EFFECTS OF SOIL ENVIRONMENT ON ABUNDANCE, DIVERSITY AND COMMUNITY STRUCTURE OF ECTOMYCORRHIZAL FUNGI IN *Pinus radiata*

by
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ABSTRACT

SARAH COVINGTON STEELE: Effects of soil environment on abundance, diversity and community structure of ectomycorrhizal fungi in *Pinus radiata*
(Under the direction of Dr. Jason Hoeksema & Dr. Megan Rúa)

Balanced and sustained nutrient cycles are critical to the success of the world's forest ecosystems. Research has shown ectomycorrhizal fungi (ECM) play a vital role in regulating these systems, as they store and mobilize cycled nutrients for the trees they colonize. Not all species of ECM allocate essential nutrients (nitrogen, phosphorous and carbon) to their host with the same efficiency. Studies have shown the soil environment is important for determining fungal distribution. To better understand the ECM fungal community of *Pinus radiata*, I explored the effects of soil characteristics (percent silt, percent clay, percent sand and soil water content) on the abundance, community structure and diversity of ECM fungi in *P. radiata* from Point Lobos State Park. Fungal species were identified from five trees using molecular methods and data analyses were completed. I found that each of the soil characteristics had an effect on raw fungal abundance, and different soil characteristics can be positively or negatively correlated with the diversity of a fungal community. Specifically, sand was negatively correlated with diversity while silt had a positive impact on diversity. This suggests soil environment, specifically soil texture, is an important variable which can alter the ECM fungal community and therefore, affect the health of the trees they colonize.

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Introduction

Nutrient cycles are critical to the success of terrestrial ecosystems as they provide energy in the form of nutrients and minerals necessary for maintaining growth and stability. In particular, the flow of nitrogen, phosphorous and carbon is essential for maintaining system stability (Read and Perez−Moreno, 2003). Unfortunately, dramatic shifts in the cycling and storage of fundamental forest nutrients have occurred over the past two centuries, primarily as a result of human activities. These alterations have caused vast nutrient depletions in certain areas of terrestrial ecosystems and toxic accumulations in others. Forest ecosystems are particularly vulnerable to such changes, as forests are aesthetically, economically and ecologically important on a global scale. Therefore, research which provides a better understanding of the natural regulation of nutrients in forest ecosystems is important for understanding the full effect of anthropogenic changes (Paul, 2014**)**.

While early studies of these cycles primarily focused on how plants directly obtain and cycle these nutrients from the organic litter in the soil, more research has emphasized the critical role of mycorrhizal fungi in sustaining these natural systems (Koide, Fernandez & Malcolm, 2014 and Smith and Read, 2008). Ectomycorrhizal fungi (ECM) are important to these cycles because they mobilize the soil nutrients nitrogen and phosphorus, which are otherwise unavailable to host plants (Averill, Turner & Finzi, 2014 and Smith and Read, 2008). ECM form mutualistic relationships with their host plant. In exchange for providing the plant with these nutrients, the colonized tree allocates photosynthetically fixed carbohydrates to ECM fungi (Smith and Read, 2008). This

symbiosis is so critical to the success of the host that studies have shown trees allotting as much as 85% of their photosynthate to their ECM in exchange for the many specific nutrients they receive (Treseder and Allen, 2000).

In addition to increasing a host plant's nutrient uptake through expansion of the surface area of the root, ectomycorrhizal fungi also improve the local soil structure, aid in seedling establishment, increase the rate of root growth and protect the plant from a range of soil pathogens and microbial parasites (Richardson et al., 2009). These mutualisms have been documented in nearly every ecosystem in the world and are found in a vast range of host species and environments (Horton and Bruns, 2001). It is estimated that between 7,000 to 10,000 species of fungi and 8,000 species of plants are capable of developing these symbiotic connections (Taylor and Alexander, 2005). Consequently, ECM fungi are ubiquitous throughout terrestrial ecosystems and are an important component for the regulation of the physical, chemical and biological processes that allow forests to grow and survive (Treseder and Allen, 2000).

Due to the important role ECM fungi play in altering terrestrial nutrient cycles, it is important to understand the environmental variables influencing their global dispersion. Fungal richness varies by environment with the greatest global fungal diversity located in the temperate regions of America, Europe and Asia (Tedersoo et al., 2010). Within the United States, the richness of fungal spores is greatest in Mississippi while the least diverse communities are found in Alaska (Glassman *et al*,. 2015). Fungal abundance also favors temperate forests (Clemmensen *et al*., 2006). These patterns of mycorrhizal distribution may be influenced by both the soil environment and the host

species (Read, 1991). Fungal diversity is altered by the environment due to temperature, disturbances, the presence or absence of another organism as well as the resources made available by the soil which vary in time and space (Bruns, 1995). Host species can alter the distribution of ECM fungi as certain fungal species, such as *Suillus* and *Rhizopogon,* may preferrentially colonize one host species (Horton and Bruns, 2001). Thus, the geographic location where the fungi are found and their relative abundance can be dependent on the hosts they colonize (Ishida, Nara & Hogetsu, 2007).

Forests dominated by pine trees are widely distributed and are a common feature of most countries. In addition to increasing the stability and productivity of forests, pines provide commercial value, recreational value and reduce atmospheric carbon dioxide. Thus, the continued success of these trees is an important concern worldwide (Moir, Geils, Benoit & Scurlock, 1997). To better understand the fungal communities which are vital to this important genus, countless studies have been conducted on the relationship between pine hosts and their fungal symbionts (Horton and Bruns, 2001). This research has shown that the ECM fungi of pines are also altered by the soil environment. The effect of soil environment on ECM fungi ranges from differences in the distribution, to changes in the entire nature of the fungal host relationship where mutually beneficial associations shift to become parasitic ones (Johnson, Graham, & Smith, 1997). Although many of these soil variables have been extensively researched, surprisingly little focus has been given to the impact of soil texture on the fungal community of *Pinus radiata* (Monterey Pine).

