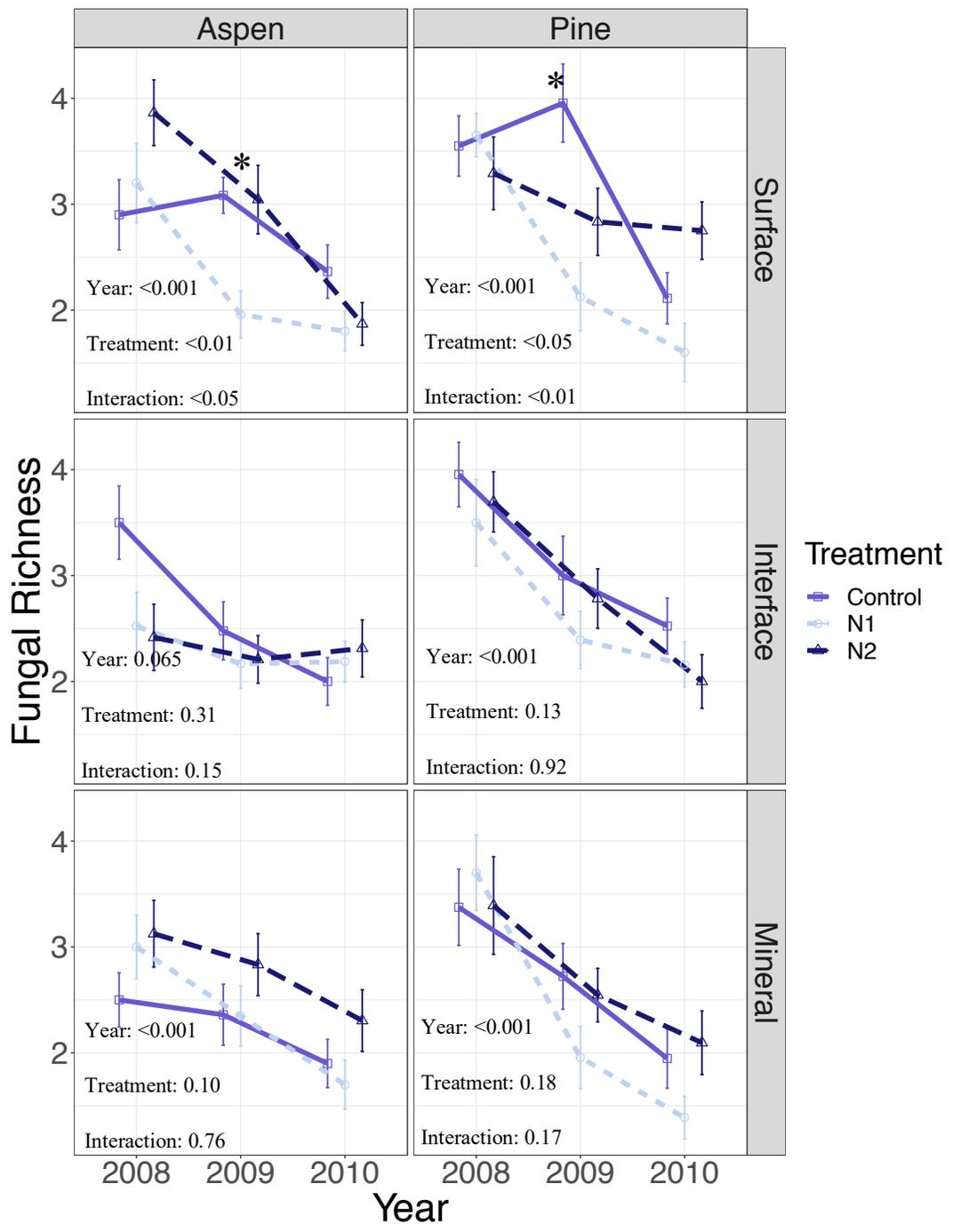**Figure 2.2** Mean and standard error of fungal richness of aspen and pine stakes over time, subset by the different stake placements. Asterisk denote a statistical difference (alpha < 0.05) among N treatments for that year. N treatment was only significantly different for stakes placed on the surface. Kruskal-Wallis and Scheirer-Ray-Hare test p-values are listed in each plot.

Additionally, *Pseudoplectania nigrella* was never present in N2 ($P<0.01$; Table A2.5); and *Lachnellula fuscosanguinea* was never present in the control, and tended to be present most often in N2 ($P<0.01$; Table A2.5).

For aspen stakes placed at the interface, we observed significant main effects of both N treatment (pseudo-$F_{2,205}$=4.20; $P<0.01$) and sampling year (pseudo-$F_{2,205}$=4.01; $P<0.01$), and a significant N treatment × sampling year interaction (pseudo-$F_{4,205}$=2.05; $P<0.01$). The interaction is likely due to significant differences between all N treatments in 2008 and 2009, but in 2010 N1 and N2 did not differ (Figure 2.3). Again, *Mollisia cinerea* was observed more often in the control treatment as compared to either N1 or N2 ($P = 0.06$; Table A2.5). Additionally, *Phialocephala fortinii* was also more often present in the control compared to the other two treatments ($P<0.001$; Table A2.5). *Phanerochaete velutina* tended to be observed more often in N1 ($P<0.01$; Table A2.5). *Acrodontium antarcticum* and *Sporothrix sp.* tended to be observed more often in N2 ($P<0.01$ and $P<0.001$, respectively; Table A2.5).

For aspen stakes placed in mineral soil, we observed significant main effects of both N treatment (pseudo-$F_{2,194}$=2.73; $P<0.01$) and sampling year (pseudo-$F_{2,194}$=3.79; $P<0.01$), and a significant N treatment × sampling year interaction (pseudo-$F_{4,194}$=1.30; $P<0.05$). The interaction is likely due to no N treatment differences observed in 2008, while in 2009 and 2010 N2 differed from both the control and N1. The control and N1 did not differ from each other across all sampling years. A *Cenococcum sp.* and *Phanerochaete velutina* tend to be observed more often in the control and N1, respectively ($P<0.01$ and $P<0.001$, respectively; Table A2.5).

For pine stakes placed on the soil surface, we observed significant main effects of both N treatment (pseudo-$F_{2,190}$=4.26; $P<0.01$) and sampling year (pseudo-$F_{2,190}$=3.89; $P<0.01$), and a significant N treatment × sampling year interaction (pseudo-$F_{4,190}$=1.71; $P<0.01$). While N treatments significantly differed across all sampling years, the interaction is likely due to divergence in communities from 2008 to 2010, especially when comparing the control and N2 to N1. *Phanerochaete velutina* and *Phanerochaete sanguinea* were observed more often in N1 ($P<0.001$ for both; Table A2.5); and *Pholiota scamba* and *Pezoloma ericae* was observed more often in N2 ($P<0.001$ and $P<0.01$, respectively; Table A2.5). Notably, *Pholiota scamba* was not observed in N1.
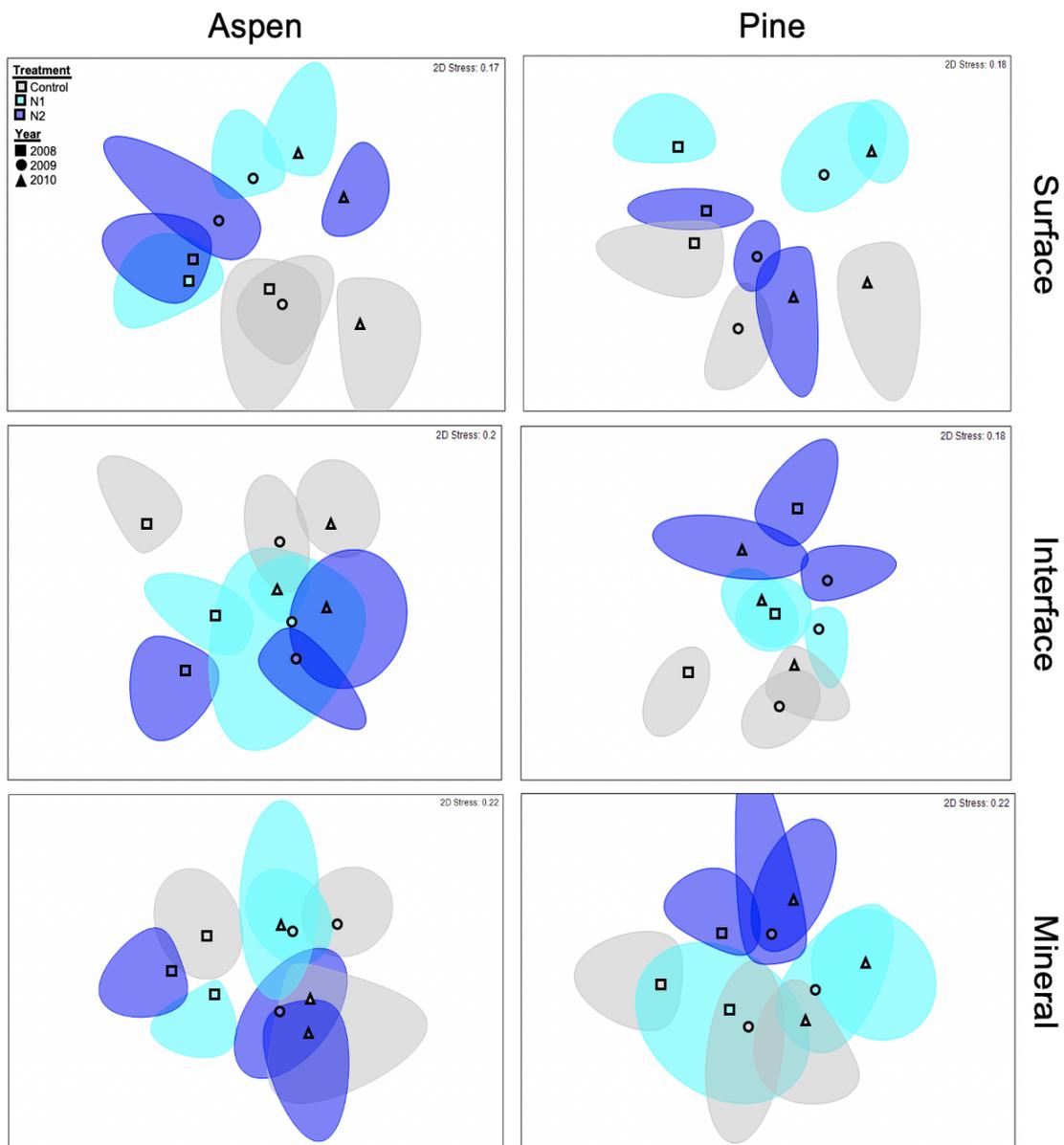
Figure 2.3 Metric multidimensional scaling (MDS) plots using Jaccard distances to assess nitrogen treatment effects for each sampling year associated with aspen and pine stakes placed at three soil depths (i.e., surface, interface, and mineral). We employed 999 bootstrap averages to determine where 95% of the centroid averages lie within multivariate space. The mean centroid is indicated by the specific symbol with 95% confidence ellipses.

For pine stakes placed at the interface, we observed significant main effects of both N treatment (pseudo-$F_{2,189}$=4.18; P<0.01) and sampling year (pseudo-$F_{2,189}$=3.07; P<0.01), and a significant N treatment × sampling year interaction (pseudo-$F_{4,189}$=1.59; P<0.01). The interaction is likely due to differences between all treatments in 2008; similar communities between the control and N1 in 2009 (N2 differed from both); and only N2 and the control differing in 2010. *Phanerochaete velutina* tended to be observed more often in the control and N1 compared to N2 (P<0.001 and P<0.05, respectively; Table A2.5). A *Coniochaeta* sp. and *Phialocephala fortinii* were observed most often in the control compared to either N1 or N2 (P<0.001 for both; Table A2.5). *Acrodontium antarcticum* and a *Sporothrix* sp. tended to be observed more often in N2 and N1 (P<0.001; Table A2.5). Notably *Phanerochaete velutina* and the Coniochaeta sp. were not observed in N2, and the *Sporothrix* sp. was not observed in the control.

For pine stakes placed in mineral soil, we observed significant main effects of both N treatment (pseudo-$F_{2,179}$=2.71; P<0.01) and sampling year (pseudo-$F_{2,179}$=2.58; P<0.01), and a significant N treatment × sampling year interaction (pseudo-$F_{4,179}$=1.50; P<0.01). This interaction is likely due to differences between all treatments in 2008, but in 2009 and 2010 the control and N1 did not differ. *Pholiota mixta* and *Acrodontium antarcticum* tended to be observed more often in N2 compared to the other two treatments (P<0.001 and P<0.01, respectively; Table A2.5). A *Sporothrix* sp. was present most often in N2, intermediate in N1, and not observed in the control (P<0.01; Table A2.5).

Discussion

This was one of few studies to examine CWD decomposition below the soil surface and accounts for several factors that control decomposition (Page-Dumroese et al., 2019; Wang et al., 2019). Specifically, we tracked changes in wood stake mass loss of two wood species, decomposer fungal community composition, fungal richness, and their responses to N treatments and soil placement over three years. We found that stake mass loss was driven to a greater extent by stake placement than by N treatment; and fungal richness was minimally affected by any treatment combination. Fungal community composition tended to respond to the different wood stake species, stake placement, and the N treatments. These

results indicate that N fertilization may have less of an effect on CWD decomposition than the microclimatic conditions associated with soil depth (Bradford et al., 2016).

Decomposition is driven by several biotic and abiotic controls. For instance, and not surprising, aspen exhibited greater decomposition than pine likely due to the higher quality of aspen CWD (i.e., lower lignin content). The role of CWD quality as a determinant of decomposition has been observed across multiple tree species (Ulyshen et al., 2020). Also, not surprising is that N amendments affected CWD decomposition. The effect of N additions on decomposition have been observed for multiple studies (Chen et al., 2016; Freedman et al., 2016; Manning et al., 2008; van der Wal et al., 2007). However, what is surprising is that N additions only impacted the decomposition of aspen stakes on the surface and at the interface. Additionally, the low N treatment resulted in greater CWD decomposition than either the control or high N treatment. This N effect could be due to a sufficient supply of N from the low N treatment resulting in energy allocation towards cellulase production, breaking down the easily accessible cellulose and hemi-cellulose components of wood (Carreiro et al., 2000; van der Wal et al., 2007). The high N treatment did not result in greater mass loss likely because fungi are only capable of using so much N at once (e.g., they reach a threshold) (Zhong et al., 2015).

Here, we found that the position of CWD in the soil profile influences wood decomposition rates. Both aspen and pine stakes placed within the mineral soil exhibited greater mass loss at the end of the three-year study than stakes placed on the forest floor or at the interface between the forest floor and mineral soil. These results are similar to those in other studies where CWD decomposition was greatest in the mineral soil compared to other locations (Page-Dumroese et al., 2019; Wang et al., 2019). This is likely due to more stable soil water and temperature conditions associated with depth of the mineral soil horizon. It also suggests that future research should focus on the dynamics of CWD decomposition in the mineral soil and potentially deeper soil layers. Research should also attempt to quantify the contribution of roots to CWD stocks in soil.

Nitrogen treatments had little effect on stake mass loss, although the low N1 treatment did result in the greatest mass loss, on average, for both aspen and pine stakes at most of the stake placement locations. Since this Norrliden field site had been treated with N

amendments for 18 years, we may be observing an interaction of the direct (immediate) and indirect (legacy) effects of N additions (Keiser et al., 2011; Manning et al., 2008; Strickland, Osburn, et al., 2009). One possible direct effect of N addition is the stimulation of initial decomposition of celluloses due to increased cellulase production by soil microorganisms that are no longer N-limited. A possible indirect effect would be long-term N deposition leading to an increase in plant concentrations of N, resulting in slower decomposition rates during the later stages of decomposition (Berg and Matzner, 1996). The Scots pine and other vegetation in the field site may be experiencing the indirect effects of these long-term N amendments, resulting in litter and debris that is higher in N. This may result in fungi that are conditioned to having higher quality substrate to decompose (Keiser et al., 2011; Strickland, Lauber, et al., 2009; Strickland, Osburn, et al., 2009). Also, with each addition of N, there may be a stimulated decomposition response (direct effect), observed with the CWD stakes. The indirect effects (i.e., decreasing C:N in wood chemistry) potentially mask the direct effects (i.e., relief of N-limitations) of these N amendments. Many of the pairwise N treatment differences were not different in regard to mass loss, though in most instances the trend indicated that N1 tended to result in greater mass on average. This may suggest—along with the fact that after 3 years the greatest mass loss was only ~30%—that a longer term study may detect significant N effects during the latter stages of CWD decomposition. However, after 3 years, N amendments had relatively little effect on mass loss except when the wood stakes were placed on the surface. The N1 treatment resulted in greater mass loss, especially for aspen stakes placed on the surface. Nitrogen was added to the surface, which may have presented those fungal communities with the initial opportunity to immobilize some N before it reached the interface or mineral stakes (Hobbie, 2008). Therefore, the communities associated with the interface and mineral stake placement locations may not have been as exposed to the N treatments and not as affected by the N amendments.

Fungal richness decreased over the course of this study. This may be due to the more easily degraded woody components such as cellulose being broken down, leaving behind lignin which is only broken down by certain groups of fungi (Rajala et al., 2012). We observed an inverse relationship with fungal richness and mass loss where a decrease in fungal richness corresponded with an increase in mass loss (Figure A2.5). In general, there was lower richness within the mineral soil, which is where greatest mass loss was observed.

Reduced fungal richness could be a result of increased dominance of fungal species more associated with the later stages of decomposition (Boddy, 2001). This could also be a reflection of time as a controlling factor on decomposition, where younger CWD material contains more labile C components (e.g., cellulose) and older CWD material contains more chemically complex components (e.g., lignin) left to breakdown. Therefore, only the fungi that can produce the necessary degrading enzymes remain (i.e., wood-degrading fungi that produce ligninolytic enzymes or white rot fungi). That is, and akin to ideas related to narrow versus broad physiological processes (Schimel, 1995), only a few fungal taxa are capable of degrading CWD during the latter stages of decomposition.

Fungal community composition tended to be more responsive to N treatments compared with mass loss and fungal richness. Some fungal species tended to be associated with the same N treatment across soil depth. For example, *Phanerochaete velutina* tended to be present more often across all stake placement locations for both aspen and pine stakes in the N1 treatment plots. *P. velutina* is a cord forming fungi and as such may be able to capitalize on increased soil N associated with the N1 treatment in order to colonize CWD (Boddy, 1993; Fukasawa & Kaga, 2020). The presence of *P. velutina* may partially explain greater decomposition of aspen stakes on the surface and interface for the N1 treatment. Another example is *Acrodontium antarcticum* which was commonly present to a higher degree in the N2 treatment plots. *A. antarcticum* is a microfungi which may be why it was only present in the interface and mineral soil layers (Fukasawa et al., 2009; Vishniac, 1996). It also tended to be present most often in the N2 treatment suggesting that greater access to N is needed for this fungal species to colonize CWD. Together these findings indicate that N shapes the composition of the CWD decomposer community but in many instances those communities are associated with similar amounts of decomposition that are determined by other abiotic factors, namely wood composition and placement within the soil profile. These communities also have the potential to alter their environmental conditions (e.g., moisture) which can affect the rate of decomposition, suggesting that community composition may have a significant role in CWD decomposition (Lindner et al., 2011). However, while species composition may ultimately play a role in determining CWD decomposition the results of our three-year study find that variation in community composition played little role in determining mass loss. This may suggest that CWD fungal communities are responsive to

environmental factors from a composition perspective but are redundant with regards to their function.