P. radiata is both an interesting pine species and ECM host due to its extremely limited natural range. The range of *P. radiata* has been restricted to only five populations; three populations along the California coast and two isolated populations on islands off the coast of Baja California, Mexico (Lavery and Mead, 2000). Previous studies from each of these sites have shown mycorrhizal associations are extremely important to the successful establishment of *P. radiata* seedlings and improve the growth and overall success of adult hosts; however, mycorrhizal fungal species differ in the degree they are able to contribute to their host (Hoeksema *et al*., 2012). Because some fungal species are more effective than others in providing benefits to *P. radiata*, it is important to understand what environmental variables impact the mycorrhizal fungi, as well as how they alter the ECM (Duñabeitia et al., 1996). In this study, we examined soil characteristics, specifically the percent sand, percent silt, percent clay and water content of the soil, to ask three questions; (1) How do these environmental variables alter fungal abundance? (2) How do these environmental variables shape the fungal community? (3) How do these environmental variables influence fungal diversity?

Methods

Site description and soil sampling

Samples were collected as part of a broader project examining ECM fungal communities at three sites across the state of California. For my project, research was conducted on soil samples collected February 22, 2014 from Point Lobos State Park. Point Lobos is located in Monterey County, California, along the north end of the Big Sur coastline. At the site, five *P.radiata* trees were selected within an area of mature forest (Table 1). The trees were assigned the follow numbers for identification: 6, 7, 8, 9, and 10. Trees were chosen to represent similar size classes as determined by their diameter at breast height (Table 1).

Tree ID	Latitude	Longitude	Elevation (feet)	Diameter
6	36° 31.003'	121° 56.708'	81	0.85
7	36° 30.953'	121° 56.565'	64	0.71
8	36° 30.939'	121° 56.567'	61	1.13
9	36° 30.942'	121° 56.576'	61	1.21
10	36° 30.914'	121° 56.684'	63	0.59

Table 1- Exact location, elevation above sea level and diameter at breast height for the five *P. radiata* trees.

Soil was obtained by collecting 20 cores, each 10 cm in diameter and 20 cm deep (excluding litter) from four locations around a focal tree. Samples were overnighted back

to the laboratory at the University of Mississippi then stored at 4° C for processing within 17 days of collection. All root samples and surrounding soil were kept intact.

Identification of ectomycorrhizal fungi in soil cores

DNA extraction, polymerase chain reaction (PCR), and direct sequencing were completed according to the Hoeksema Lab protocol. Within two weeks of harvest, the roots of each sample were collected and prepared for visualization and morphotyping using a dissecting (Leica) microscope. This was accomplished by separating the roots from the soil using a soil sieve, rinsing them with water, and cutting them into shorter segments approximately 1mm in length. Root lengths were then placed in a petri dish and under the microscope, the composition of each fungal community was recorded. To do this, fungal species were identified and assigned temporary names according to visual characteristics such as color, texture, branching and shape. The number of fungal tips which matched each morphotype were counted and recorded. The following data was also collected for each sample: (1) total number of root tips, (2) dry root weight, and (3) root length. Root length was approximated according to Equation 1:

$$RL = \pi NA / 2h$$

Equation 1

where N is the number of root intersections, A is the area of the petri dish (63.617cm), and h is the total length of the grid lines (173.2cm). To obtain the number of root intersections, a 1 cm grid paper was placed under the petri dish and the total number of

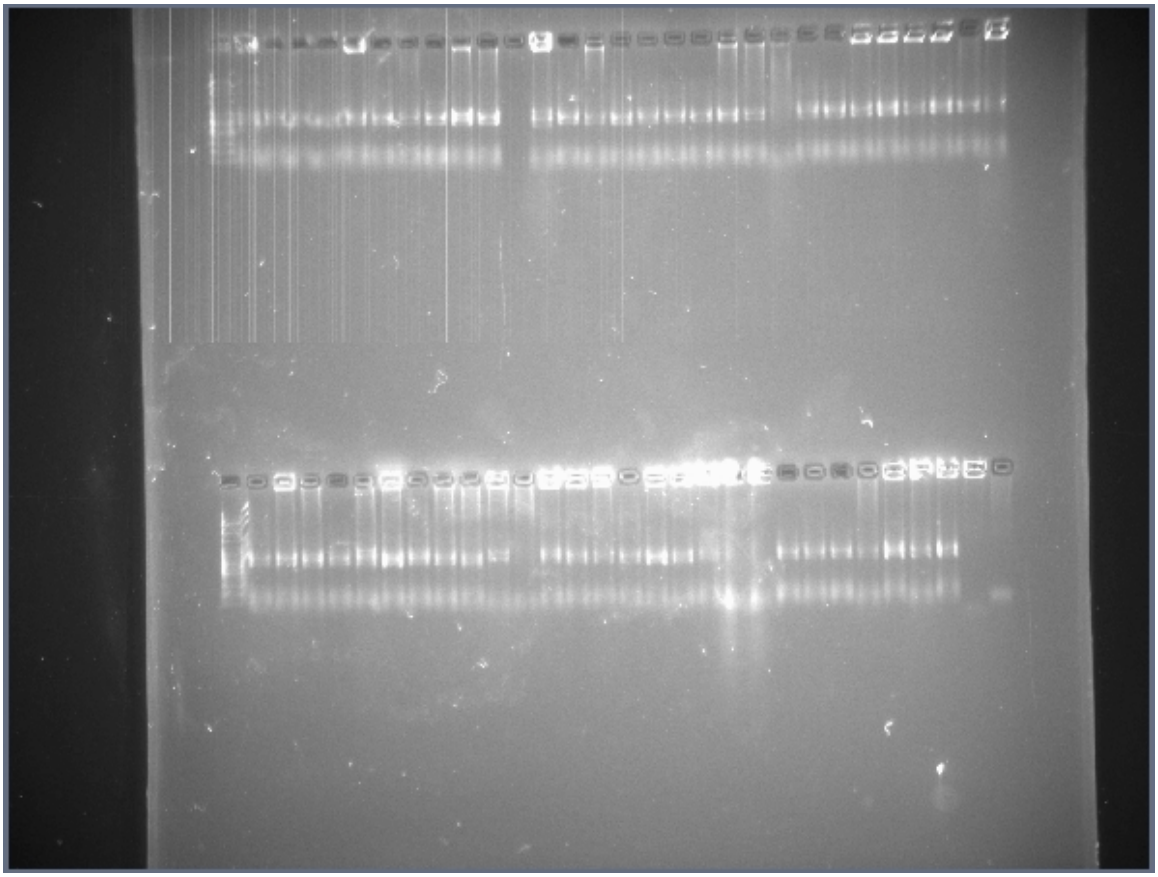
root, grid line intersections was recorded. The dry root weight was measured for each soil core using a Mettler Toledo scale.

For each observed morphotype, two root tips were extracted and placed into individual wells of a 96 well extraction plate for molecular identification. DNA extraction was completed on the same day as morphotyping and ectomycorrhizal root tip collection. DNA was obtained from each root tip using Extraction Buffer and Neutralization Solution from a Sigma Extract-N-Amp Tissue Kit. After 10 µl of the Sigma Extraction Buffer was added to each sample, the plate was placed in a Techne Genius thermocycler at 65 degrees C for 10 minutes and at 95 degrees C for 10 minutes. Upon completion of the cycle, 30 µl of Neutralization Solution was added to each well along with 160 µl of PCR-grade water to dilute DNA concentration to 20%. All samples were stored for approximately one month at -20º C following DNA extraction.

The fungal specific primers ITS1F and ITS4, which correspond to an 800 base pair internal transcribed spacer region specific to fungi, were used for DNA amplification via PCR (Gardes and Bruns, 1993). A PCR master-mix was created using 2.2 µl water, 4 µl of 2XRed Taq Premix (Apex, Inc.), and 0.4 µl of each of the two primers per each root tip sample. 7 µl of this master-mix was then combined in a new well with a 1 µl sample of each tip's DNA extract. The PCR process consisted of five steps which took place on an Applied Biosystems thermocycler. Initial denaturation began with 1 cycle at 94°C for 3 minutes. Next, there were 30 cycles of denaturing at 94°C for 45 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for 72 seconds. A final extension took place for 1 cycle at 72°C for 10 minutes. PCR amplification was inspected via electrophoresis

of a gel made of 1% agarose, a sodium borate buffer and SYBR® Safe DNA gel stain (Molecular Probes, Eugene, OR, USA). 2.5 µl of the PCR product was loaded into the gel which ran at 200 volts for 18 minutes. Gels were visualized using Quantity One UV software (Fig. 1). Excess primer and unincorporated nucleotides were removed enzymatically using ExoSAP-IT (USB Corporation Cleveland, OH, USA).

Figure 1- 1% Agarose gel for amplification of fungal DNA via PCR



5 µl of a master-mix, composed of 0.05 µl ExoI enzyme, 0.2 µl AP enzyme, and 4.75 µl of water per reaction, was combined in a new well with 5 µl of each PCR product. The

plate was then run on the Applied Biosystems thermocycler at 37°C for 30 minutes, at 80°C for 20 minutes, and at 4°C for 5 minutes.

Following clean-up, the ECM DNA was sequenced using the forward primer ITS5 (Gardes and Bruns, 1993) and the Big Dye Terminator Sequencing Kit (v3.1, Invitrogen Corp.).. A master-mix was created using 0.4 µl Big Dye Reaction Premix, 1.8 µl 5X Big Dye Sequencing Buffer, 0.5 µl Primer 5, and 6.3 µl of water per each sample. 9 µl of the master-mix was combined with 1 µl of each cleaned PCR product in a sequencing plate. This plate was then run on a four part sequencing program of an Applied Biosystems thermocycler. Initial denaturation took place for 1 cycle at 96°C for 1 minute. This was followed by 45 cycles of denaturation at 95°C for 20 seconds, annealing at 50°C for 20 seconds, and extension at 50°C for 4 minutes. The reactions were then dried with a vacufuge at 45°C for 30 minutes and shipped overnight to the DNA Lab at Arizona State University, where Big Dye reactions were purified and read on a capillary genetic analyzer.

Following sequencing, the raw DNA sequences were visually processed using Geneous Software (Biomatter Ltd.). Sequences which were less than 200 base pairs long, or had higher than 3% in base pair ambiguity, were eliminated. The remaining sequences were then assembled into operational taxonomic units (OTU) at 97% similarity using the CAP3 software (Huang & Madan, 1999) via the University of Alaska Bioinformatics Pipeline as described previously Taylor *et al.* 2007 using default settings except the following: maximum overhang percent length = 60, match score factor = 6, overlap percent identity cut-off = 96, clipping range = 6. The consensus fungal sequences from

each OTU were submitted using BLAST (nucleotide) searches on the International Nucleotide Sequence Database (INSD) and User-Friendly Nordic ITS Ectomycorrhizal (UNITE) databases October 28, 2014 to obtain best matches for taxonomic affiliation of OTUs. Sequences >97% similar in composition to database sequences from named, cultured fungi were considered the same OTU (hereafter, 'species'). Sequences with a 95% > 97% match were assigned a name according to genus. Finally, sequences with a 90% > 94% match were identified at the family level. No match below 90% was accepted. Because the blast identified the root tips at various levels of scientific classification, ranging from the species to the family level, a final species name was assigned to each of the samples at the most specific level possible. If sequence matches among the two sequence repositories showed equal affinity or similarity to multiple genera within a family, priority was given to the vouchered specimens residing on the UNITE. Any species known to be strictly non-mycorrhizal was eliminated from the data set.