Conclusion

Wood stake species, placement, and N fertilization each shaped the decomposition of CWD in this study. However, disentangling the complex relationships among these factors that influence CWD decomposition will require future studies. For example, further research is needed to identify mechanisms to explain the different effects of N fertilization, stake placement, and stake species on CWD decomposition rates. In addition, future work should attempt to confirm and explain our observation that N additions only affect CWD decomposition on the surface of the forest floor. Importantly, because we found that stake placement was the most important factor controlling CWD decomposition, there should be increased investigation of sub-surface CWD decomposition with continuous measurements of microclimate conditions (i.e., soil temperature and moisture for each stake placement location). Finally, effects of nitrogen fertilization on decomposition of wood across a broad range of lignin:N would further our understanding of how exogenous N influences CWD decomposition and potentially unveil the optimum amount of N for wood decomposition. This study emphasized the importance of incorporating multiple factors in a field-experiment to fully understand the decomposition process. It also highlights that although factors interact to affect a response, some factor may be the main driver of that response. Here we found that stake placement and stake species were the main drivers of CWD mass loss and N treatment was the main driver of fungal community composition.

Chapter 3: Leaf Litter Decomposition Microcosm Study

The effect of various microbial communities on leaf litter decomposition and volatile organic compound production

Introduction

Forest floor leaf litter decomposition is a fundamental process in terrestrial ecosystems. Leaf litter decomposition has been widely studied because of its importance in carbon and nutrient cycling (Gougoulias et al., 2014). Many of these studies have primarily focused on the mass-loss of litter and release of soluble compounds (Du et al., 2020; Hart et al., 1992; Remsburg & Turner, 2006; Strickland, Lauber, et al., 2009; Strickland, Osburn, et al., 2009). However, this focus overlooks another potentially important product of leaf litter decomposition, volatile organic compounds (VOCs).

Forest floor litter accumulates when foliage falls from trees, shrubs, and other plants. This occurs seasonally for some plants (deciduous), but can also be a result of old age, defoliating pests, and strong winds. Once on the ground, this litter is decomposed by a suite of organisms. Leaf litter decomposition adds nutrients into the soil and can result in soil organic matter accumulation (Cotrufo et al., 2013; Hobbie, 2015; Sayer & Tanner, 2010; Vesterdal et al., 2008). The rate of leaf litter decomposition is determined by several factors including: leaf litter chemistry, climate (both on micro and global spatial scales) (Bradford et al., 2016; Strickland et al., 2015), and microbial community composition (Keiser et al., 2014; Strickland, Lauber, et al., 2009; Strickland, Osburn, et al., 2009). The function of the microbial community is shaped by the environment and resource availability, which is a function of leaf litter chemistry. Leaf litter chemistry varies across plant species, eliciting different rates of decomposition (Rosenfield et al., 2020).

Microbial communities influence decomposition by driving differences in respiration and mass loss. Microbial community composition is important in determining litter decomposition rate and how carbon compounds are degraded (Glassman et al., 2018). Under the same environmental conditions, different microbial communities have been shown to be functionally distinct (Cleveland et al., 2014; Strickland, Lauber, et al., 2009; Strickland, Osburn, et al., 2009), demonstrating the importance of community composition as a control on litter decomposition. Specifically, microbial communities sourced from various locations

elicited different rates of carbon mineralization for varied litter sources (Strickland, Lauber, et al., 2009; Strickland, Osburn, et al., 2009). As part of their metabolic processes, microbial communities also have the potential to produce different types and quantitates of VOCs as a product of leaf litter decomposition. Volatile organic compounds are carbon containing compounds, usually associated with an odor, and can be measured as they are produced from organisms such as microbes.

More widely studied however, are the natural VOC profiles of different plant and litter species (Gray et al., 2010; Greenberg et al., 2012; Hakala et al., 2002). It has been acknowledged that there are distinct VOC profiles among a variety of plants. Could microbial communities also produce distinct VOC profiles or amounts of VOCs? Volatiles of single fungal and bacterial strains have been identified (Choudoir et al., 2019; Lemfack et al., 2018; Tyc et al., 2017), however microbial community composition and function have not been widely correlated to a VOC profile or total VOCs produced (McNeal & Herbert, 2009; Stahl & Parkin, 1996). It is also understood that VOCs impact microbial community composition, as VOCs are used as a source of C (McBride et al., 2020) and are also used as infochemical molecules to communicate with other microorganisms (Schmidt et al., 2016). Volatile organic compounds have the potential to unveil new information about microbial community structure and function.

Studying the VOC profile of various microbial communities can potentially enhance our understanding of community function along with some insight about community composition (McNeal & Herbert, 2009). Currently, microbial communities are identified via metabolites, DNA fingerprinting, sequencing, and other laboratory methods (Fierer et al., 2009; Fierer & Jackson, 2006; Graham et al., 2016). These processes require extensive and expensive lab procedures along with destructive harvesting of samples. The potential to identify microbial communities via VOC profile enables measurements to be taken in the field without destructive sampling and would be much more cost-effective than current methods. Genomics methods also only show one snapshot of the microbial community at a specific time point under the current conditions. VOC profile analysis would allow multiple measurements to be taken over time, showing changes in community composition or function on a narrower timescale. The level at which organisms could be identified with high

confidence is still to be determined. However, community diversity metrics have already demonstrated to be effective in predicting C and N cycling rates and other ecosystem processes (Graham et al., 2016). Volatile organic compounds can further our understanding of microbial processes and enhance our current methods for studying microbial community composition.

To understand how differences in microbial community composition drives changes in leaf litter decomposition and VOC production, we conducted a 12-week microcosm experiment. We tested seven different soil microbial communities sourced from different soils with different plant cover and their effect on ponderosa pine (*Pinus ponderosa*) litter decomposition. Mass loss, respiration, and total VOC measurements were recorded weekly. Microbial community composition was analyzed for weeks 0, 4, 8, and 12 via a separate set of destructively sampled microcosms. We hypothesized that mass loss and VOC production would vary among soil microbial communities sourced from different ecosystems.

Materials and Methods

Sampling

I collected *Pinus ponderosa* (Ponderosa pine) litter samples from the University of Idaho Experimental Forest in the spring of 2019. Litter was ground using a Waring blender, air dried, and sterilized via autoclaving in two sessions 24 hours apart (121°C, 30 min). Soil samples were taken at five individual plots in each of seven locations: the University of Idaho Experimental Forest Ponderosa pine stand where litter was collected (control, PP), a remnant Palouse Prairie (RPP) in Moscow, Idaho, a hardwood stand in a University of Massachusetts forest (MAH), a conifer stand near Waltham, Massachusetts (MAC), a mesic hardwood hammock in Florida (HHF), a garden soil in Florida (HHG), and a conifer stand in Salem, Virginia (SVA) following soil sampling protocol outlined in Figure B3.1. Soil properties and locations are listed in Table 3.1. Soil samples from each location were thoroughly homogenized, sieved to 4.75mm and kept at 4°C until use. A subsample was reserved at -80°C for initial microbial community analysis (week 0). Water holding capacity (WHC) and gravimetric moisture content (GVM) were determined for soil and litter samples. Additionally, the pH of the soil samples was determined.

Microcosm Preparation

Soil from each location was incubated with the Ponderosa pine litter. The 7 different soils were crossed with the 1 litter type, replicated 4 times each to yield 28 experimental units. Microcosms were established in pint glass jars and included 10.0 grams of dry-weight soil and 25.0 grams of dry-weight litter with water added to reach 50% WHC. Microcosms were incubated between 22 and 24ºC at 100% humidity to reduce moisture loss. A separate set of microcosms in 50ml centrifuge tubes was also established for microbial community analysis. At 3 times points over the course of the experiment (weeks 4, 8, and 12), three microcosms representing each of the 7 treatments were harvested (21 microcosms per time point). These soils were stored at -80ºC until analysis. The experiment was conducted over a 12-week period with mass loss, total VOC concentrations, and CO_2 flux taken once a week.

Soil Properties: Table 3.1

Water holding capacity (WHC) was determined by adding 1-5g fresh soil to a funnel lined with Whatman #1 filter paper and saturating the soil with deionized water. The soil was allowed to drain for 2 hours and was then transferred to a pre-weighed tin tray where the mass of the tin and soil was taken. The soil samples were placed in a 105°C oven for 24 hours to dry completely then placed in a desiccator to cool and be reweighed. This was done for all soil samples in replicates of 3 per soil sample. Gravimetric moisture content was determined similarly to WHC. The only difference was that the initial mass measurement of the soil was its field weight, not its saturated weight. Soils were dried in an oven at 105°C for 24 hours, cooled and reweighed as above. Soil pH (1:1, soil:H2O by volume) was determined using airdried soil samples and a benchtop pH meter.

Table 3.1 Soil site information.

Soil Sample	Average GVM (%)	Average WHC (%)	pH	Coordinate Location (Approx.)	Soil Type
PP	28.18	45.01	5.79	46.842018, -116.828992	Carlinton-Carrico-Kruse complex
RPP	14.96	28.74	6.19	46.674587, -116.980387	Schumacher-Libertybutte complex
MAC	23.63	45.98	3.93	42.380151, -72.439859	Gloucester
MAH	48.64	66.17	3.95	42.370929, -72.429967	Gloucester
SVA	27.63	40.83	6.37	37.293533, -80.054681	Sequoia
HHF	4.71	31.16	4.28	29.655103, -82.291931	Millhopper Sand
HHG	18.02	29.96	7.63	29.656917, -82.289292	Millhopper Sand

Microbial Community Analysis

Microbial community analysis was performed with an initial soil sample from each location (week 0), and for weeks 4, 8, and 12 using the separate set of microcosm tubes prepared. Microbial community composition was assessed using 16S/ITS metabarcoding protocol, described by Lucas et al. (2020). Briefly, DNA from each sample was extracted using the MoBio© PowerSoil kit (MoBio Laboratories, Inc., Carlsbad, CA, USA), according to the manufacturer's protocols. A 2-step PCR was used to amplify the ribosomal marker genes. We used the ITS1/ITS2 and 515F/806R primer pairs for fungi and bacteria, respectively, according to the Earth Microbiome Project protocol (www.earthmicrobiome.org). Sequences were cleaned using ExoSAP-IT™ PCR clean-up reagent (Affymetrix Inc., Santa Clara, CA, USA) after the first round of PCR and unique barcoded primers were attached to each sample during the second round of PCR. Samples were then cleaned and normalized using SequelPrep™ 96-well plates (Invitrogen, Carlsbad, CA, USA). We pooled equimolar amounts of DNA and sequenced our amplicon pools on an Illumina MiSeq instrument using 2 x 300 bp sequencing kits at the Genomics Resource Core,

IBEST sequencing facility at the University of Idaho. Controls were used throughout these procedures. Raw sequences were demultiplexed by the University of Idaho's Genomics Resource Core using the dbcAmplicons program (Uribe-Convers et al., 2016) which also removed barcodes and primers. Paired sequences were processed using the DADA2 pipeline (Callahan et al., 2016) and then trimmed to uniform lengths, dereplicated, and denoised using the 'dada' function. Errors were accounted for throughout the model generated with the 'learnErrors' command. We then merged paired-end sequences, removed chimeras, and determined taxonomy assignments based on the Silva (ver. 138, Quast et al., 2013) and UNITE dynamic release (ver. 10.10.2017, Abarenkov et al., 2010) databases for bacteria and fungi, respectively. To account for differences in sequencing depths, we rarefied samples to 2226 and 1765 sequences per sample for bacteria and fungi, respectively.

Microcosm Respiration

A LI-8100A infrared gas analyzer (IRGA) CO_2/H_2O gas-multiplexer system (LI-COR Biosciences, Lincoln, Nebraska, USA) was used to sample headspace gas from each microcosm. Air was pulled from each microcosm through a 0.2 m tube, where a gas multiplexer and gas-analyzer monitored CO_2 production rates. Each microcosm was sampled for a total time of 2 minutes 15 seconds with a 45 second initial dead-band to allow balance from one sample to the next. CO_2 flux measurements from each microcosm were taken once a week for 12 weeks to show a trend in respiration over time (Table A3.1).

Microcosm VOC Production

A MSEM 160 sensor (Sensigent, Baldwin Park, California, USA) was used to record the total VOCs of each microcosm weekly for 12 weeks (Table B3.1). VOC measurements were taken using the PID sample configuration in Triggered Mode. Triggered Mode includes three cycles: pre-sample purge, sample, and post-sample purge. Each cycle ran for 60 seconds with the microcosm attached for the initial purge and sample cycles, then removed after 120 seconds to allow completion of the post-sample purge. The purge cycles avoid cross-contamination between samples.

Statistical Analyses

Statistical analyses were performed in R (R Core Team, 2019) using the 'lme4' and 'emmeans' packages (Bates et al., 2015; Lenth, 2020). To assess the effect of the different soil treatments and time (i.e., treatment and week) on the response variables (i.e., respiration, total VOCs, and mass loss), we used generalized linear model mixed effects models ('glmer' function, gamma distribution and log-link function, lme4 package). The mixed effects models included 'jar number' as a categorical random effect, which allowed us to account for repeated measurements of response variables from the same jars over time. We included 'week' as a fixed effect in these models, which allowed us to conduct pairwise comparisons (Tukey's HSD) between all treatments within each week using the 'emmeans' package ('emmeans' and 'contrast' functions).

Shannon diversity ('diversity' function) was calculated for both fungi and bacteria after sequences were rarefied ('rrarefy' function) using the 'vegan' package (Oksanen et al., 2020). We used a generalized linear model mixed effects model again to test the Shannon diversity response to the different soil treatments with 'week' included as a fixed effect in order to conduct pairwise comparisons between all treatments within each week.

We assessed the bacterial and fungal community composition using the 'phyloseq' package (McMurdie and Holmes, 2013). Bray-Curtis dissimilarity was determined ('vegdist' function) and used to perform a permutational MANOVA (perMANOVA 'adonis' function). To visualize results, PCoA was used and plotted with means and standard error.

We used linear models ('lm' function) to perform regression analyses to assess the effect of the present bacterial and fungal classes on the response variables. To assess the relationship of bacterial and fungal community composition on the response variables, we regressed PCoA axis values against the response variables using linear models.

Results

Microbial community composition of the soil treatments over time

Bacterial communities within each treatment remained compositionally distinct (pseudo-*P*=0.001; Figure 3.1) throughout the experiment (pseudo-*P*=0.001; Figure 3.1). Treatment and week also interacted to affect bacterial community composition (pseudo-*P*=0.001; Figure 3.1). This interaction is likely due to bacterial communities within each treatment becoming more similar with each subsequential week as represented by clustering in the PCoA (Figure 3.1). Shannon diversity of bacterial communities tended to decrease with week (Figure 3.2A) and was affected by the soil treatments (*P*<0.001; Figure B3.2A) and week (*P*<0.001; Figure B3.2A). The class α-proteobacteria tended to increase in relative abundance for all soil treatments, decreasing evenness at the class level (Figure 3.2B).

Fungal communities within each treatment also remained compositionally distinct (pseudo-*P*=0.001; Figure 3.3) throughout the experiment (pseudo-*P*=0.001; Figure 3.3). Treatment and week also interacted to affect fungal community composition (pseudo-*P*=0.001; Figure 3.3). This interaction is likely due to fungal communities within each treatment becoming more similar with each subsequential week as represented by clustering in the PCoA (Figure 3.3). Shannon diversity of fungal communities appeared to decrease by week 4, increase by week 8, and decrease again by week 12 (Figure 3.4A). Diversity was affected by the soil treatments (*P*<0.001; Figure B3.2B) and week (*P*<0.001; Figure B3.2B). Soil treatment and week interacted to affect Shannon diversity (*P*<0.01; Figure B3.2B).
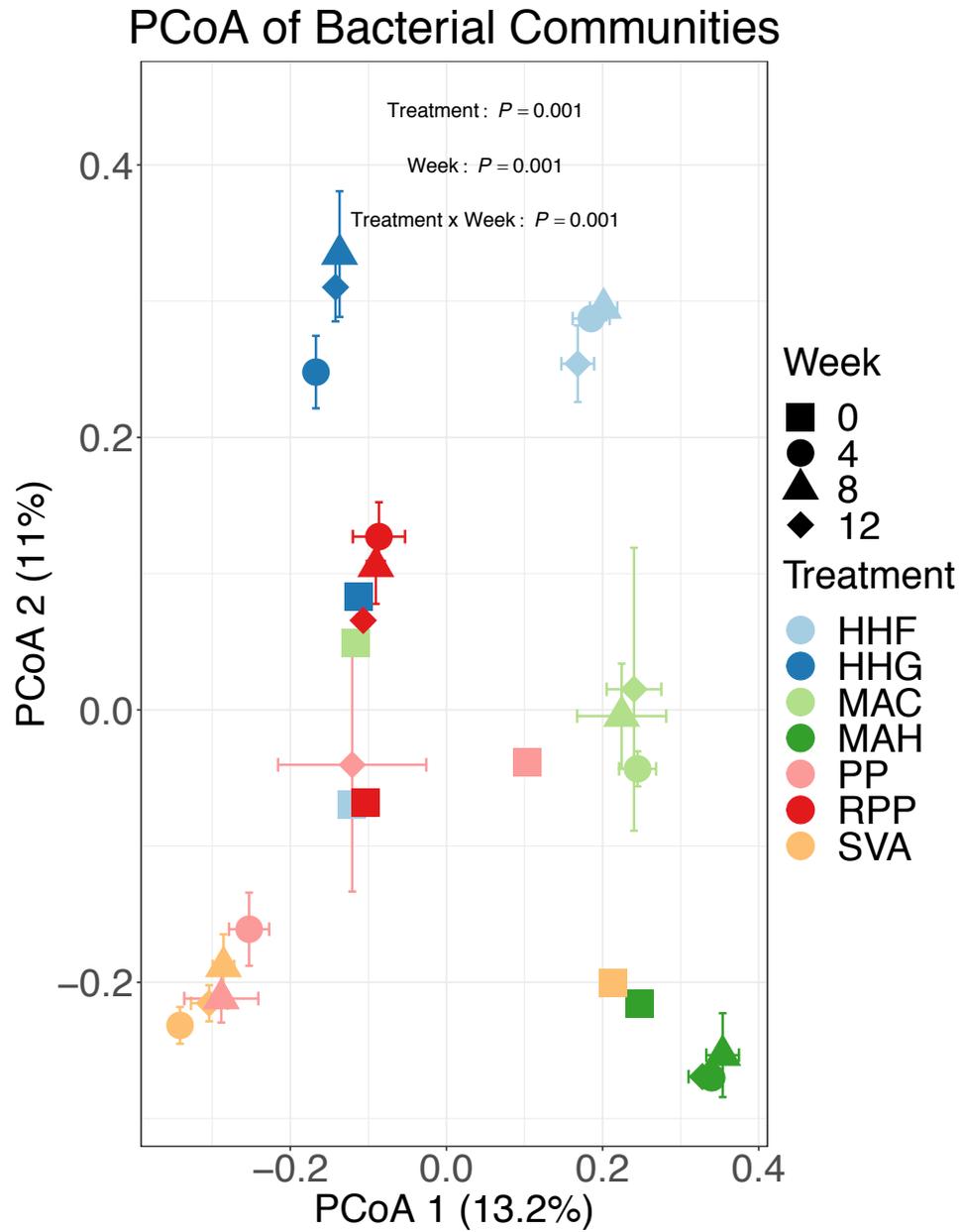
Figure 3.1 PCoA of bacterial communities plotted with means and standard error by treatment and week. PerMANOVA p-values are reported in the figure.
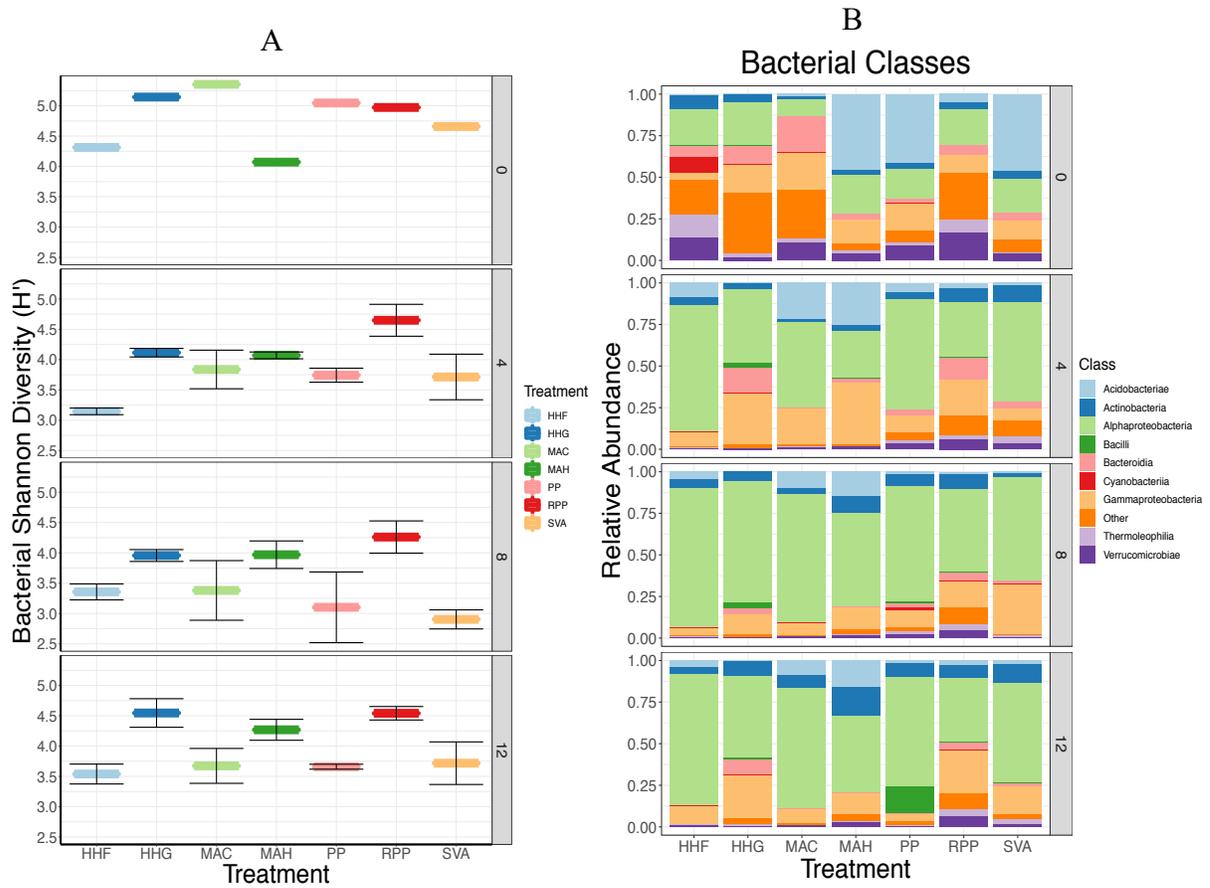
Figure 3.2 Shannon diversity of bacteria within each treatment subset for weeks 0, 4, 8, and 12 (A) and relative abundance of bacterial classes subset for weeks 0, 4, 8, and 12 (B).
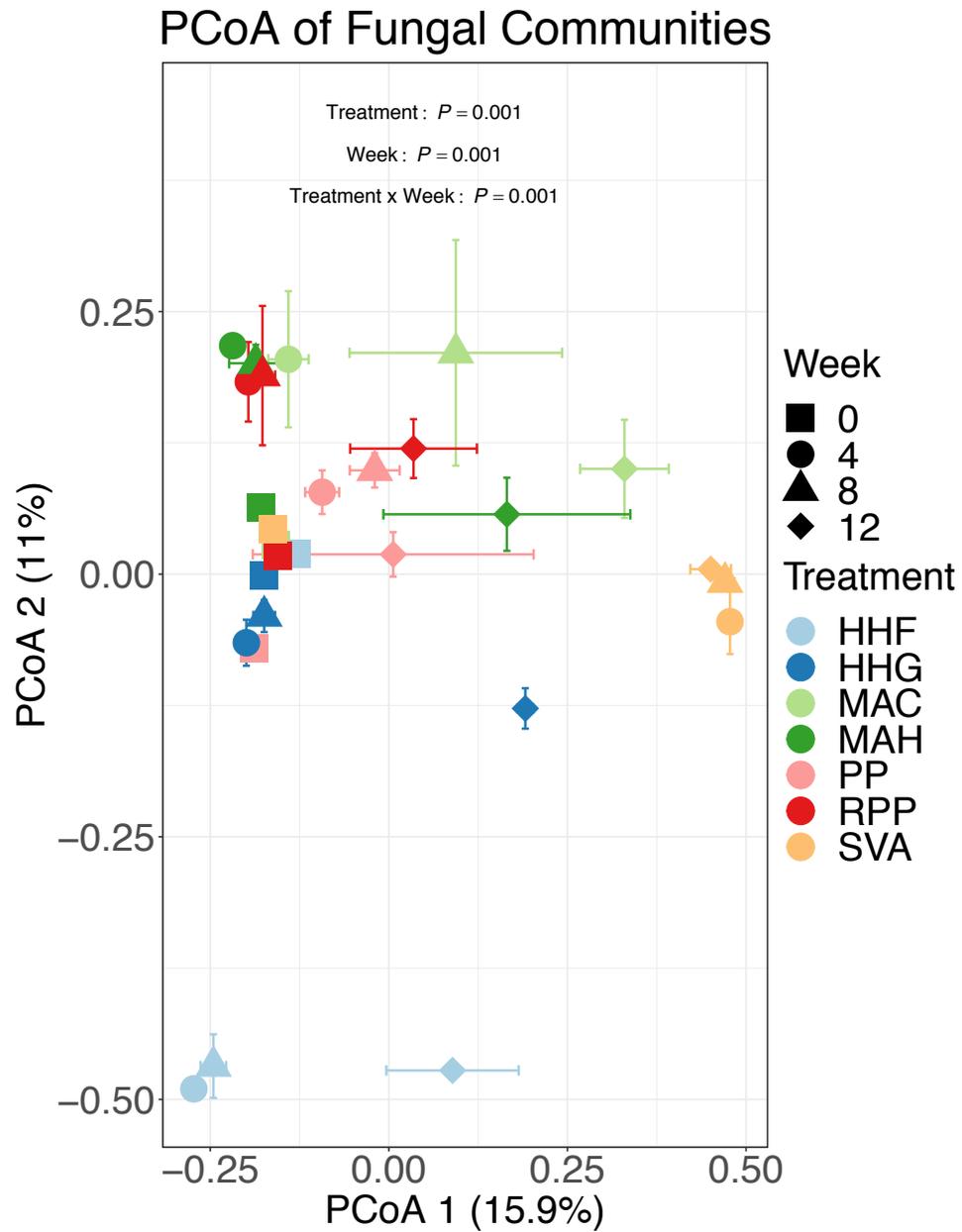
Figure 3.3 PCoA of fungal communities plotted with means and standard error
by treatment and week. PerMANOVA p-values are reported in the figure.

A

B
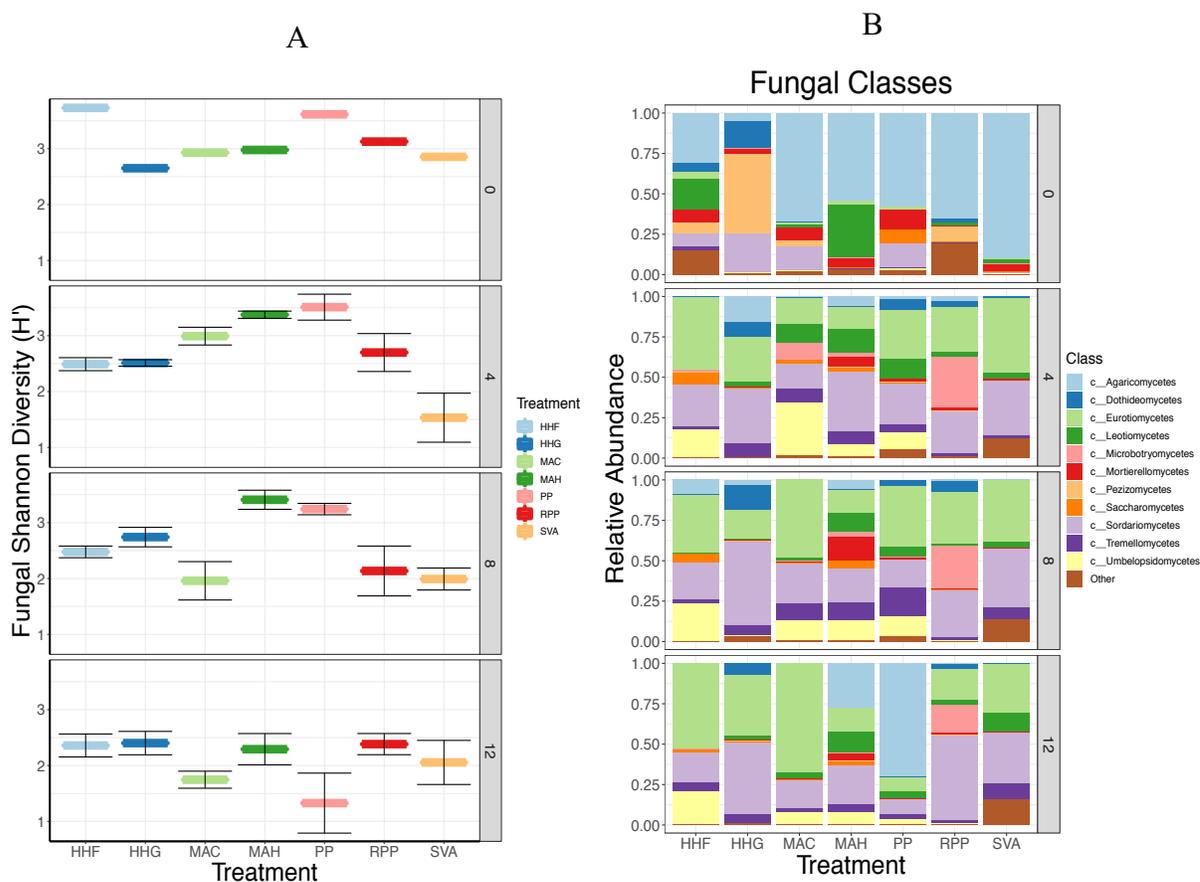
Figure 3.4 Shannon diversity of fungi within each treatment subset for weeks 0, 4, 8, and 12 (A) and relative abundance of fungal classes subset for weeks 0, 4, 8, and 12 (B).

Mass loss dynamics after 12 weeks

Not surprisingly, mass loss increased with week ($P<0.0001$) and mass loss was affected by the different soil treatments ($P<0.05$). The different soil treatments and week accounted for 96% of the variation in mass loss based on trigamma R-squared estimates. Week and soil treatment also interacted to affect mass loss ($P<0.0001$). This interaction is likely driven by the divergence in percent mass loss towards the final weeks of the experiment (Figure 3.5A). The treatments began with relatively similar mass loss values during initial weeks and became increasingly dissimilar as time went on. The control (PP) treatment tended to have intermediate mass loss throughout the experiment compared to the

other treatments. Treatment HHG tended to have the greatest mass loss, especially toward the end of the experiment compared to all other treatments (Table B3.3, Figure 3.5A).

Respiration dynamics after 12 weeks

Respiration tended to decrease with week ($P<0.0001$) and was affected by the different soil treatments ($P<0.0001$). The different soil treatments and week accounted for 77% of the variation in respiration based on trigamma R-squared estimates. Week and soil treatment also interacted to affect respiration ($P<0.0001$). This interaction is likely due to the decrease in respiration for all treatments after week 1 but great spike in respiration during week 6 for treatment HHG. This spike in respiration is in line with its increase in mass loss (Figure 3.5B). Treatment MAH tended to have greater respiration toward the end of the experiment compared to the other treatments and also had greater mass loss during that time (Figure 3.5B). Cumulative respiration was affected by treatment ($P<0.0001$) and week ($P<0.0001$). Week and soil treatment also interacted to affect cumulative respiration ($P<0.01$). Average cumulative respiration values for each treatment are displayed in Figure B3.3. Treatment HHG had much greater cumulative respiration compared to all other treatments.

VOC dynamics after 12 weeks

Total VOCs produced tended to fluctuate each week ($P<0.0001$) and were affected by the different soil treatments ($P<0.0001$; Figure 3.5C). The different soil treatments and week accounted for 86% of the variation in total VOC production based on trigamma R-squared estimates. Week and soil treatment also interacted to affect total VOC production ($P<0.0001$). This interaction is likely driven by soil treatments having greater or lesser total VOCs each week. For example, during week 2, treatment RPP appeared to produce the most VOCs compared to all other treatments, but during weeks 5, 6, 8, 9, and 10, treatment PP produced the most VOCs.
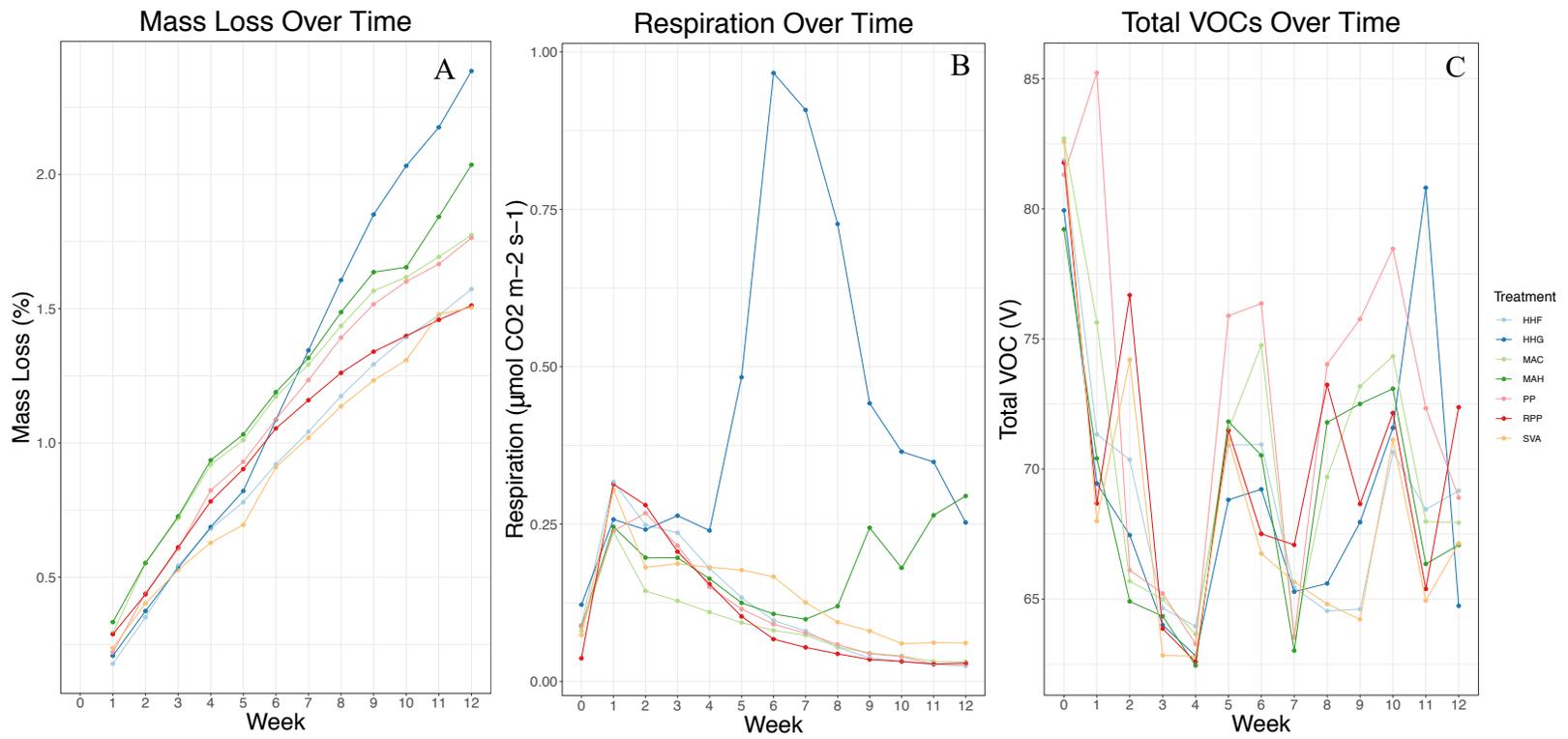
Figure 3.5 Mass loss (A), respiration (B), and total VOCs produced (C) for each treatment per week.