Soil Analysis

Each of the soil samples was analyzed for texture and soil water content. Soil texture was characterized using a Lamotte soil texture kit. Soil water content was determined by assessing the difference in weight of fresh soil compared to the same soil which was dried for three days at 65ºC

Fungal Analysis

All data analyses were completed using R v 3.1.2 (R Core Team, 2015). To examine the soil environment, a correlation was run on the soil predictor variables: percent silt, percent sand, percent clay and soil water content. This was accomplished using the c*or* function from the *Base* package (R Core Team, 2015) Variation in fungal abundance was analyzed in two ways. First, the impact of the soil environment on tip abundance was examined using a Generalized Linear Model (GLM) with family, poisson. A GLM was chosen as the raw abundance represented count data which is not normally distributed and thus does not meet the assumptions of a Linear Model. Abundance was also studied as a function of root length (colonization), which was determined by dividing the raw number of root tips by the root length. This allowed the data to be standardized with regard to root length. While colonization was also studied using a GLM, the family quasipoisson was chosen rather than a poisson due to overdispersion of the data. All generalized linear models were created using the *glm* function from the *stats* package (R Core Team, 2015) Next, the impact of the environmental variables on the shape of the fungal community was explored using the *adonis* function, which is a multi-factor permanova, from the *vegan* package (Oksanen *et al.,* 2015). In this analysis, the Bray Curtis method was specifically chosen as it quantifies community data through compositional dissimilarity based on counts at each site. For visualization purposes, non dimensional scaling was used and the three most abundant species were mapped. To consider the effect of particular species in driving community interactions, an indicator

species analysis was run using the *multipatt* function from the *indicspecies* package and 999 permutations (De Caceres and Legendre, 2015). Fungal diversity was calculated using the *diversity* function from the *vegan* package for both Shannon and Simpson diversity. The impact of the environmental variables on fungal diversity was studied using the *lme* function of the *nlme* package (Pinheiro *et al*., 2015).

Results

Environmental Variables

The correlation analysis for the soil characteristics revealed two important results: a strong, negative correlation (-0.91) between percent sand and percent silt and a weak, negative correlation (-0.51) between percent sand and percent clay (Fig. 2 and Fig. 3)

Figure 2- Circle of correlation for environmental soil variables.

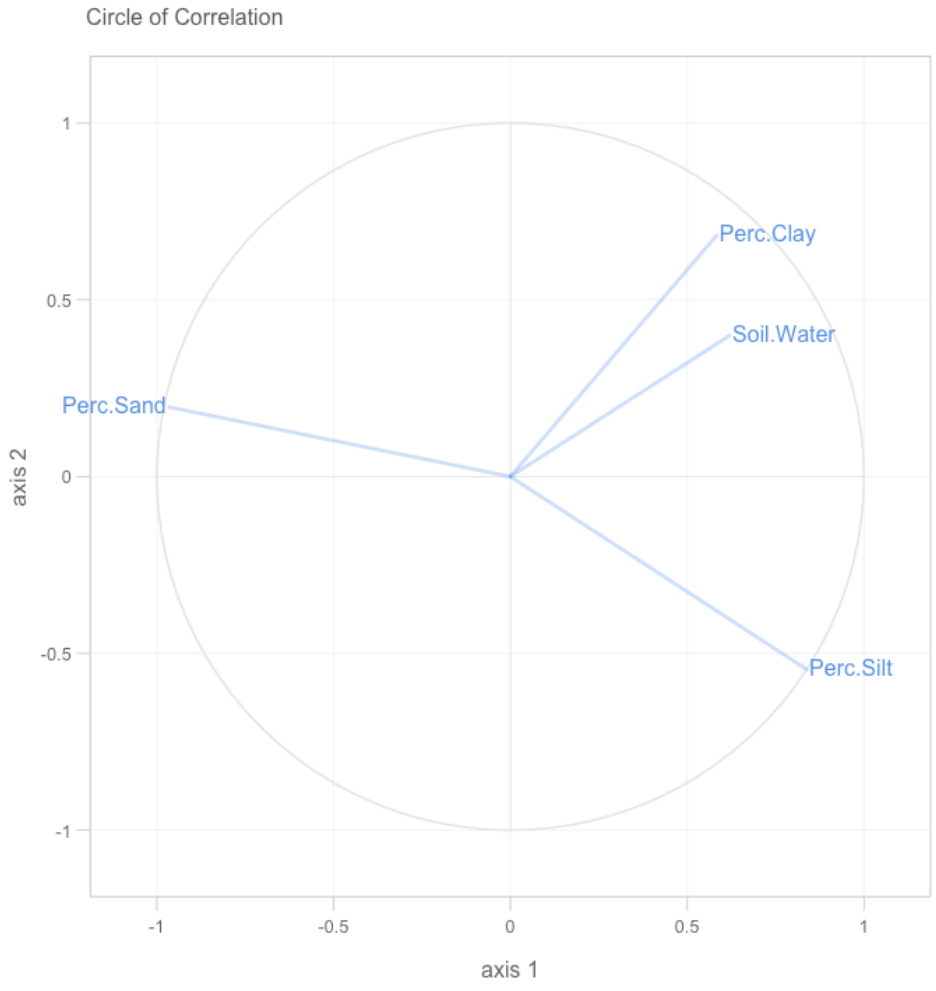
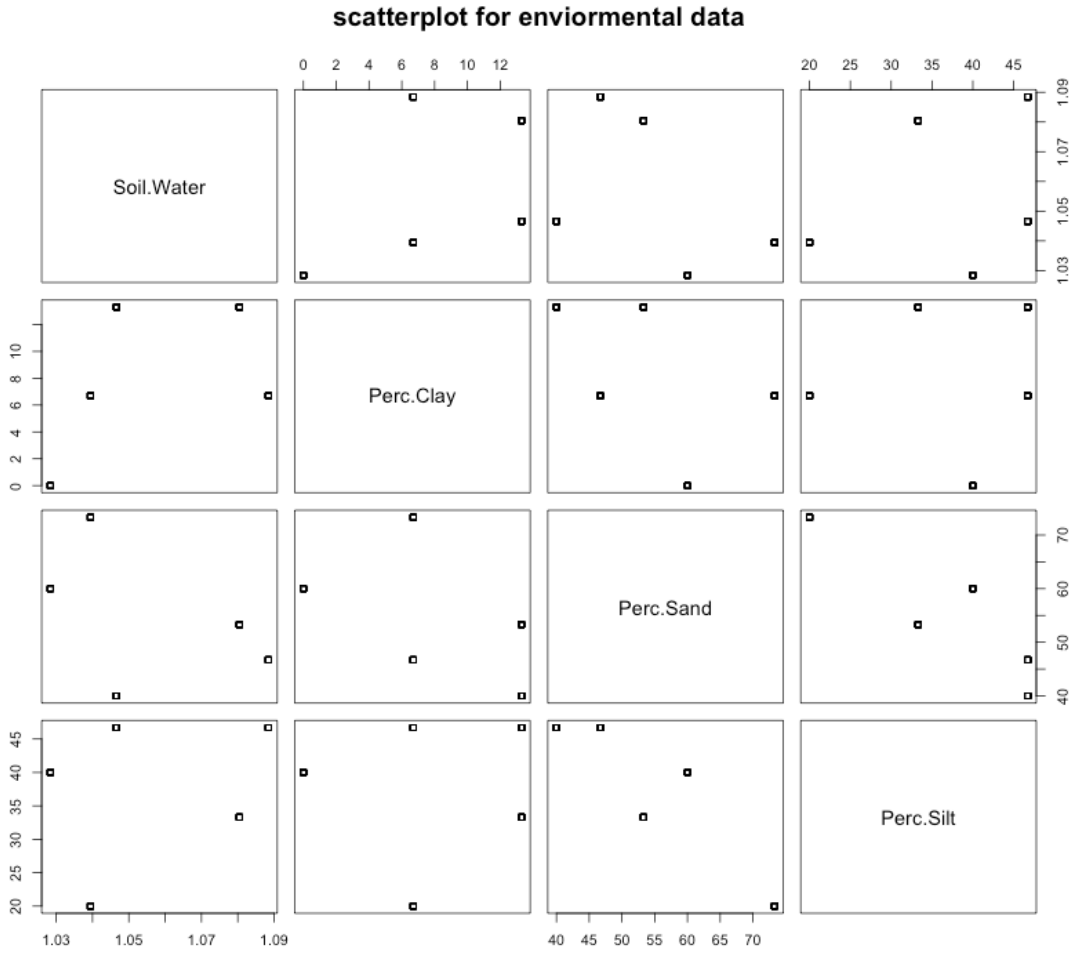


Figure 3- Scatterplot visualizing the relationship of environmental variables to one another.



Ectomycorrhizal Fungi

Fungal abundance significantly increased in response to every environmental variable: percent clay, percent silt, percent sand and soil water content (Table 2).

	Estimate	Std. Error	t value	Pr(>|z|)
(Intercept)	-140.3087	40.3658	-3.476	0.000509 ***
Soil.Water	4.6709	0.9239	5.056	4.29e-07 ***
Perc.Clay	1.3877	0.4110	3.377	0.000734 ***
Perc.Sand	1.3912	0.4073	3.416	0.000636 ***
Perc.Silt	1.3912	0.4060	3.426	0.000612 ***

Table 2- Poisson ANOVA table for the effect of soil characteristics on raw abundance.

Although the soil environment had an impact on fungal abundance, fungal colonization, which takes into account root length, was not affected by any of the environmental variables (Table 3); however, the degree of colonization varied among the fungal OTUs, with Cortinarius , Cenococcum and Cortinarius 1 exhibiting the highest levels of colonization (Fig. 4).

	Estimate	Std. Error	t value	Pr(>|z|)
(Intercept)	251.210	288.038	0.872	0.386
Soil.Water	-2.953	6.575	-0.449	0.655
Perc.Clay	-2.484	2.917	-0.852	0.398
Perc.Sand	-2.499	2.894	-0.864	0.391
Perc.Silt	-2.489	2.884	-0.863	0.391

Table 3- Quasipoisson ANOVA table for the effect of soil characteristic on colonization.

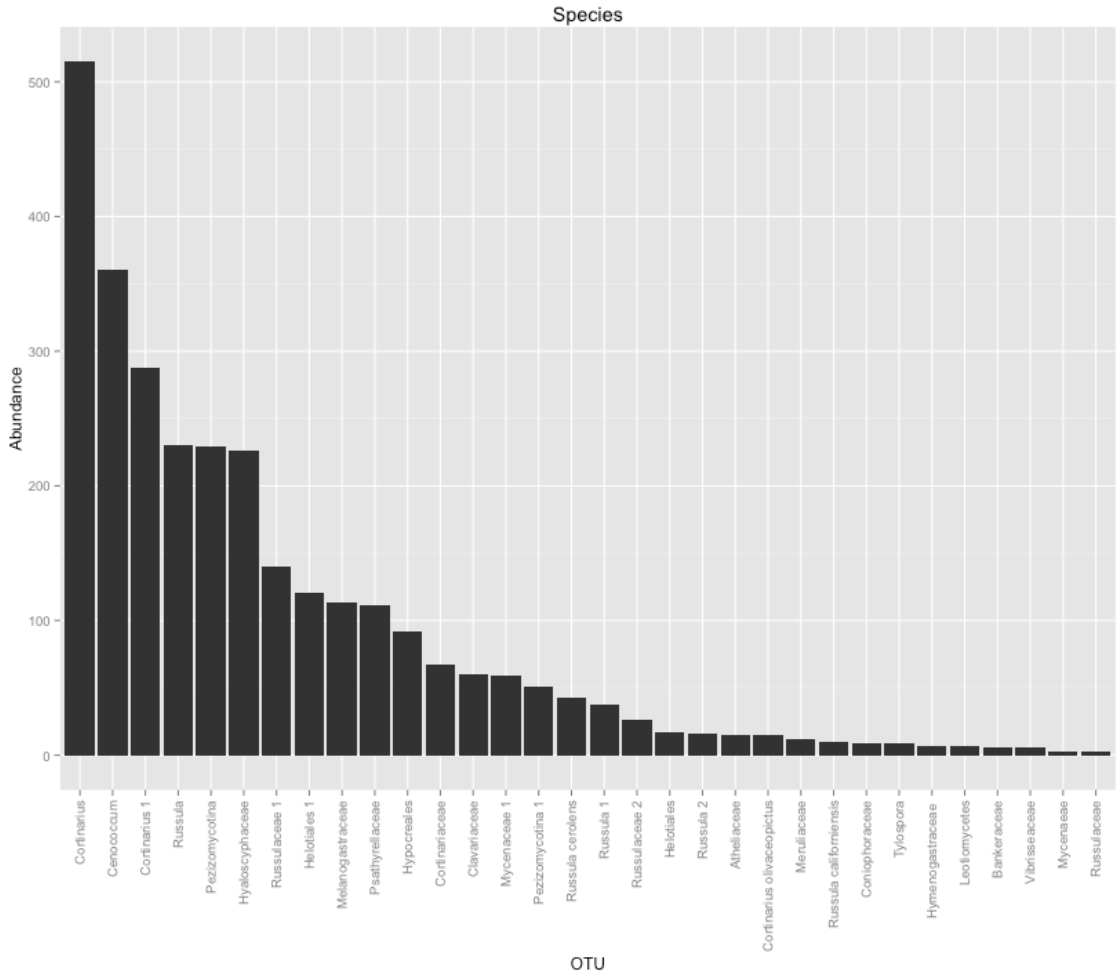


Figure 4- Abundance for each of the fungal OTUs.