Mass loss associated with soil treatment and bacterial and fungal communities

Mass loss appeared to be driven to a greater extent by fungal communities than bacterial communities as mass loss was only related to PCoA axis 2 of fungal communities ($P<0.05$) when PCoA axis values were regressed against mass loss values (Figure 3.6A). Actinobacteria was the only bacterial class to show a positive relationship to mass loss ($P<0.01$; Figure 3.6B). For fungi, the class Umbelopsidomycetes showed a negative relationship to mass loss ($P<0.05$; Figure 3.6C).
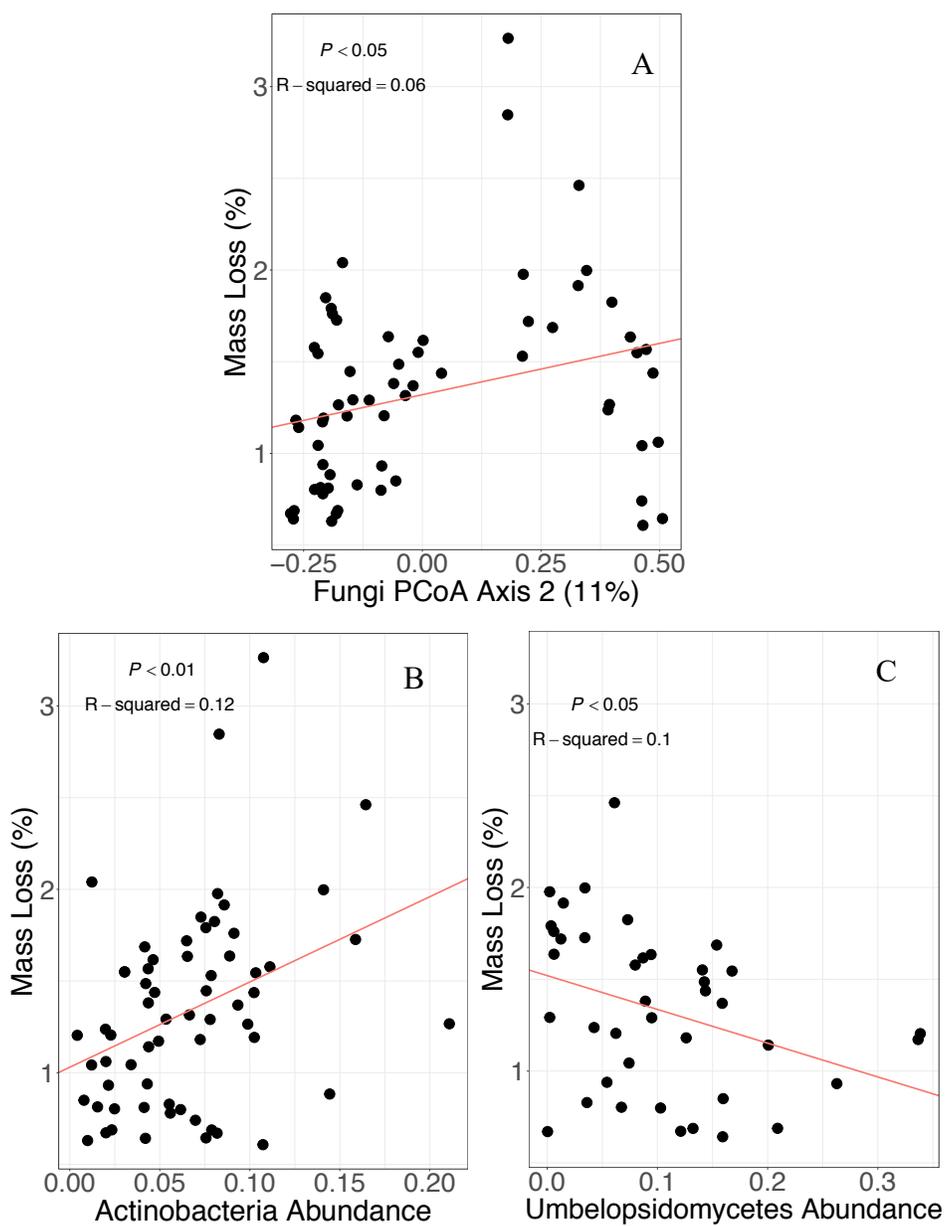
Figure 3.6 Linear regression of fungal PCoA axis 2 values (A), Actiniobacteria abundance (B), Umbelopsidomycetes abundance (C), and mass loss.

Respiration associated with soil treatment and bacterial and fungal communities

Respiration was neither related to the fungal nor the bacterial communities as a whole or to any specific bacterial class as determined by PCoA axis regressions. Respiration was however positively related to the fungal classes Sordariomycetes (*P*<0.01) and Dothideomycetes (*P*<0.001; Figure 3.7A and B).
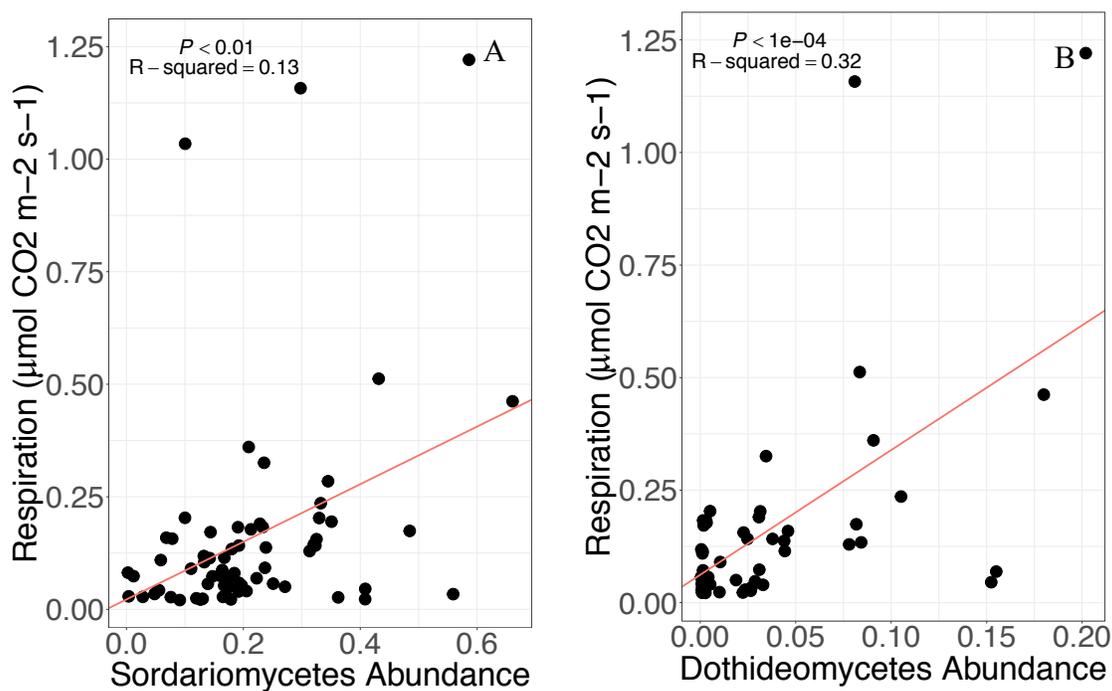
Figure 3.7 Linear regression of Sordariomycetes abundance (A), Dothideomycetes abundance (B), and respiration.

Total VOCs associated with soil treatment and bacterial and fungal communities

Total VOCs were not driven by bacterial or fungal communities as determined by PCoA axis regressions. However, total VOCs were positively related to the class Agaricomycetes (*P* < 0.01 Figure 3.8A) and negatively related to the class Sordariomycetes (*P* < 0.05; Figure 3.8B).
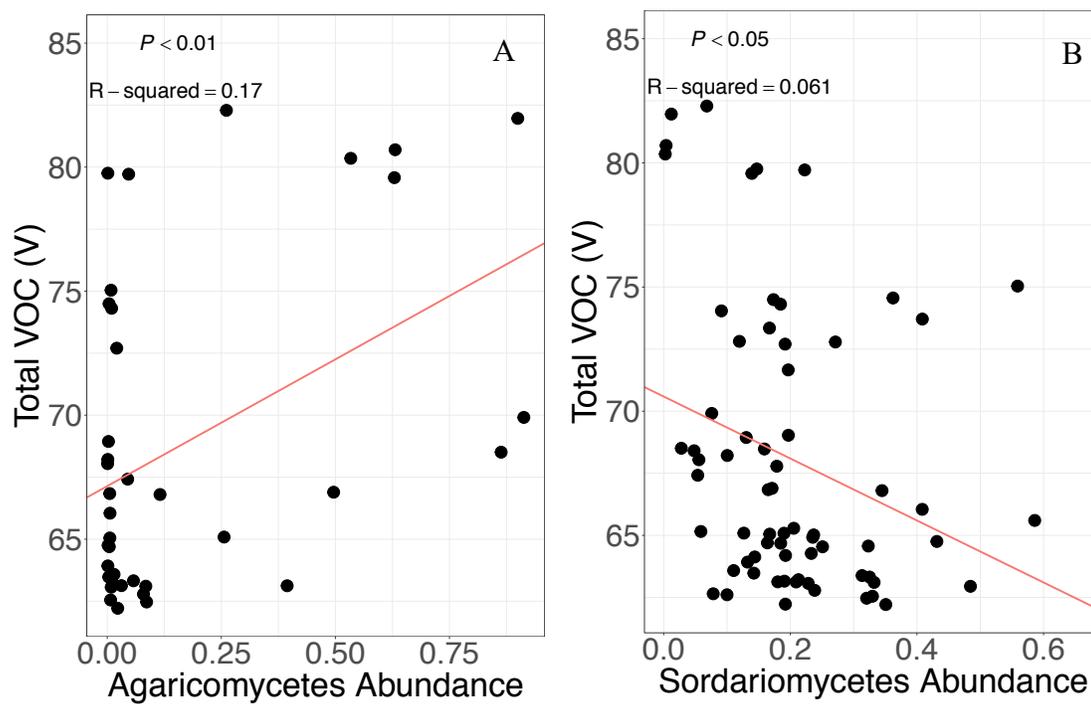
Figure 3.8 Linear regression of Agaricomycetes abundance (A) and Sordariomycetes abundance (B) and total VOCs.

Discussion

Understanding the dynamics of microbial communities during decomposition is important in expanding our knowledge of C and nutrient cycling and soil C storage. The methods by which microbial communities are studied however, are often time consuming and expensive. Here, we explored a new method for studying microbial community composition and function. Microbes produce VOCs as metabolites during decomposition and we aimed to link these VOCs to microbial community composition using a microcosm experiment.

Microbial community composition shifted overtime and Shannon diversity decreased for both bacteria and fungi. The treatment communities remained distinct throughout the experiment even though the same litter was used as a substrate. This demonstrates that microbial communities are both compositionally and functionally dissimilar (Keiser et al., 2014; Strickland, Lauber, et al., 2009; Strickland, Osburn, et al., 2009). This also highlights the potential to use VOCs and respiration measurements as indicators of microbial community composition and function, as those measurements were dependent on the microbial community throughout the experiment. Volatile organic compound profiles have been studied for certain bacterial and fungal strains (Choudoir et al., 2019; Tyc et al., 2017); however more work is needed to build a repository of microbial VOC profiles to better study microbial community composition and function.

We found that mass loss increased over time and that soil treatment (i.e., different microbial communities) resulted in different amounts of mass loss even though the same type of litter was used as the decomposition substrate. Mass loss was positively related to the class Actinobacteria. Actinobacteria have been identified in other studies to be important in decomposition as actinobacteria tend to encode the most genes for carbohydrate-active enzymes (Bao et al., 2021; Wang et al., 2016). Respiration and cumulative respiration generally decreased over time for all treatments, however we found respiration varied depending on the treatment. These results are similar to other studies where cumulative respiration was different for various soil inocula and litter combinations (Keiser et al., 2011; Strickland, Lauber, et al., 2009; Strickland, Osburn, et al., 2009). Respiration tends to decrease over time as labile compounds are used during the beginning of decomposition,

leaving more recalcitrant compounds for the later stages of decomposition (Berg, 2014). Respiration appeared to be driven more greatly by fungal communities than bacterial communities. This is in line with the metabolic differences of bacteria and fungi. Fungi tend to be less metabolically diverse than bacteria (Glassman et al., 2018), leading to a greater amount of carbon mineralized to CO_2 rather than other compounds. Whereas bacteria may have the metabolic ability to produce a larger variety of VOCs than fungi (Stahl & Parkin, 1996). Sordariomycetes are one of the largest classes of Ascomycota (Zhang et al., 2006) which is likely the reason we see a relationship with respiration and total VOC production.

Evidence linking VOC profiles to microbial communities is sparse (McNeal & Herbert, 2009; Stahl & Parkin, 1996). Volatile organic compound production is usually studied as emissions from soil and litter samples (Asensio et al., 2012; Greenberg et al., 2012; Insam & Seewald, 2010; Leff et al., 2008; Rossabi et al., 2018). Here, we examined total VOCs produced from various soil microbial communities as they decomposed the same litter source. Previous studies have shown that bacteria produced more VOCs than fungi and VOCs can serve as an indicator of community composition (McNeal & Herbert, 2009; Stahl & Parkin, 1996). Due to laboratory constraints, we only studied the total amount of VOCs produced from each community during decomposition. A better link between microbial community composition and VOC production may be made with identification of individual VOCs. However, we were able to demonstrate differences between bacterial and fungal VOC production even with total VOC measurements.

Conclusion

Volatile organic compounds produced by microbial communities are a promising approach to studying microbial community composition and function. Total VOCs produced by different soil microbial communities broadly correlated to community composition; however, future work should examine the entire VOC profile to create better links to community composition. After analysis of VOC profiles has been determined sufficient in studying microbial community composition, development of a method to study VOC profiles in the field will be next. This will remove the need to destructively harvest samples for genomics methods, reducing labor and costs. As we continue to study soil microbes, we will

also expand our knowledge on the roles they play in C and nutrient cycling and can potentially link specific VOCs to specific microbial processes.

Chapter 4: Conclusion

Conclusion

Both studies, presented in this thesis, dove into the complex interacting factors that control decomposition. As decomposition is dependent on several biotic and abiotic factors, the process is variable across ecosystems, but also at local scales. Understanding the dynamics of decomposition is critical to understanding soil C and nutrient dynamics. This also has the potential to influence land management decisions as soil C storage is becoming increasingly important due to climate change. Yet, while decomposition has been widely studied, there is still much that we do not understand. Here we focused on forest and prairie ecosystems with the aim of understanding the factors that control coarse woody debris decomposition and how variation in microbial communities drive leaf litter decomposition and its products.

Our course wood debris decomposition study was based in Norrliden, Sweden. The study site had previously been fertilized with ammonium nitrate for 18 consecutive years. This study was designed to investigate the potential long-term effects of N fertilizer applications. We found that mass loss of wood stakes was most influenced by stake placement and wood species, with aspen and pine stakes placed in the mineral soil having the greatest mass loss. We also found that the N treatments applied did not significantly affect wood stake decomposition or fungal richness except for stakes placed on the surface of the forest floor. This suggests that microclimate may be a main driving factor of CWD decomposition as climate conditions are likely more favorable with depth along a soil profile. Nitrogen treatments did affect fungal community composition, but this variation in community composition had little effect on CWD decomposition. However, longer-term studies may better describe the effects that change in community composition may have on CWD decomposition.

Our leaf litter decomposition study encapsulated how varying soil microbial communities sourced from areas with different vegetation cover decompose the same litter source. We also aimed to show the potential of using VOCs to analyze microbial community composition and function. We found that after 12 weeks of being kept in the same environment, these microbial communities remained compositionally distinct and were

related to differences in decomposition, respiration, and total VOC production. This refutes the long-time hypothesis that microbial communities are functionally redundant and demonstrates the importance of maintaining microbial diversity and understanding microbial functions. If climate or management change impact soil microbial communities, this may have consequences for decomposition, which ultimately affects soil organic matter formation and soil C storage. The diversity in microbial function observed also demonstrates the great potential to use microbially produced VOCs to study microbial community structure and function since different microbial communities produced different amounts of VOCs throughout this study.

Although both studies focused on organic material decomposition, there are a few significant differences between the two studies to highlight. The CWD decomposition study focused on how site characteristics influence CWD decomposition and did not focus on the initial microbial decomposer community. The fungal community composition and richness were analyzed as a response to the stake species, stake placement, and N fertilizer treatments during decomposition. The leaf litter decomposition study focused on how different microbial communities influence litter decomposition while keeping site characteristics the same. Decomposition (i.e., mass loss, respiration, and VOCs) was analyzed as a response to the different soil microbial communities. Both studies found important interacting effects of the various treatments employed and expanded our knowledge on CWD and leaf litter decomposition. Of note, however, is the observation that microbial community composition affected leaf litter decomposition but not CWD decomposition. Barring the fact that a longer-term study may elucidate community effects on CWD decomposition, this result may indicate that community composition plays a less important role in the decomposition of more recalcitrant organic matter.

Future research should continue exploring the dynamics and drivers of organic material decomposition and the microbial communities at play. Anthropogenic influences such as N fertilizer application, soil disturbance that induces mixing of organic materials into soil, and seedling plantations that may change leaf litter inputs should be studied. Studies focused on these concepts will corroborate our findings here and further our understanding of the importance of decomposition on C and nutrient cycling. Longer term CWD

decomposition studies are needed to understand various treatment effects as CWD takes longer to colonize and decompose. To continue the method of using VOCs to study microbial community composition and function, studies exploring more detailed VOC profiles are needed. Gas chromatography-mass spectroscopy is one method we think will be most useful in studying VOC profiles of microbial communities.

This thesis contributes to our overall understanding of the decomposition process and CWD and leaf litter specifically. We studied how microbial communities interact with various biotic and abiotic factors to influence the decomposition process. Microbes are diverse and their role in decomposition and SOM formation are important. Therefore, it is important we continue to study biotic and abiotic factors that affect their composition and function. We paved the way to another method for studying microbial communities via VOC profiles. More work is needed to fully develop this method; however, we demonstrated the potential for this alternative method to work and be more efficient than current methods. We also evaluated how long-term anthropogenic N fertilization affects CWD decomposition and fungal communities. This is important as N fertilization is common practice and its input could alter the decomposition process. Overall, we highlight key differences between the CWD and leaf litter decomposition processes and will be able to use these findings in future decomposition research as we continue to develop methods to study microbial communities in the field.

Appendix A: Chapter 2 Supplemental Material

Mass Loss of Aspen Stakes
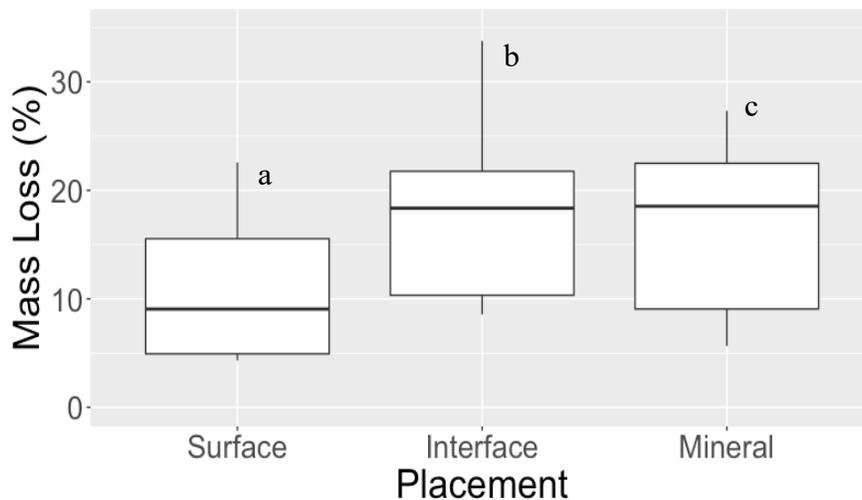
Figure A2.1 Mean and standard errors of mass loss of aspen stakes at the different placement locations averaged across all sample years and N treatments.

Mass Loss of Pine Stakes
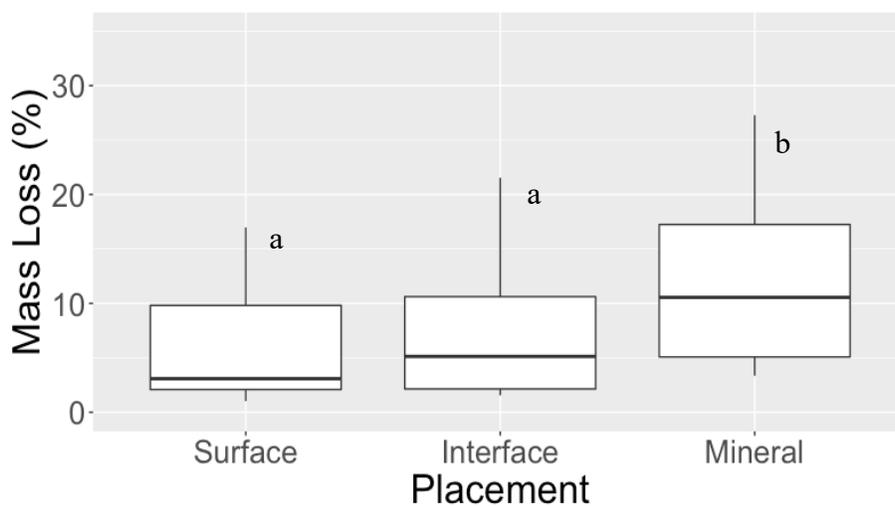
Figure A2.2 Mean and standard errors of mass loss of pine stakes at the different placement locations averaged across all sample years and N treatments.