A permanova utilizing Bray Curtis distance measures was used to examine fungal community relationships and to determine if communities were shaped by any of the environmental variables. Results showed the fungi did not significantly cluster based on the soil environment (Fig. 5, Table 4).

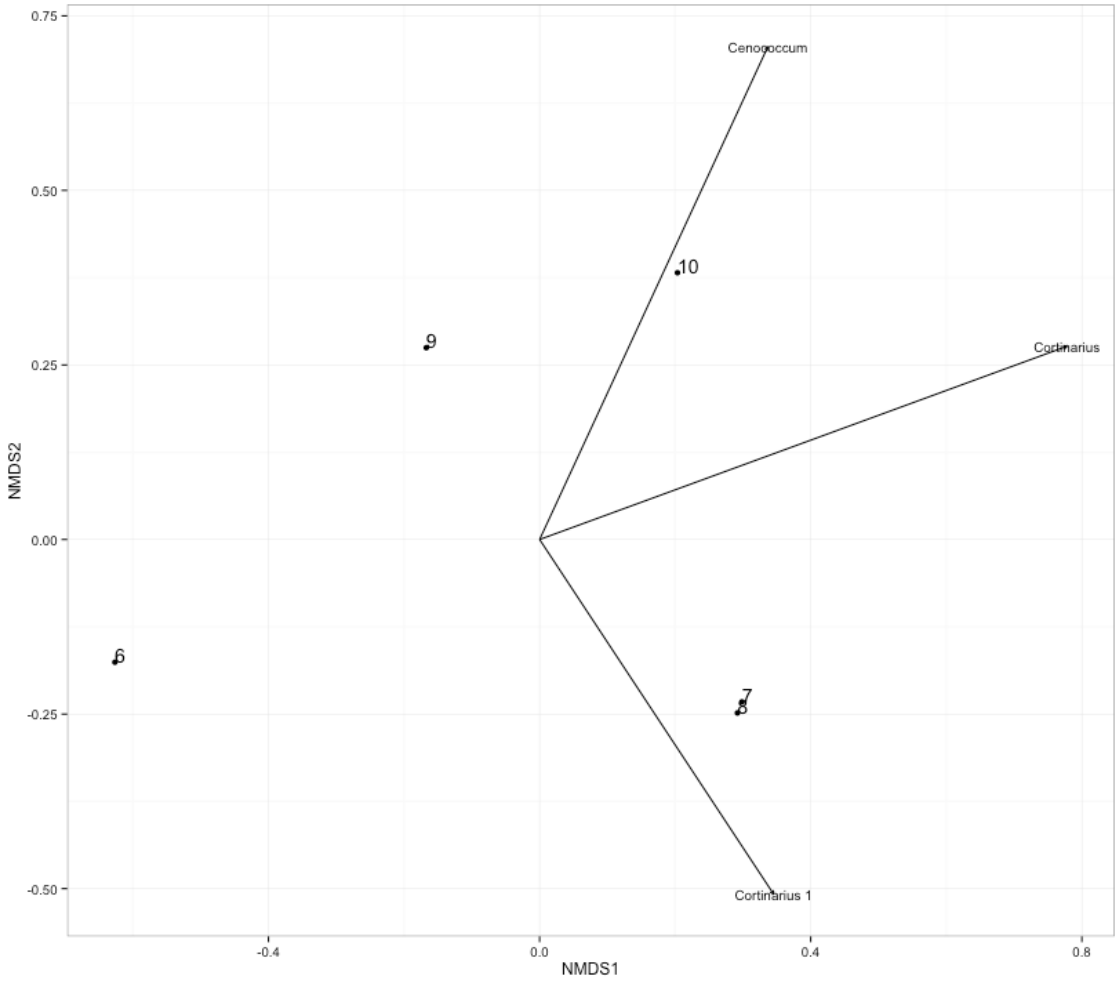


Figure 5- Non-metric multidimensional scaling ordination for each of the five trees and the three most abundant fungal OTUs.

	Df	SS	Mean SS	F	R2	Pr(>F)
Soil.Water	1	0.31082	0	0	0.20978	1
Perc.Clay	1	0.54950	1	0	0.37087	1
Perc.Sand	1	0.33906	0	0	0.22884	1
Perc.Silt	1	0.28227	0	0	0.19051	1
Residuals	0	0.00000	-Inf		0.00000	
Total	4	1.48165			1.00000	

Table 4- PERMAVOVA table for the effect of soil characteristics on fungal community structure.

An indicator species analysis was run to determine if any species of fungi was associated with a specific soil characteristic. No fungal species was more common with a particular soil characteristic.

Finally, the diversity of the fungal community was examined as a function of tree identity and soil characteristics. Simpson and Shannon Diversity Indices were created for each tree. Despite variation in the number of species present at each tree, Shannon and Simpson Diversity indicated there was no significant effect on the diversity of the fungal community due to tree ($F_{1,3}$=0.011, p=0.922-Shannon) ($F_{1,3}$=0.0882, p=0.7859-Simpson). Tree 7 had the greatest number of different fungal species and was followed, in decreasing order, by the communities of Trees 9, 10, 6 and 8. The number of species observed at each tree ranged from 16 species present in the soil of Tree 7 to 10 species observed at Tree 8 (Fig. 6).

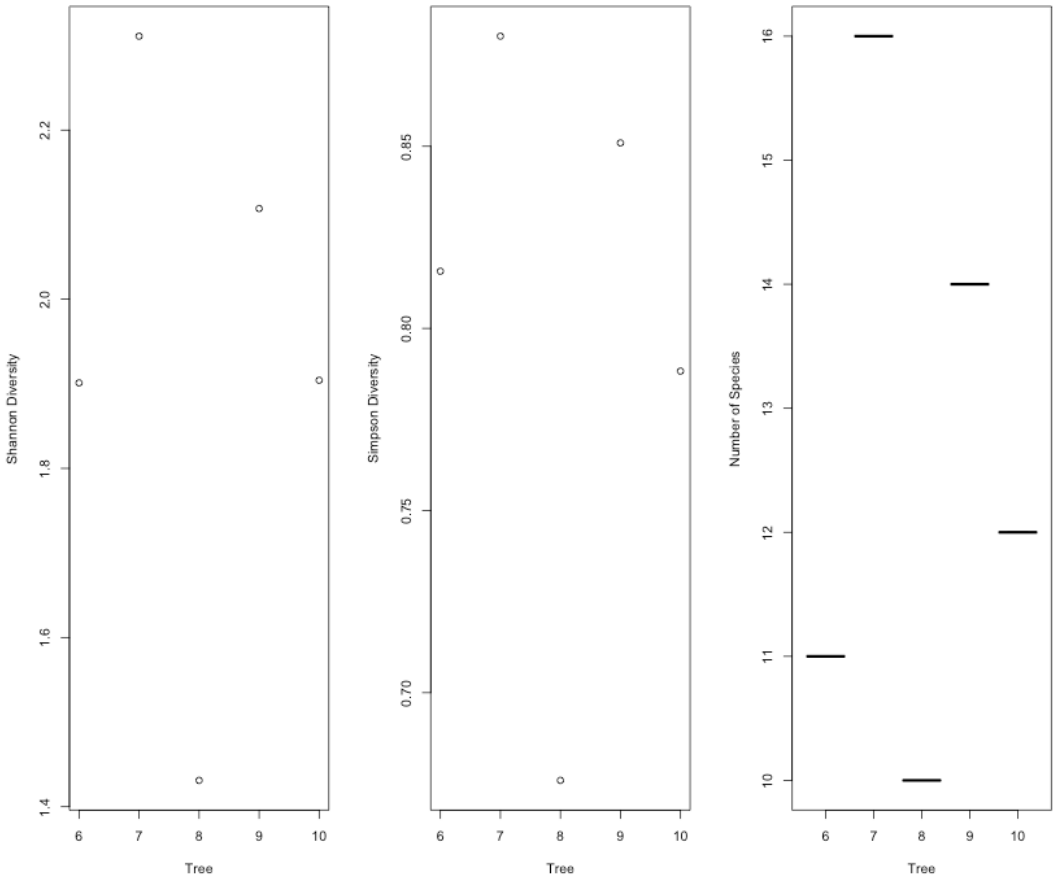


Figure 6- Number of fungal species, Shannon and Simpson Diversity indices for each tree.

Diversity analyses were also against soil characteristics. These analyses revealed that soil water content ($F_{1,3}$=0.438, p=0.556) and percent clay had no significant effect on fungal diversity ($F_{1,3}$=0.388, p=0.578) but percent sand and percent silt significantly influenced the diversity of the community. Percent sand exhibited a significant negative effect on fungal diversity ($F_{1,3}$=86.93, p=0.003), such that high soil percent sand had a lower fungal richness than low soil percent sand (Fig. 7). The results of sand soil composition were very different than those of silt. Increased percent silt exhibited a

positive effect on fungal diversity ($F_{1,3}$=35.81, p= 0.009). Specifically, fungal diversity increased with increasing percent silt (Fig. 8).

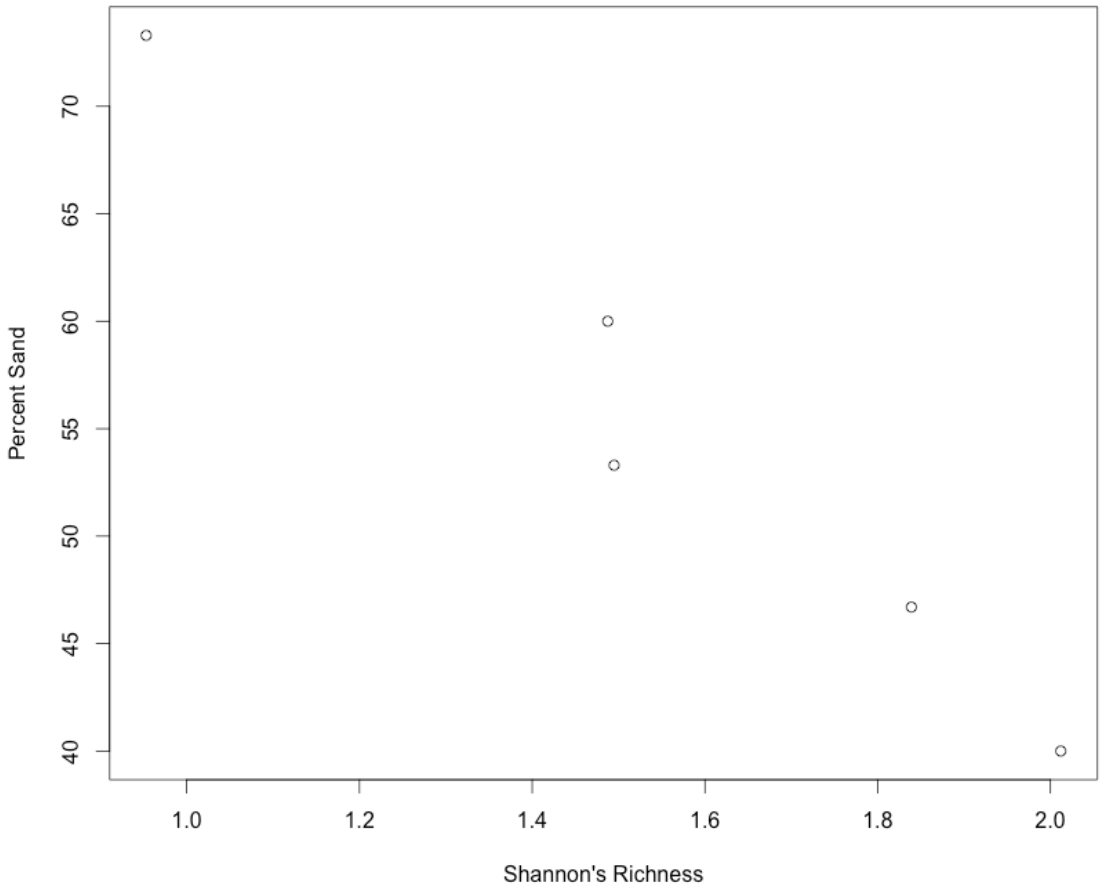


Figure 7- Relationship between percent sand and fungal diversity.