Fungal Richness of Aspen Stakes
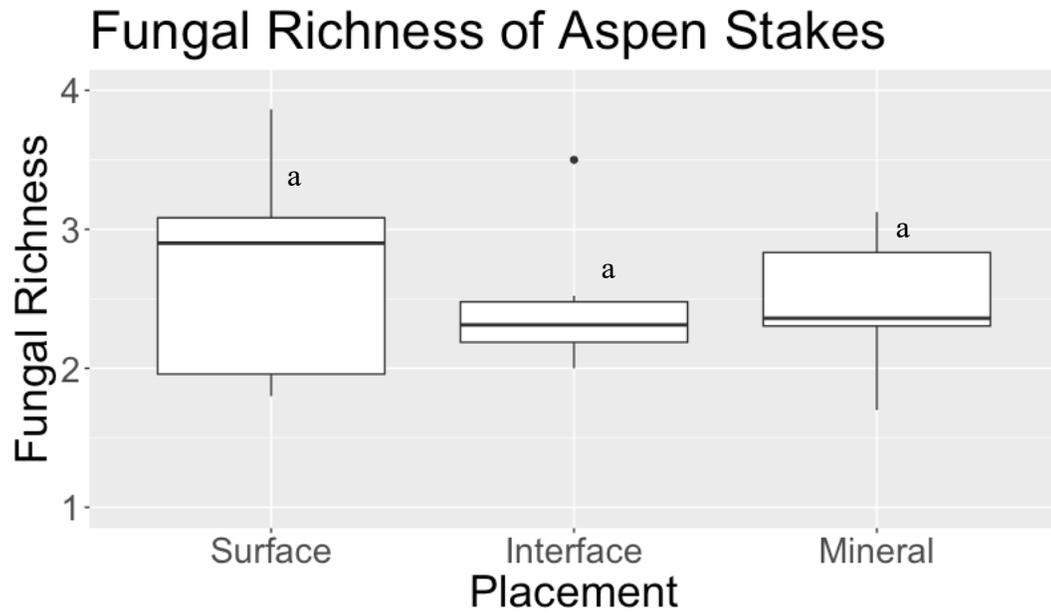
Figure A2.3 Mean and standard errors of fungal richness of aspen stakes at the different placement locations averaged across all sample years and N treatments.

Fungal Richness of Pine Stakes
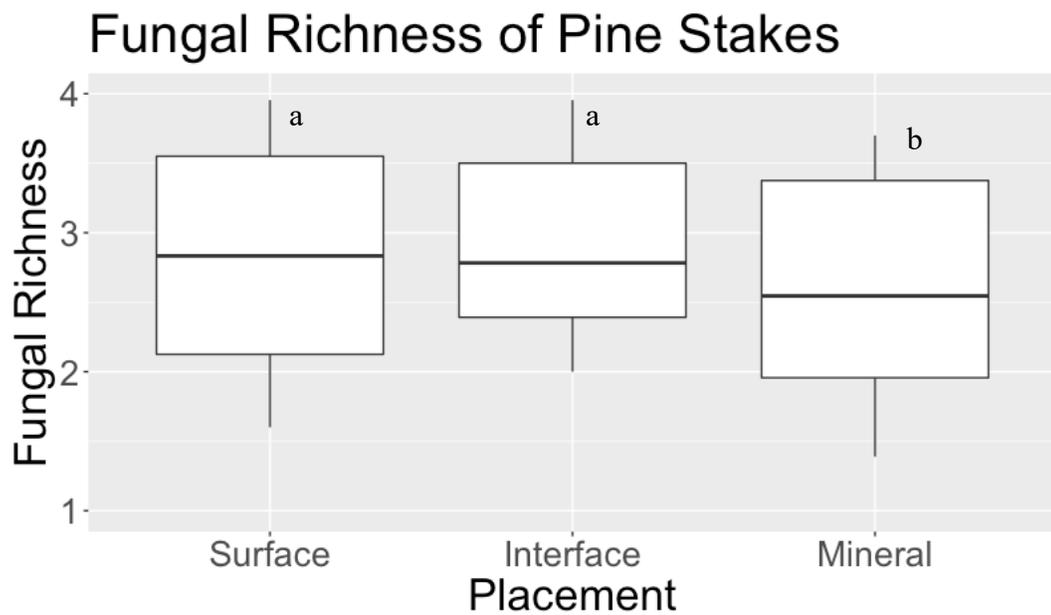
Figure A2.4 Mean and standard errors of fungal richness of pine stakes at the different placement locations averaged across all sample years and N treatments.

Table A2.1 P-value results from Kruskal-Wallis and Scheirer-Ray-Hare test results with mass loss as the response variable. Aspen and pine stakes were analyzed separately and further subset by the difference stake placement locations. Year was significant for all subsets. N treatment (treatment) was only significant for aspen stakes at surface and interface locations. There was not a significant year by treatment interaction.

| | | Aspen | | | | | | | | | Pine | | | | | | | | |
| | | Surface | | | Interface | | | Mineral | | | Surface | | | Interface | | | Mineral | | |
Factor	DF	P-Value	SS	X^2	P-Value	SS	X^2	P-Value	SS	X^2	P-Value	SS	X^2	P-Value	SS	X^2	P-Value	SS	X^2
Year	2	<0.001	-	59.65	<0.001	-	68.65	<0.001	-	57.66	<0.001	-	53.81	<0.001	-	73.56	<0.001	-	80.54
Treatment	2	<0.001	-	20.52	0.012	-	0.012	0.35	-	2.12	0.19	-	3.35	0.35	-	2.10	0.11	-	4.37
Year X Treatment	4	0.067	27666	-	0.86	5028	-	0.22	17562	-	0.38	13191	-	0.83	4456	-	0.82	4422	-

Table A2.2 P-value results from Kruskal-Wallis and Scheirer-Ray-Hare test results with fungal richness as the response variable. Aspen and pine stakes were analyzed separately and further subset by the difference stake placement locations. Year was significant for all subsets except aspen stakes placed at the interface. N treatment (treatment) was only significant for aspen and pine stakes placed on the surface. There was a significant year by treatment interaction for aspen and pine stakes placed on the surface.

| | | Aspen | | | | | | | | | Pine | | | | | | | | |
| | | Surface | | | Interface | | | Mineral | | | Surface | | | Interface | | | Mineral | | |
Factor	DF	P-Value	SS	X^2	P-Value	SS	X^2	P-Value	SS	X^2	P-Value	SS	X^2	P-Value	SS	X^2	P-Value	SS	X^2
Year	2	<0.001	84615	-	0.065	-	5.46	<0.001	-	15.11	<0.001	77464	-	<0.001	-	27.49	<0.001	-	31.99
Treatment	2	0.0084	29665	-	0.31	-	2.37	0.10	-	4.61	0.015	26566	-	0.13	-	4.13	0.18	-	3.40
Year X Treatment	4	0.018	36794	-	0.15	24108	-	0.76	6136	-	0.0037	49622	-	0.92	2894	-	0.17	17930	-

Table A2.3 P-value results from Dunn-test pairwise N treatment differences with mass loss as the response variable. Aspen and pine stakes were analyzed separately and further subset by the difference stake placement locations and sample years. Most pairwise treatment differences were observed from aspen stakes placed on the surface.

| | Aspen | | | | | | | | | Pine | | | | | | | | |
| | Surface | | | Interface | | | Mineral | | | Surface | | | Interface | | | Mineral | | |
Factor	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010
Control-N1	1	<0.001	0.014	0.45	0.11	0.087	0.15	0.30	0.98	0.045	0.91	0.045	0.84	1	0.36	0.96	0.28	0.055
Control-N2	0.79	0.040	0.037	0.62	0.16	0.33	0.30	0.35	1	0.45	0.52	0.99	1	0.80	0.75	1	0.29	0.21
N1-N2	0.73	0.0055	0.60	0.53	0.63	0.53	0.45	0.081	1	0.12	0.89	0.061	0.84	1	0.53	1	0.69	0.34

Table A2.4 P-value results from Dunn-test pairwise N treatment differences with fungal richness as the response variable. Aspen and pine stakes were analyzed separately and further subset by the difference stake placement locations and sample years. Significant treatment differences were sparse, with statistical differences only for stakes placed on the surface for aspen and pine stakes.

| | Aspen | | | | | | | | | Pine | | | | | | | | |
| | Surface | | | Interface | | | Mineral | | | Surface | | | Interface | | | Mineral | | |
Factor	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010
Control-N1	0.60	0.0015	0.32	0.070	1	0.66	0.35	0.97	0.52	0.71	<0.001	0.11	1	0.77	0.39	0.77	0.11	0.17
Control-N2	0.11	0.47	0.19	0.071	0.81	0.87	0.58	0.49	0.57	0.78	0.067	0.15	0.93	0.81	0.64	0.82	0.76	0.68
N1-N2	0.18	0.0099	0.89	0.81	0.86	0.66	0.95	0.27	0.37	0.87	0.10	0.002	0.64	0.56	0.76	1	0.088	0.13

Table A2.5 The mean presence for the most abundant fungal taxa for each stake species at each soil depth.

Aspen - Surface	Control	N1	N2	*P-value*
Mollisia cinerea	0.30^a	0.00^b	0.07^b	*P<0.001*
Sistotrema brinkmannii	0.09	0.12	0.15	*ns*
Lachnellula fuscosanguinea	0.00^a	0.11^a	0.18^b	*P<0.01*
Phanerochaete velutina	0.03^a	0.20^b	0.06^a	*P<0.01*
Ascocoryne cylichnium	0.04^a	0.06^{ab}	0.18^b	*P<0.05*
Pseudoplectania nigrella	0.14^a	0.12^a	0.00^b	*P<0.01*

Aspen - Interface	Control	N1	N2	*P-value*
Acrodontium antarcticum	0.21^a	0.38^{ab}	0.47^b	*P<0.01*
Phanerochaete velutina	0.06^a	0.27^b	0.10^a	*P<0.01*
Mollisia cinerea	0.17^a	0.05^b	0.09^{ab}	*P=0.06*
Sporothrix sp.	0.01^a	0.06^a	0.23^b	*P<0.001*
Phialocephala fortinii	0.19^a	0.05^b	0.00^b	*P<0.001*
Phialocephala fortinii	0.17^a	0.03^b	0.03^b	*P<0.001*

Aspen - Mineral	Control	N1	N2	*P-value*
Acrodontium antarcticum	0.18	0.24	0.32	*ns*
Cenococcum sp.	0.29[a]	0.14[ab]	0.07[b]	*P<0.01*
Phanerochaete velutina	0.05[a]	0.24[b]	0.06[a]	*P<0.001*
Coniochaeta sp	0.06	0.14	0.14	*ns*
Phialocephala fortinii	0.11	0.13	0.08	*ns*
Phialocephala fortinii	0.10	0.05	0.11	*ns*

Pine - Surface	Control	N1	N2	*P-value*
Infundichalara minuta	0.21	0.14	0.13	*ns*
Phanerochaete velutina	0.12[ab]	0.24[a]	0.01[b]	*P<0.001*
Hamamotoa lignophila	0.15	0.14	0.06	*ns*
Pezoloma ericae	0.11[ab]	0.01[a]	0.18[b]	*P<0.01*
Phanerochaete sanguinea	0.02[a]	0.24[b]	0.04[a]	*P<0.001*
Ascocoryne cylichnium	0.13	0.04	0.06	*ns*
Helotiaceae sp.	0.11	0.03	0.10	*ns*
Helotiales sp.	0.03	0.07	0.08	*ns*
Pholiota scamba	0.02[a]	0.00[a]	0.15[b]	*P<0.001*

Pine - Interface	Control	N1	N2	P-value
Acrodontium antarcticum	0.11[a]	0.38[b]	0.51[b]	*P<0.001*
Phanerochaete velutina	0.21[a]	0.22[a]	0.00[b]	*P<0.001*
Phialocephala fortinii	0.20[a]	0.07[b]	0.06[b]	*P<0.05*
Sporothrix sp.	0.00[a]	0.13[b]	0.19[b]	*P<0.001*
Hyaloscypha sp.	0.03	0.11	0.12	*ns*
Coniochaeta sp	0.24[a]	0.01[b]	0.00[b]	*P<0.001*

Pine - Mineral	Control	N1	N2	P-value
Acrodontium antarcticum	0.11[a]	0.10[a]	0.29[b]	*P<0.01*
Pholiota mixta	0.11[a]	0.07[a]	0.29[b]	*P<0.001*
Phanerochaete velutina	0.16	0.19	0.08	*ns*
Cenococcum sp.	0.14	0.13	0.08	*ns*
Phialocephala fortinii	0.07	0.08	0.09	*ns*
Sporothrix sp.	0.00[a]	0.07[ab]	0.13[b]	*P<0.01*

Soil sampling and analysis: Table A2.6

   To characterize soil bulk density, pH, organic matter, carbon, and nitrogen contents when the wood stakes were installed, we collected mineral soil samples from each plot and replicate for analysis (Table A2.6). Soil was collected from the 0-20 cm depth using the excavation-foam core method (Lindner et al., 2011). Samples were frozen prior to shipment. Samples were shipped to the USDA Forest Service Soil Analytical Laboratory in Moscow, ID, USA where they were dried at 105º C for 24 h, weighed, and sieved through a 2-mm

mesh screen to remove rocks, which were weighed to calculate soil fine-fraction bulk density. The sieved soil was analyzed for organic matter by weight loss on ignition at 375º C for 16 h, total carbon and nitrogen (Leco Corp, St. Joseph, MI, USA), and pH on a 2:1 water to soil paste.

Soil temperature

Temperature measurements were recorded periodically throughout the study. Unfortunately, due to equipment failure we were unable to get consistent, year-long soil temperature data. However, it is unlikely that fertilizer application altered soil temperature significantly and we assume they were similar for the duration of our study.

Table A2.6 Average soil properties in two fertilizer treatments and a control at Norrliden, Sweden.

Treatment	Total bulk density	Fine fraction bulk density	Rock content	pH	Organic matter	Carbon	Nitrogen
	Mg m^{-3}		%		Mg/ha		kg/ha
Control	1.09	0.85	44	4.3	90.9	12.9	515.8
N1	1.04	0.85	27	4.1	115.3	15.0	596.9
N2	1.42	1.12	40	4.1	107.7	17.1	763.5
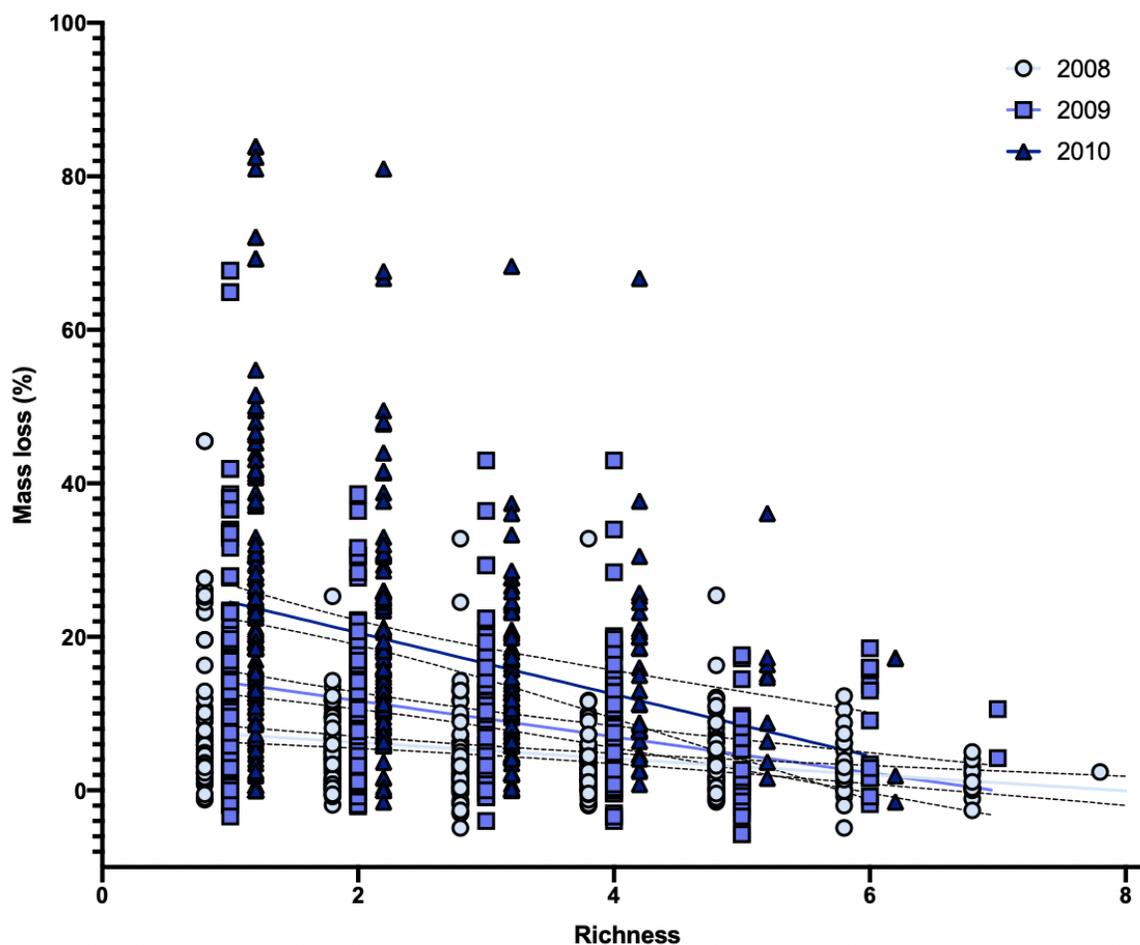
Figure A2.5 Regression analysis plot of fungal richness and stake mass loss. For each sample year a negative relationship was observed, with lower fungal richness associated with mass loss. This relationship tended to be less steep in 2008 (y = -1.05x + 8.35; r^2 = 0.07; *P*<0.0001), intermediate in 2009 (y = -2.35x + 16.36; r^2 = 0.09; *P*<0.0001), and most steep in 2010 (y = -4.02x + 28.56; r^2 = 0.08; *P*<0.0001). These observed relationships are likely driven by declines in fungal richness due to more advanced stages of CWD decomposition.

Appendix B: Chapter 3 Supplemental Material

Plot 2

10 cm

3 meters

90°

90°

Plot 5

Plot 1

Plot 3

90°

90°

Plot 4
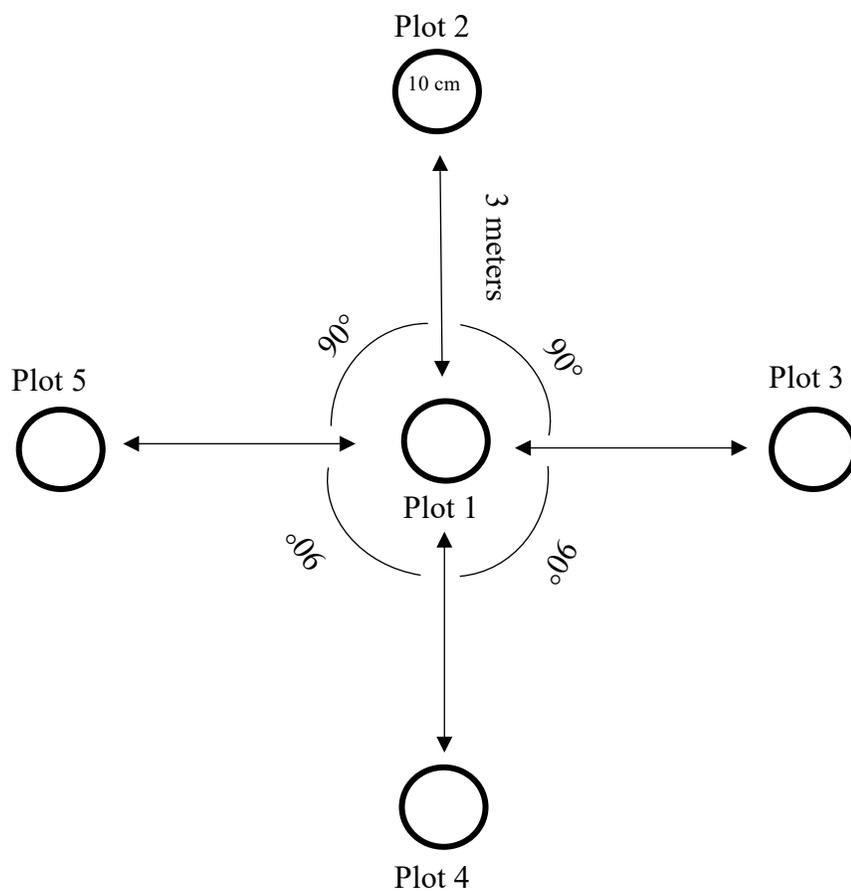
Figure B3.1 Representation and specification of soil collection plots. Soil was collected from 5 plots within a designated area. Plots were 3 meters apart with a 10cm diameter. The first 5-10 cm of mineral soil was collected after removing surface litter and coarse debris.

Table B3.1 Dates of experimental respiration, VOC, mass loss and microbial community measurements. 'X' denotes measurements were taken.

Week	Date	Respiration	VOCs	Mass Loss	Microbial Community
0	06/29/2020	X	X	X	X
1	07/06/2020	X	X	X	
2	07/13/2020	X	X	X	
3	07/20/2020	X	X	X	
4	07/27/2020	X	X	X	X
5	08/03/2020	X	X	X	
6	08/10/2020	X	X	X	
7	08/17/2020	X	X	X	
8	08/24/2020	X	X	X	X
9	08/31/2020	X	X	X	
10	09/07/2020	X	X	X	
11	09/14/2020	X	X	X	
12	09/21/2020	X	X	X	X

Figure B3.2 Bacterial Shannon diversity (A) and fungal Shannon diversity (B) for weeks 0, 4, 8, and 12. P-values are displayed in the figures.
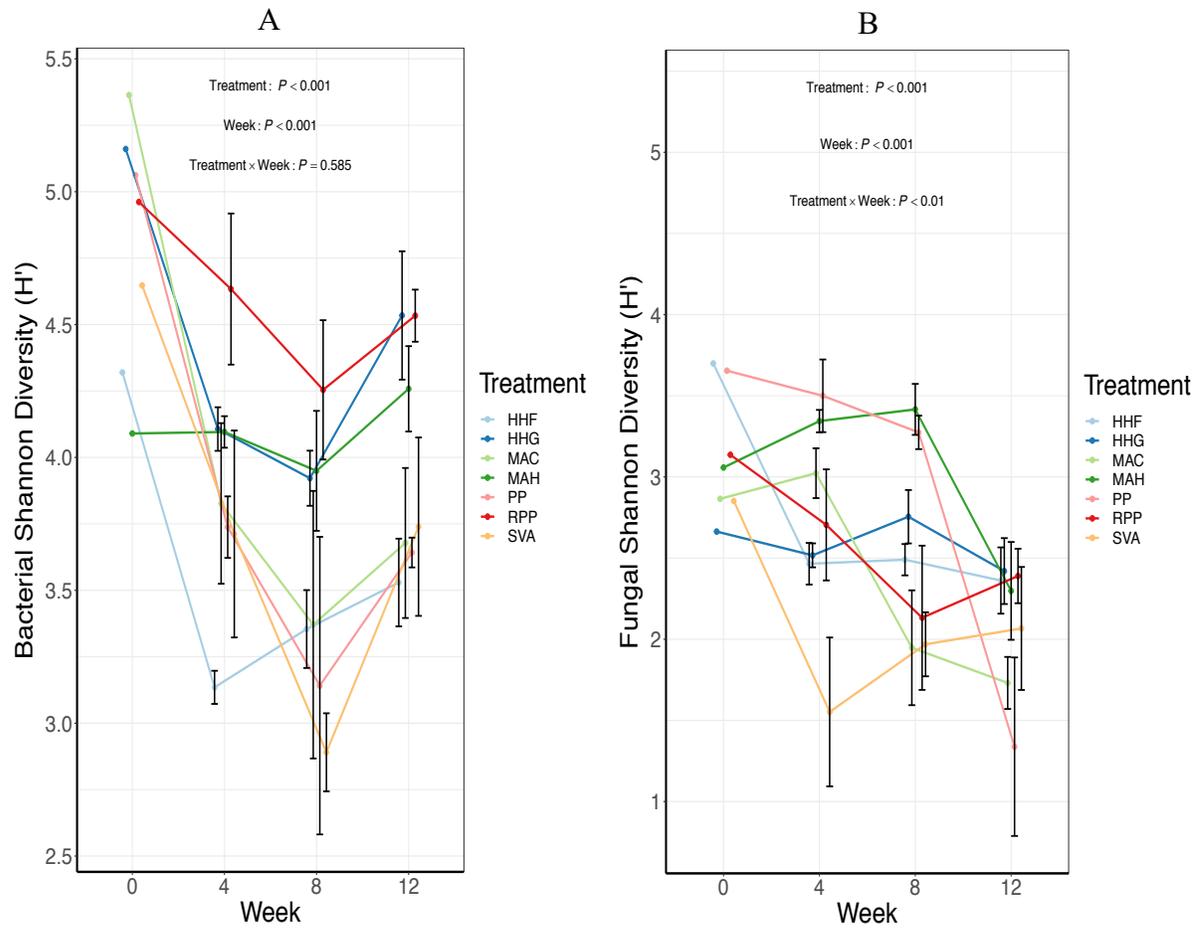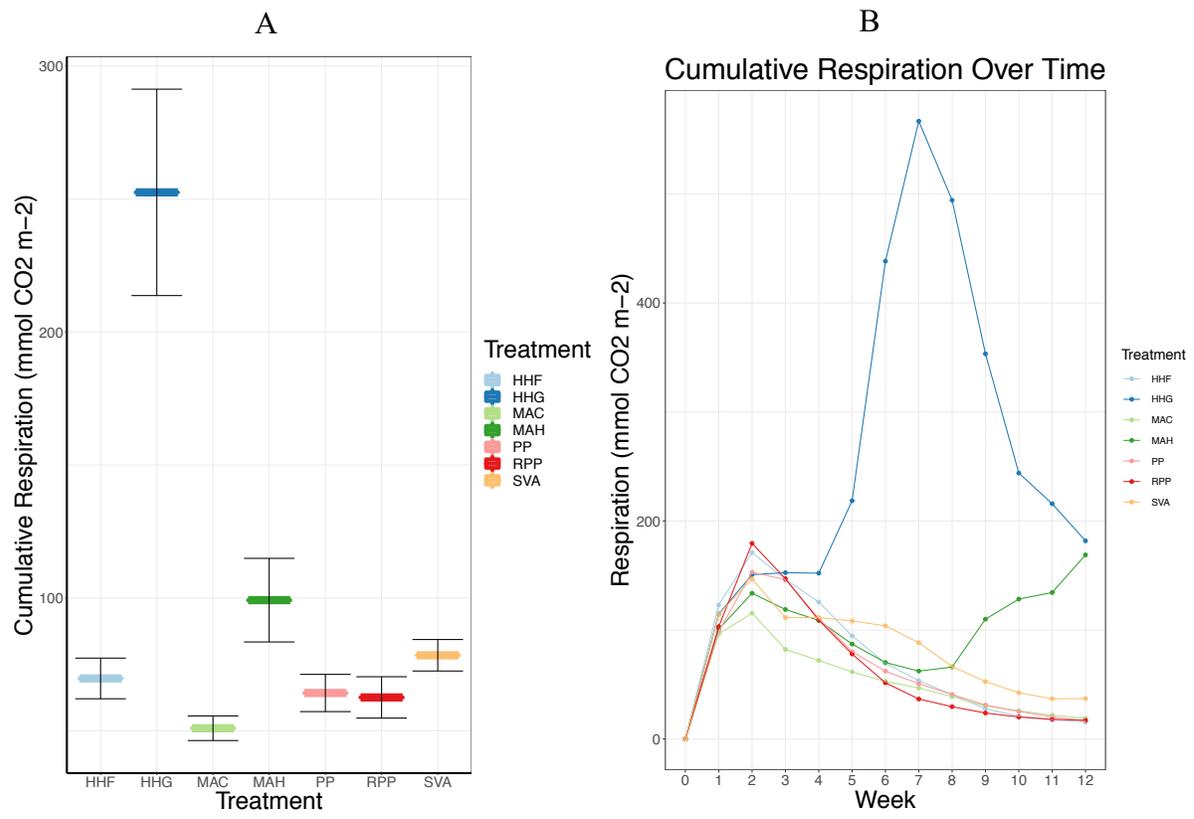
Figure B3.3 Mean and standard error of cumulative respiration for each treatment (A) and cumulative respiration over time (B).

Table B3.2 Pairwise treatment differences with mass loss as the response variable to treatment per week.

Pairwise Treatments / Week	0	1	2	3	4	5	6	7	8	9	10	11	12
HHG-HHF	0	0.93	0.99	1	1	0.99	0.87	0.48	0.25	0.12	0.09	0.077	<0.05
MAC-HHF	0	<0.01	<0.01	0.34	0.25	0.4	0.48	0.61	0.69	0.72	0.9	0.92	0.96
MAH-HHF	0	<0.001	<0.01	0.22	0.14	0.26	0.36	0.49	0.5	0.5	0.84	0.6	0.42
PP-HHF	0	0.54	0.49	0.97	0.72	0.77	0.82	0.81	0.82	0.86	0.93	0.96	0.97
RPP-HHF	0	<0.01	0.55	0.96	0.92	0.89	0.92	0.98	0.99	1	1	1	0.99
SVA-HHF	0	0.21	0.92	1	0.99	0.97	1	1	1	0.99	0.99	1	0.99
MAC-HHG	0	0.071	<0.05	0.22	0.23	0.67	0.99	1	0.99	0.94	0.72	0.63	0.41
MAH-HHG	0	<0.05	<0.05	0.14	0.13	0.49	0.98	1	0.99	0.99	0.8	0.94	0.95
PP-HHG	0	0.99	0.73	0.92	0.69	0.94	1	0.99	0.97	0.84	0.66	0.52	0.35
RPP-HHG	0	0.06	0.79	0.89	0.91	0.98	1	0.95	0.61	0.23	0.093	0.057	<0.05
SVA-HHG	0	0.87	0.99	1	0.99	0.85	0.84	0.37	0.14	<0.05	<0.05	0.067	<0.05
MAH-MAC	0	0.89	1	1	1	1	1	1	1	0.99	1	0.99	0.95
PP-MAC	0	0.35	0.69	0.89	0.99	0.99	0.99	0.99	1	1	1	1	1
RPP-MAC	0	1	0.63	0.91	0.91	0.98	0.99	0.98	0.95	0.97	0.9	0.89	0.84
SVA-MAC	0	0.71	0.22	0.21	<0.05	0.053	0.43	0.5	0.5	0.45	0.59	0.91	0.81
PP-MAH	0	<0.05	0.56	0.78	0.96	0.98	0.99	0.99	0.99	0.99	1	0.99	0.93
RPP-MAH	0	0.92	0.49	0.82	0.79	0.94	0.96	0.95	0.85	0.69	0.85	0.52	0.23
SVA-MAH	0	0.086	0.14	0.13	<0.05	<0.05	0.32	0.38	0.32	0.26	0.51	0.56	0.2
RPP-PP	0	0.31	1	1	0.99	1	1	0.99	0.98	0.96	0.93	0.94	0.88
SVA-PP	0	0.99	0.99	0.92	0.28	0.21	0.78	0.71	0.65	0.63	0.66	0.95	0.86
SVA-RPP	0	0.67	0.99	0.89	0.55	0.35	0.9	0.94	0.98	0.99	0.99	1	1

Table B3.3 Pairwise treatment differences with respiration as the response variable to treatment per week.

Pairwise Treatments / Week	0	1	2	3	4	5	6	7	8	9	10	11	12
HHG-HHF	0.82	1	1	0.98	0.99	0.21	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
MAC-HHF	0.99	0.99	0.78	0.68	0.86	0.97	0.99	1	1	0.99	0.99	0.99	0.99
MAH-HHF	1	0.99	0.99	1	1	1	0.99	0.99	0.82	<0.05	<0.05	<0.01	<0.0001
PP-HHF	1	0.99	1	1	0.99	0.99	1	1	1	0.99	0.99	1	1
RPP-HHF	0.27	1	0.99	0.99	0.99	0.99	0.96	0.94	0.99	1	1	1	0.99
SVA-HHF	0.99	1	0.98	0.99	1	0.99	0.81	0.9	0.77	0.42	0.63	0.3	0.21
MAC-HHG	0.61	0.99	0.76	0.19	0.39	<0.05	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
MAH-HHG	0.93	1	0.99	0.95	0.99	0.25	<0.001	<0.0001	<0.0001	0.22	0.22	0.63	0.93
PP-HHG	0.78	0.99	1	0.94	0.87	0.077	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
RPP-HHG	<0.01	1	1	0.9	0.9	<0.05	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SVA-HHG	0.5	1	0.98	0.78	0.99	0.66	<0.01	<0.001	0.0001	<0.01	<0.01	<0.01	<0.05
MAH-MAC	0.99	0.99	0.93	0.79	0.85	0.95	0.97	0.98	0.82	0.11	0.14	<0.01	<0.01
PP-MAC	1	1	0.7	0.82	0.99	0.99	0.99	1	1	1	1	0.99	0.99
RPP-MAC	0.46	0.99	0.59	0.87	0.98	1	0.99	0.99	0.99	0.99	0.99	0.99	1
SVA-MAC	1	0.99	0.99	0.96	0.84	0.62	0.5	0.77	0.77	0.7	0.93	0.57	0.57
PP-MAH	0.99	0.99	0.99	1	0.99	0.99	0.99	0.99	0.9	0.11	0.13	<0.01	<0.001
RPP-MAH	0.15	0.99	0.99	1	0.99	0.99	0.8	0.69	0.46	<0.05	<0.05	<0.01	<0.001
SVA-MAH	0.99	1	0.99	0.99	1	0.99	0.96	0.99	1	0.93	0.76	0.5	0.29
RPP-PP	0.3	0.99	1	1	1	1	0.98	0.97	0.99	0.99	0.99	1	1
SVA-PP	0.99	0.99	0.96	0.99	0.9	0.91	0.71	0.84	0.86	0.7	0.92	0.34	0.35
SVA-RPP	0.57	1	0.92	1	0.99	0.78	0.21	0.28	0.4	0.31	0.62	0.36	0.46

Table B3.4 Pairwise treatment differences with VOCs as the response variable to treatment per week.

Pairwise Treatments / Week	0	1	2	3	4	5	6	7	8	9	10	11	12
HHG-HHF	0.94	0.89	0.49	0.99	0.98	0.82	0.93	1	0.99	0.26	0.99	<0.0001	<0.05
MAC-HHF	0.99	0.12	0.034	1	1	0.99	0.22	0.86	<0.01	<0.0001	0.25	0.99	0.99
MAH-HHF	0.76	0.99	0.0057	1	0.94	0.99	1	0.62	<0.0001	<0.0001	0.73	0.81	0.82
PP-HHF	1	<0.0001	0.079	0.99	0.99	<0.05	<0.05	0.84	<0.0001	<0.0001	<0.0001	0.17	1
RPP-HHF	1	0.62	0.0025	0.99	0.96	0.99	0.28	0.93	<0.0001	0.091	0.96	0.39	0.4
SVA-HHF	0.99	0.33	0.21	0.86	0.98	1	0.091	1	1	1	0.99	0.22	0.84
MAC-HHG	0.73	<0.01	0.9	0.99	0.99	0.6	<0.01	0.9	0.52	<0.05	0.62	<0.0001	0.32
MAH-HHG	0.99	0.99	0.6	1	1	0.46	0.98	0.7	0.097	0.058	0.97	<0.0001	0.69
PP-HHG	0.97	<0.0001	0.97	0.98	0.99	<0.001	<0.001	0.89	<0.01	<0.0001	<0.001	<0.0001	0.077
RPP-HHG	0.95	0.99	<0.0001	1	1	0.61	0.92	0.89	<0.001	0.99	0.99	<0.0001	<0.0001
SVA-HHG	0.77	0.97	0.0004	0.98	1	0.76	0.66	1	1	0.14	1	<0.0001	0.66
MAH-MAC	0.46	<0.05	0.99	0.99	0.98	1	0.12	0.99	0.84	0.99	0.99	0.93	0.99
PP-MAC	0.99	<0.0001	0.99	1	1	0.11	0.96	1	0.1	0.71	0.19	0.081	0.99
RPP-MAC	0.99	<0.001	<0.0001	0.98	0.98	1	<0.0001	0.2	0.28	0.066	0.84	0.59	0.072
SVA-MAC	1	<0.0001	<0.0001	0.74	0.99	1	<0.0001	0.78	<0.001	<0.0001	0.43	0.39	0.99
PP-MAH	0.86	<0.0001	0.98	0.99	0.99	0.18	<0.001	0.99	0.83	0.43	<0.05	<0.01	0.89
RPP-MAH	0.79	0.92	<0.0001	0.99	1	1	0.44	0.078	0.97	0.19	0.99	0.99	<0.05
SVA-MAH	0.5	0.71	<0.0001	0.94	1	0.99	0.17	0.52	<0.0001	<0.0001	0.89	0.96	1
RPP-PP	1	<0.0001	<0.0001	0.97	0.99	0.12	<0.0001	0.19	0.99	<0.001	<0.01	<0.0001	0.3
SVA-PP	0.99	<0.0001	<0.0001	0.64	0.99	0.058	<0.0001	0.76	<0.0001	<0.0001	<0.001	<0.0001	0.91
SVA-RPP	0.99	0.99	0.78	0.99	1	1	0.99	0.96	<0.0001	<0.05	0.99	0.99	<0.05
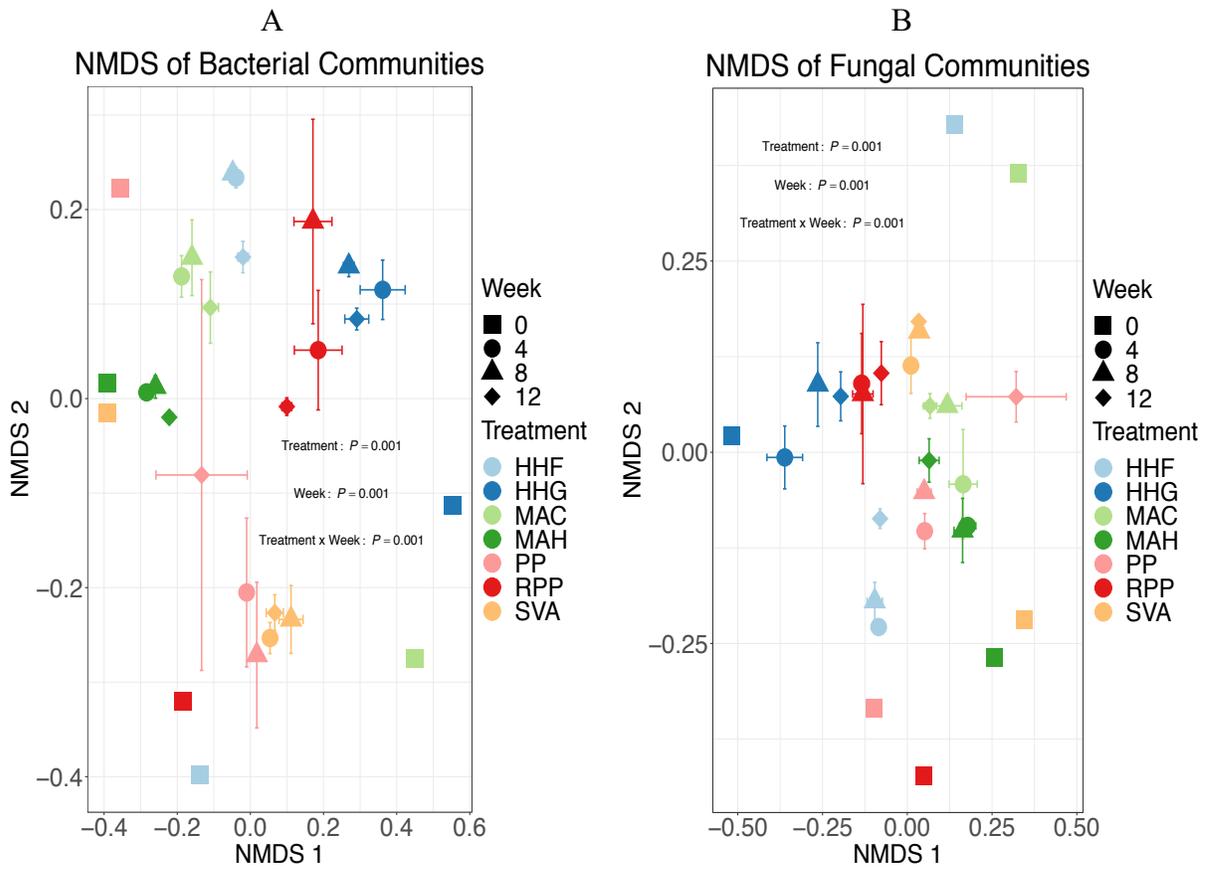
Figure B3.4 NMDS of bacterial communities (A) and fungal communities (B) plotted with means and standard error by treatment and week for weeks 0, 4, 8, and 12. P-values are displayed in the figures.

Figure B3.5 Relative abundances of bacterial (A) and fungal (B) phyla subset for weeks 0, 4, 8, and 12.

Functional Responses

Figure B3.6 PCA of mean values for the functional responses (i.e., mass loss, respiration, and VOCs) for each treatment per week.

Literature Cited

Abarenkov, K., Nilsson, R.H., Larsson, K.-H., Alexander, I.J., Eberhardt, U., Erland, S., Hoiland, K., Kjoller, R., Larsson, E., Pennanen, 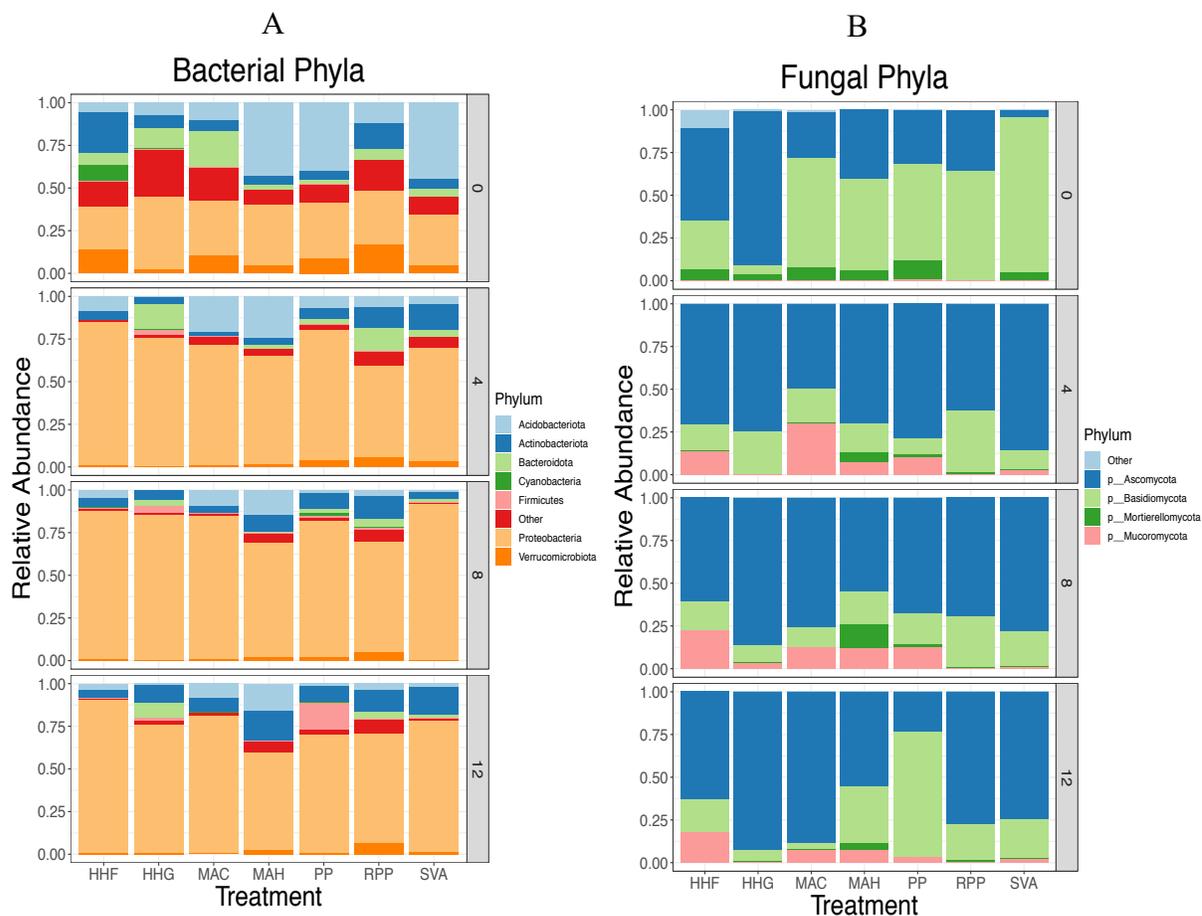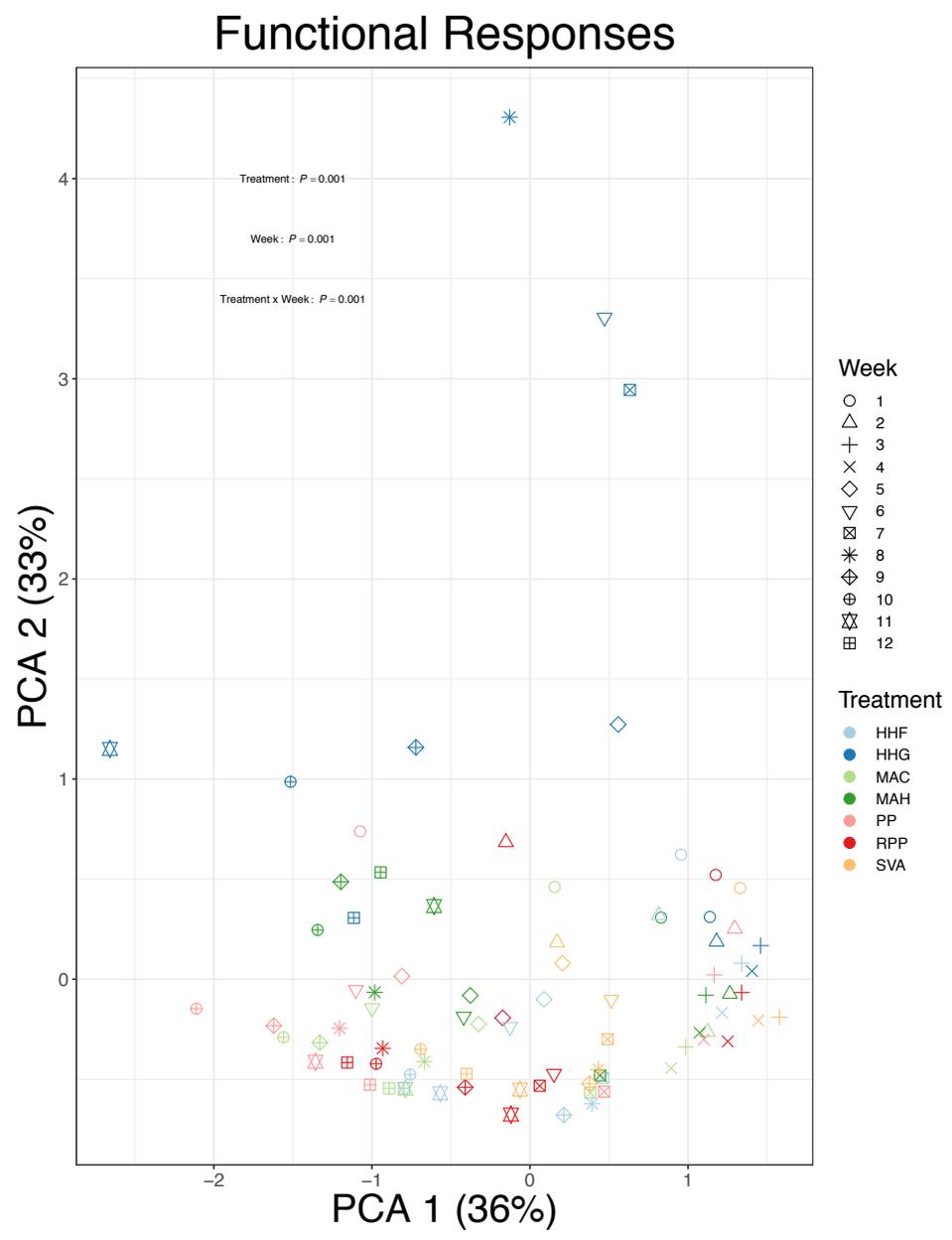T., Sen, R., Taylor, A.F.S., Tedersoo, L., Ursing, B.M., Vralstad, T., Liimatainen, K., Peintner, U., Koljalg, U. (2010). The UNITE database for molecular identification of fungi - recent updates and future perspectives. New Phytologist 186, 281–285.

Aerts, R. (1997). Climate , Leaf Litter Chemistry and Leaf Litter Decomposition in Terrestrial Ecosystems: A Triangular Relationship Author (s): Rien Aerts Published by: Wiley on behalf of Nordic Society Oikos Stable URL : https://www.jstor.org/sta. *Nordic Society Oikos*, *79*(3), 439–449.

Amaral, J. A., Ekins, A., Richards, S. R., & Knowles, R. (1998). Effect of Selected Monoterpenes on Methane Oxidation, Denitrification, and Aerobic Metabolism by Bacteria in Pure Culture. *Applied and Environmental Mircobiology,* 64(2), 520-525.

Anderson, M., Gorley, R., Clarke, K., 2008. PerMANOVA+ for Primer: Guide to Software and Statistical Methods. Primer-E Ltd, Plymouth, United Kingdom.

Asensio, D., Yuste, J. C., Mattana, S., Ribas, À., Llusià, J., & Peñuelas, J. (2012). Litter VOCs induce changes in soil microbial biomass C and N and largely increase soil CO_2 efflux. *Plant and Soil*, *360*(1–2), 163–174. https://doi.org/10.1007/s11104-012-1220-9

Bates, D., Maechler, M., Bolker, B., Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1-48. doi:10.18637/jss.v067.i01.

Bao, Y., Dolfing, J., Guo, Z., Chen, R., Wu, M., Li, Z., Lin, X., & Feng, Y. (2021). Important ecophysiological roles of non-dominant Actinobacteria in plant residue decomposition, especially in less fertile soils. *Microbiome*, *9*(1), 84. https://doi.org/10.1186/s40168-021-01032-x

Bebber, D. P., Watkinson, S. C., Boddy, L., & Darrah, P. R. (2011). Simulated nitrogen deposition affects wood decomposition by cord-forming fungi. *Oecologia*, *167*(4), 1177–1184. https://doi.org/10.1007/s00442-011-2057-2

Berg, B. (2014). Decomposition patterns for foliar litter – A theory for influencing factors. *Soil Biology and Biochemistry*, *78*, 222–232. https://doi.org/10.1016/j.soilbio.2014.08.005

Berg, B., & Matzner, E. (1996). Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. *Environmental Reviews*, 5:1-25.

Boddy, L. (2001). Fungal community ecology and wood decomposition processes in angiosperms: from standing tree to complete decay of coarse woody debris. *Ecological Bulletins*, 49: 43-56.

Boddy, L. (1993). Saprotrophic cord-forming fungi: Warfare strategies and other ecological aspects. *Mycological Research*, *97*(6), 641–655. https://doi.org/10.1016/S0953-7562(09)80141-X

Bosatta, E., & Ågren, G. I. (1999). Soil organic matter quality interpreted thermodynamically.
Soil Biology and Biochemistry, *31*(13), 1889–1891. https://doi.org/10.1016/S0038-0717(99)00105-4

Bot, A., & Benites, J. (2005). *The importance of soil organic matter: Key to drought-resistant soil and sustained food and production*. Food and Agriculture Organization of the United Nations.

Bradford, M. A., Berg, B., Maynard, D. S., Wieder, W. R., & Wood, S. A. (2016). Understanding the dominant controls on litter decomposition. *Journal of Ecology*, *104*(1), 229–238. https://doi.org/10.1111/1365-2745.12507

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. Nature Methods 13, 581.

Carreiro, M. M., Sinsabaugh, R. L., Repert, D. A., & Parkhurst, D. F. (2000). Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *81*(9), 7.

Chen, Y., Sayer, E. J., Li, Z., Mo, Q., Li, Y., Ding, Y., Wang, J., Lu, X., Tang, J., & Wang, F. (2016). Nutrient limitation of woody debris decomposition in a tropical forest: Contrasting effects of N and P addition. *Functional Ecology*, *30*(2), 295–304. https://doi.org/10.1111/1365-2435.12471

Choudoir, M., Rossabi, S., Gebert, M., Helmig, D., & Fierer, N. (2019). A Phylogenetic and Functional Perspective on Volatile Organic Compound Production by Actinobacteria. *MSystems*, *4*(2), 1–15. https://doi.org/10.1128/msystems.00295-18

Cleveland, C. C., Reed, S. C., Keller, A. B., Nemergut, D. R., O'Neill, S. P., Ostertag, R., & Vitousek, P. M. (2014). Litter quality versus soil microbial community controls over decomposition: A quantitative analysis. *Oecologia*, *174*(1), 283–294. https://doi.org/10.1007/s00442-013-2758-9

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? *Global Change Biology*, *19*(4), 988–995. https://doi.org/10.1111/gcb.12113

Du, N., Li, W., Qiu, L., Zhang, Y., Wei, X., & Zhang, X. (2020). Mass loss and nutrient release during the decomposition of sixteen types of plant litter with contrasting quality under three precipitation regimes. *Ecology and Evolution*, *10*(7), 3367–3382. https://doi.org/10.1002/ece3.6129

Eilers, K. G., Debenport, S., Anderson, S., & Fierer, N. (2012). Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. *Soil Biology and Biochemistry*, *50*, 58–65. https://doi.org/10.1016/j.soilbio.2012.03.011

Fierer, N., & Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences*, *103*(3), 626–631. https://doi.org/10.1073/pnas.0507535103

Fierer, N., Strickland, M. S., Liptzin, D., Bradford, M. A., & Cleveland, C. C. (2009). Global patterns in belowground communities. *Ecology Letters*, *12*(11), 1238–1249. https://doi.org/10.1111/j.1461-0248.2009.01360.x

Finér, L., Jurgensen, M., Palviainen, M., Piirainen, S., & Page-Dumroese, D. (2016). Does clear-cut harvesting accelerate initial wood decomposition? A five-year study with standard wood material. *Forest Ecology and Management*, *372*, 10–18. https://doi.org/10.1016/j.foreco.2016.03.060

Fissore, C., Jurgensen, M. F., Pickens, J., Miller, C., Page-Dumroese, D., & Giardina, C. P. (2016). Role of soil texture, clay mineralogy, location, and temperature in coarse wood decomposition—A mesocosm experiment. *Ecosphere*, *7*(11). https://doi.org/10.1002/ecs2.1605

Freedman, Z. B., Upchurch, R. A., Zak, D. R., & Cline, L. C. (2016). Anthropogenic N Deposition Slows Decay by Favoring Bacterial Metabolism: Insights from Metagenomic Analyses. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.00259

Freschet, G. T., Weedon, J. T., Aerts, R., van Hal, J. R., & Cornelissen, J. H. C. (2012). Interspecific differences in wood decay rates: Insights from a new short-term method to study long-term wood decomposition. *Journal of Ecology*, *100*(1), 161–170. https://doi.org/10.1111/j.1365-2745.2011.01896.x

Fukasawa, Y., & Kaga, K. (2020). Effects of wood resource size and decomposition on hyphal outgrowth of a cord-forming basidiomycete, Phanerochaete velutina. *Scientific Reports*, *10*(1), 21936. https://doi.org/10.1038/s41598-020-79058-8

Fukasawa, Y., Osono, T., & Takeda, H. (2009). Microfungus communities of Japanese beech logs at different stages of decay in a cool temperate deciduous forest. *Canadian Journal of Forest Research*, *39*(8), 1606–1614. https://doi.org/10.1139/X09-080

Gruber, N., & Galloway, J. N. (2008). An Earth-system perspective of the global nitrogen cycle. *Nature*, *451*(7176), 293–296. https://doi.org/10.1038/nature06592

Garrett, L., Davis, M., & Oliver, G. (2007). Decomposition of coarse woody debris, and methods for determining decay rates. *New Zealand Journal of Forestry Science*, *37*(2), 227–240.

Glassman, S. I., Weihe, C., Li, J., Albright, M. B. N., Looby, C. I., Martiny, A. C., Treseder, K. K., Allison, S. D., & Martiny, J. B. H. (2018). Decomposition responses to climate depend on microbial community composition. *Proceedings of the National Academy of Sciences*, *115*(47), 11994–11999. https://doi.org/10.1073/pnas.1811269115

Gougoulias, C., Clark, J. M., & Shaw, L. J. (2014). The role of soil microbes in the global carbon cycle: Tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. *Journal of the Science of Food and Agriculture*, *94*(12), 2362–2371. https://doi.org/10.1002/jsfa.6577

Graham, E. B., Knelman, J. E., Schindlbacher, A., Siciliano, S., Breulmann, M., Yannarell, A., Beman, J. M., Abell, G., Philippot, L., Prosser, J., Foulquier, A., Yuste, J. C., Glanville, H. C., Jones, D. L., Angel, R., Salminen, J., Newton, R. J., Bürgmann, H., Ingram, L. J., … Nemergut, D. R. (2016). Microbes as Engines of Ecosystem Function: When Does Community Structure Enhance Predictions of Ecosystem Processes? *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.00214

Gray, C. M., Monson, R. K., & Fierer, N. (2010). Emissions of volatile organic compounds during the decomposition of plant litter. *Journal of Geophysical Research: Biogeosciences*, *115*(3). https://doi.org/10.1029/2010JG001291

Greenberg, J. P., Asensio, D., Turnipseed, A., Guenther, A. B., Karl, T., & Gochis, D. (2012). Contribution of leaf and needle litter to whole ecosystem BVOC fluxes. *Atmospheric Environment*, *59*, 302–311. https://doi.org/10.1016/j.atmosenv.2012.04.038

Hakala, M. A., Lapveteläinen, A. T., & Kallio, H. P. (2002). Volatile compounds of selected strawberry varieties analyzed by purge-and-trap headspace GC-MS. *Journal of Agricultural and Food Chemistry*, *50*(5), 1133–1142. https://doi.org/10.1021/jf0111256

Harmon, M. E., Franklin, J. F., Swanson, F. J., Sollins, P., Gregory, S. V., Lattin, J. D., Anderson, N. H., Cline, S. P., Aumen, N. G., Sedell, J. R., Lienkaemper, G. W., Cromack, K., & Cummins, K. W. (2004). Ecology of Coarse Woody Debris in Temperate Ecosystems. In *Advances in Ecological Research* (Vol. 34, pp. 59–234). Elsevier. https://doi.org/10.1016/S0065-2504(03)34002-4

Hart, S. C., Firestone, M. K., & Paul, E. A. (1992). Decomposition and nutrient dynamics of ponderosa pine needles in a Mediterranean-type climate. *Canadian Journal of Forest Research*, *22*(3), 306–314. https://doi.org/10.1139/x92-040

He, W., Zhang, M., Jin, G., Sui, X., Zhang, T., & Song, F. (2021). Effects of Nitrogen Deposition on Nitrogen-Mineralizing Enzyme Activity and Soil Microbial Community Structure in a Korean Pine Plantation. *Microbial Ecology*, *81*(2), 410–424. https://doi.org/10.1007/s00248-020-01595-6

Henriksen, T. M., & Breland, T. A. (1999). Nitrogen availability e ects on carbon mineralization, fungal and bacterial growth, and enzyme activities during decomposition of wheat straw in soil. *Soil Biology and Biochemistry*, 14.

Hobbie, S. E. (2008). Nitrogen effects on decomposition: a five year experiment in eight temperate sites. *Ecology*, *89*(9), 2633–2644. https://doi.org/10.1890/07-1119.1

Hobbie, S. E. (2015). Plant species effects on nutrient cycling: Revisiting litter feedbacks. *Trends in Ecology and Evolution*, *30*(6), 357–363. https://doi.org/10.1016/j.tree.2015.03.015

Högberg, P., Fan, H., Quist, M., Binkley, D., & Tamm, C. O. (2006). Tree growth and soil acidification in response to 30 years of experimental nitrogen loading on boreal forest. *Global Change Biology*, *12*(3), 489–499. https://doi.org/10.1111/j.1365-2486.2006.01102.x

Högberg P. (1990). Forests losing large quantities of nitrogen have elevated ^{15}N:^{14}N ratios. *Oecologia*, 84: 229-231.

Holmen, H., Nilsson, A., Popovic, B., Wiklander, G. (1976). The optimum nutrition experiment Norrliden. A brief description of an experiment in a young stand of Scots pine *(Pinus sylvestris* L.). Res Note No 26, Dep For Ecol & For Soils, Sch For, Stockholm, Sweden

Hope, R. M. (2013). Rmisc: Rmisc: Ryan Miscellaneous. R package version 1.5. https://CRAN.R-project.org/package=Rmisc

Hu, Z., Michaletz, S. T., Johnson, D. J., McDowell, N. G., Huang, Z., Zhou, X., & Xu, C. (2018). Traits drive global wood decomposition rates more than climate. *Global Change Biology*, *24*(11), 5259–5269. https://doi.org/10.1111/gcb.14357

Insam, H., & Seewald, M. S. A. (2010). Volatile organic compounds (VOCs) in soils. *Biology and Fertility of Soils*, *46*(3), 199–213. https://doi.org/10.1007/s00374-010-0442-3

Jia-bing, W., De-xin, G., Shi-jie, H., Mi, Z., & Chang-jie, J. (2005). Ecological functions of coarse woody debris in forest ecosystem. *Journal of Forestry Research*, *16*(3), 247–252. https://doi.org/10.1007/BF02856826

Jurgensen M. F., Reed D, Page-Dumroese D, Laks P, [et al.]. (2006). Wood strength loss as a measure of decomposition in northern forest mineral soil. *Eur. J. Soil Biol.*, 42: 23-31.

Kaarik, A. A. (1974). Decomposition of wood. In C. H. Dickinson, & G. J. F. Pugh (Eds.), *Biology of plant litter decomposition* (pp. 129– 174). London, UK: Academic Press.

Keiser, A. D., Keiser, D. A., Strickland, M. S., & Bradford, M. A. (2014). Disentangling the mechanisms underlying functional differences among decomposer communities. *Journal of Ecology*, *102*(3), 603–609. https://doi.org/10.1111/1365-2745.12220

Keiser, A. D., Strickland, M. S., Fierer, N., & Bradford, M. A. (2011). The effect of resource history on the functioning of soil microbial communities is maintained across time. *Biogeosciences*, *8*(6), 1477–1486. https://doi.org/10.5194/bg-8-1477-2011

Keyser, P. (1978). Ligninolytic Enzyme System of Phanerochaete chrysosporium: Synthesized in the Absence of Lignin in Response to Nitrogen Starvation. *J. BACTERIOL.*, *135*, 8.

Laiho, R., & Prescott, C. E. (2004). Decay and nutrient dynamics of coarse woody debris in northern coniferous forests: A synthesis. *Canadian Journal of Forest Research*, *34*(4), 763– 777. https://doi.org/10.1139/x03-241

Leff, J. W. & Fierer, N. (2008). Volatile organic compound (VOC) emissions from soil and litter samples. *Soil Biology and Biochemistry*, *40*(7), 1629–1636. https://doi.org/10.1016/j.soilbio.2008.01.018

Lemfack, M. C., Gohlke, B. O., Toguem, S. M. T., Preissner, S., Piechulla, B., & Preissner, R. (2018). MVOC 2.0: A database of microbial volatiles. *Nucleic Acids Research*, *46*(D1). https://doi.org/10.1093/nar/gkx1016

Lenth, R. V. (2020). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.3. https://CRAN.R-project.org/package=emmeans

Lindner, D. L., & Banik, M. T. (2009). Effects of cloning and root-tip size on observations of fungal ITS sequences from *Picea glauca* roots. *Mycologia*, *101*(1), 157–165. https://doi.org/10.3852/08-034

Lindner, D. L., Vasaitis, R., Kubartová, A., Allmér, J., Johannesson, H., Banik, M. T., & Stenlid, J. (2011). Initial fungal colonizer affects mass loss and fungal community development in Picea abies logs 6yr after inoculation. *Fungal Ecology*, *4*(6), 449–460. https://doi.org/10.1016/j.funeco.2011.07.001

Lorch, J.M., Lindner, D.L., Gargas, A., Muller, L.K., Minnis, A.M. and Blehert, D.S., (2013). A culture-based survey of fungi in soil from bat hibernacula in the eastern United States and its implications for detection of Geomyces destructans, the causal agent of bat white-nose syndrome. *Mycologia*, *105*(2), pp.237-252.

Lucas, J. M., McBride, S. G., & Strickland, M. S. (2020). Trophic level mediates soil microbial community composition and function. *Soil Biology and Biochemistry*, *143*, 107756. https://doi.org/10.1016/j.soilbio.2020.107756

Mangiafico, S. (2021). rcompanion: Functions to Support Extension Education Program Evaluation. R package version 2.3.27. https://CRAN.R-project.org/package=rcompanion

Manning, P., Saunders, M., Bardgett, R. D., Bonkowski, M., Bradford, M. A., Ellis, R. J., Kandeler, E., Marhan, S., & Tscherko, D. (2008). Direct and indirect effects of nitrogen deposition on litter decomposition. *Soil Biology and Biochemistry*, *40*(3), 688–698. https://doi.org/10.1016/j.soilbio.2007.08.023

McBride, S. G., Choudoir, M., Fierer, N., & Strickland, M. S. (2020). Volatile organic compounds from leaf litter decomposition alter soil microbial communities and carbon dynamics. *Ecology*, *101*(10). https://doi.org/10.1002/ecy.3130

McBride, S. G., Osburn, E. D., Barrett, J. E., & Strickland, M. S. (2019). Volatile methanol and acetone additions increase labile soil carbon and inhibit nitrification. *Biogeochemistry*, *145*(1–2), 127–140. https://doi.org/10.1007/s10533-019-00595-0

McMurdie, P. J., and Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8(4):e61217.

McNeal, K. S., & Herbert, B. E. (2009). Volatile Organic Metabolites as Indicators of Soil Microbial Activity and Community Composition Shifts. *Soil Science Society of America Journal*, *73*(2), 579–588. https://doi.org/10.2136/sssaj2007.0245

Moroni, M. T., Morris, D. M., Shaw, C., Stokland, J. N., Harmon, M. E., Fenton, N. J., Merganičová, K., Merganič, J., Okabe, K., & Hagemann, U. (2015). Buried Wood: A Common Yet Poorly Documented Form of Deadwood. *Ecosystems*, *18*(4), 605–628. https://doi.org/10.1007/s10021-015-9850-4

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H., Szoecs, E., & Wagner, H. (2020). vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan

Page-Dumroese, D. S., Jurgensen, M. F., Miller, C. A., Pickens, J. B., & Tirocke, J. M. (2019). Wildfire alters belowground and surface wood decomposition on two national forests in Montana, USA. *International Journal of Wildland Fire*, *28*(6), 456. https://doi.org/10.1071/WF18218

Palviainen, M., & Finér, L. (2015). Decomposition and nutrient release from Norway spruce coarse roots and stumps – A 40-year chronosequence study. *Forest Ecology and Management*, 11.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Gl€ockner, F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41, D590–D596.

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rajala, T., Peltoniemi, M., Pennanen, T., & Mäkipää, R. (2012). Fungal community dynamics in relation to substrate quality of decaying Norway spruce (Picea abies [L.] Karst.) logs in boreal forests. *FEMS Microbiology Ecology*, *81*(2), 494–505. https://doi.org/10.1111/j.1574-6941.2012.01376.x

Remsburg, A. J., & Turner, M. G. (2006). Amount, position, and age of coarse wood influence litter decomposition in postfire Pinus contorta stands. *Canadian Journal of Forest Research*, *36*(9), 2112–2123. https://doi.org/10.1139/X06-079

Rosenfield, M. V., Keller, J. K., Clausen, C., Cyphers, K., & Funk, J. L. (2020). Leaf traits can be used to predict rates of litter decomposition. *Oikos*, *129*(10), 1589–1596. https://doi.org/10.1111/oik.06470

Rossabi, S., Choudoir, M., Helmig, D., Hueber, J., & Fierer, N. (2018). Volatile Organic Compound Emissions From Soil Following Wetting Events. *Journal of Geophysical Research: Biogeosciences*, *123*(6), 1988–2001. https://doi.org/10.1029/2018JG004514

Sayer, E. J., & Tanner, E. V. J. (2010). Experimental investigation of the importance of litterfall in lowland semi-evergreen tropical forest nutrient cycling: Tropical forest litterfall and nutrients. *Journal of Ecology*, *98*(5), 1052–1062. https://doi.org/10.1111/j.1365-2745.2010.01680.x

Schimel, J. P. (1995). Ecosystem consequences of microbialdiversity and community structure. Pages 239–244 in F. S. Chapin, III and C. Korner, editors. Arctic and Alpine biodiversity: patterns, causes, and ecosystem consequences. Springer-Verlag, Berlin, Germany

Schmidt, R., Etalo, D. W., de Jager, V., Gerards, S., Zweers, H., de Boer, W., & Garbeva, P. (2016). Microbial small talk: Volatiles in fungal-bacterial interactions. *Frontiers in Microbiology*, *6*(JAN), 1–12. https://doi.org/10.3389/fmicb.2015.01495

Shorohova, E., & Kapitsa, E. (2014). Influence of the substrate and ecosystem attributes on the decomposition rates of coarse woody debris in European boreal forests. *Forest Ecology and Management*, *315*, 173–184. https://doi.org/10.1016/j.foreco.2013.12.025

Sinsabaugh, R. L., Hill, B. H., & Follstad Shah, J. J. (2009). Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature*, *462*(7274), 795–798. https://doi.org/10.1038/nature08632

Stahl, P. D., & Parkin, T. B. (1996). Microbial production of volatile organic compounds in soil microcosms. *Soil Science Society of America Journal*, *60*(3), 821–828. https://doi.org/10.2136/sssaj1996.03615995006000030020x

Strickland, M. S., Keiser, A. D., & Bradford, M. A. (2015). Climate history shapes contemporary leaf litter decomposition. *Biogeochemistry*, *122*(2–3), 165–174. https://doi.org/10.1007/s10533-014-0065-0

Strickland, M. S., Lauber, C., Fierer, N., & Bradford, M. A. (2009). Testing the functional significance of microbial community composition. *Ecology*, *90*(2), 441–451. https://doi.org/10.1890/08-0296.1

Strickland, M. S., Osburn, E., Lauber, C., Fierer, N., & Bradford, M. A. (2009). Litter quality is in the eye of the beholder: Initial decomposition rates as a function of inoculum characteristics. *Functional Ecology*, *23*(3), 627–636. https://doi.org/10.1111/j.1365-2435.2008.01515.x

Tamm, C.O., Aronsson, A., Popovic, B., Flower-Ellis, J.G.K. (1999). Optimum nutrition and nitrogen saturation in Scots pine stands. *Studia Forestalia Suecica*, 106. 126pp. ISSN 0039-3150. ISBN 91-576-5958-3

Thiessen, S., Gleixner, G., Wutzler, T., & Reichstein, M. (2013). Both priming and temperature sensitivity of soil organic matter decomposition depend on microbial biomass – An incubation study. *Soil Biology and Biochemistry*, *57*, 739–748. https://doi.org/10.1016/j.soilbio.2012.10.029

Tyc, O., Song, C., Dickschat, J. S., Vos, M., & Garbeva, P. (2017). The Ecological Role of Volatile and Soluble Secondary Metabolites Produced by Soil Bacteria. *Trends in Microbiology*, *25*(4), 280–292. https://doi.org/10.1016/j.tim.2016.12.002

Ulyshen, M. D., Horn, S., Brownie, C., Strickland, M. S., Wurzburger, N., & Zanne, A. (2020). Comparison of decay rates between native and non-native wood species in invaded forests of the southeastern U.S.: A rapid assessment. *Biological Invasions*, *22*(8), 2619–2632. https://doi.org/10.1007/s10530-020-02276-8

Uribe-Convers, S., M.L. Settles, D.C. Tank. (2016). A phylogenomic approach based on PCR target enrichment and high throughput sequencing: resolving the diversity within the South American species of Bartsia L. (Orobanchaceae). PLoS One 11, e0148203.

van der Wal, A., de Boer, W., Smant, W., & van Veen, J. A. (2007). Initial decay of woody fragments in soil is influenced by size, vertical position, nitrogen availability and soil origin. *Plant and Soil*, *301*(1–2), 189–201. https://doi.org/10.1007/s11104-007-9437-8

Van Veen, J. A., & Kuikman, P. J. (1990). Soil structural aspects of decomposition of organic matter by micro-organisms. *Biogeochemistry*, *11*(3), 213–233. https://doi.org/10.1007/BF00004497

Vesterdal, L., Schmidt, I. K., Callesen, I., Nilsson, L. O., & Gundersen, P. (2008). Carbon and nitrogen in forest floor and mineral soil under six common European tree species. *Forest Ecology and Management*, *255*(1), 35–48. https://doi.org/10.1016/j.foreco.2007.08.015

Vishniac, H. S. (1996). Biodiversity of yeasts and filamentous microfungi in terrestrial Antarctic ecosystems. *Biodiversity & Conservation*, *5*(11), 1365–1378. https://doi.org/10.1007/BF00051983

Wadman, W. P., & de Haan, S. (1997). *Decomposition of organic matter from 36 soils in a long-term pot experiment*. *189*, 289–301.

Wang, C., Dong, D., Wang, H., Müller, K., Qin, Y., Wang, H., & Wu, W. (2016). Metagenomic analysis of microbial consortia enriched from compost: New insights into the role of Actinobacteria in lignocellulose decomposition. *Biotechnology for Biofuels*, *9*(1), 22. https://doi.org/10.1186/s13068-016-0440-2

Wang, W., Lindner, D. L., Jusino, M. A., Page-dumroese, D., Palmer, J. M., Banik, M. T., Jurgensen, M., Draeger, K., & Liu, Y. (2020). Forest Ecology and Management Wood-colonizing fungal community response to forest restoration thinnings in a Pinus tabuliformis plantation in northern China. *Forest Ecology and Management*, *476*, 118459. https://doi.org/10.1016/j.foreco.2020.118459

Wang, W., Page-Dumroese, D., Jurgensen, M., Miller, C., Walitalo, J., Chen, X., & Liu, Y. (2019). Restoration thinning impacts surface and belowground wood decomposition. *Forest Ecology and Management*, *449*, 117451. https://doi.org/10.1016/j.foreco.2019.117451

Zhang, N., Castlebury, L. A., Miller, A. N., Huhndorf, S. M., Schoch, C. L., Seifert, K. A., Rossman, A. Y., Rogers, J. D., Kohlmeyer, J., Volkmann-Kohlmeyer, B., & Sung, G.-H. (2006). An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. *Mycologia*, *98*(6), 1076–1087. https://doi.org/10.1080/15572536.2006.11832635

Zhong, Y., Yan, W., & Shangguan, Z. (2015). Impact of long-term N additions upon coupling between soil microbial community structure and activity, and nutrient-use efficiencies. *Soil Biology and Biochemistry*, *91*, 151–159. https://doi.org/10.1016/j.soilbio.2015.08.030

Zhou, L., Dai, L., Gu, H., & Zhong, L. (2007). Review on the decomposition and influence factors of coarse woody debris in forest ecosystem. *Journal of Forestry Research*, *18*(1), 48–54. https://doi.org/10.1007/s11676-007-0009-9