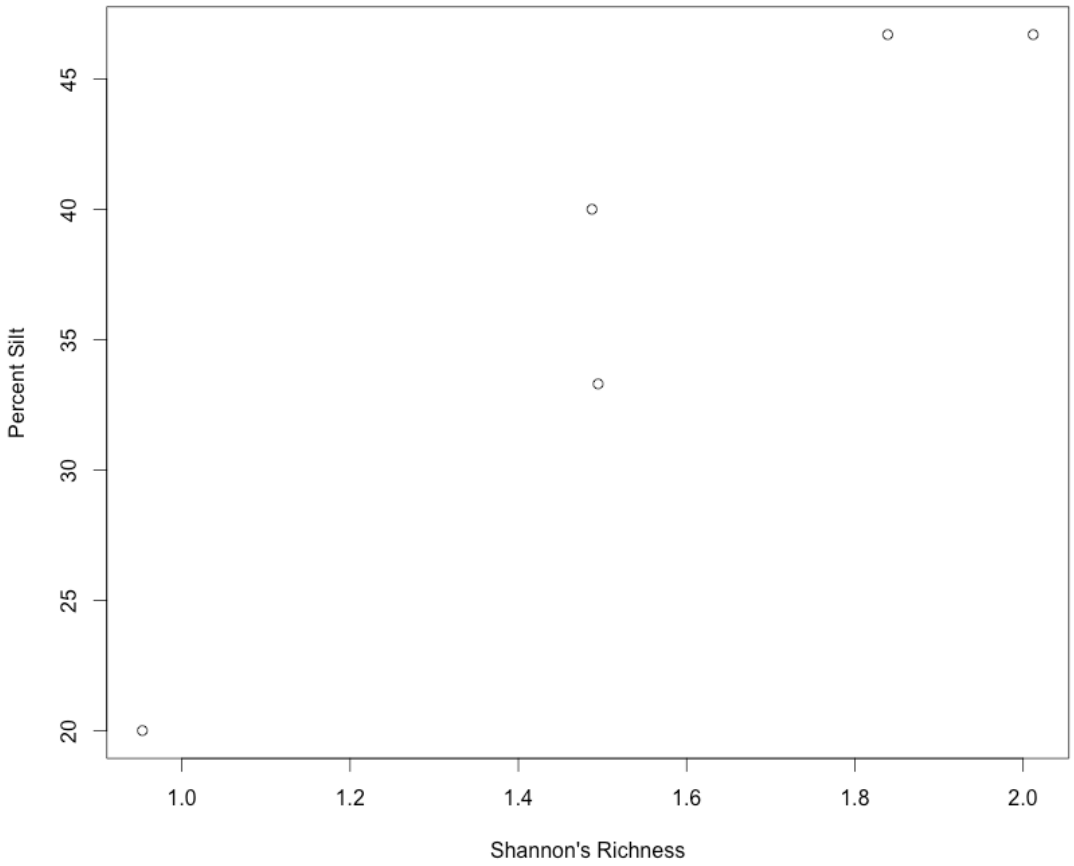


Figure 8- Relationship between percent silt and fungal diversity.

Discussion

Previous research studies have shown that both abiotic and biotic aspects of the soil, from erosion to soil mineral content, can alter the structure and composition of the ectomycorrhizal fungal community (Hartley, Cairney, & Meharg, 1997). My results echo these results. Specifically, I found that all soil characteristics (soil texture, percent sand, percent silt, percent clay and soil water content), significantly increased fungal abundance. However, none of the soil variables significantly affected colonization. I also found that no soil texture measure significantly shaped the fungal community. Finally, I discovered correlations between the texture of the soil and the diversity of the ECM community. While percent sand had a negative effect on fungal diversity, percent silt had a positive effect. Correlations between these environmental soil variables were also independently studied. Results showed a strong, negative correlation between soil percent sand and soil percent silt, as well as a weak, negative correlation between percent sand and percent clay.

Previous research on soil characteristics which influence the community structure of ECM fungi indicates these variables can be incredibly important. Examination has ranged from large scale studies on the impacts of latitude on fungal communities and their associations with host pines (Hoeksema & Thompson, 2007 and Glassman *et al*,. 2015) to small scale investigations of community differences along a specific environmental nutrient gradient (Gehring, Theimer, Whitham & Keim, 1998). The data from these investigations suggests that fungal distribution is strongly correlated with soil characteristics, which may be a result of differences between ECM species in their

effectiveness in obtaining and providing hosts with necessary nutrients (Toljander *et al*., 2006). Heterogeneous distributions of ECM fungi may also result because of the selectivity of ECM fungi for certain environments, with as little as 20% of ECM species being able to withstand a vast range of environmental conditions (Kranabetter, Durall, and MacKenzie, 2009). In studies which examined the effect of soil moisture on fungal distribution, decreasing soil moisture had a significant negative impact on the EM composition for most species, with the exception of a few drought-tolerant ECM fungal species such as *C. geophilum*, (Toljander *et al*., 2006). As a result of these findings, we hypothesized that soil texture, which impacts soil water, would affect the community structure; however, our Bray-Curtis permanova revealed no significant effect by any of the environmental variables on the ECM fungal community. One possible explanation for these findings is a lack of adequate sample sizes to detect soil texture's impact on the ECM distribution. Because the mycorhizae of many species are patchily dispersed underground (Horton & Bruns, 2001), it is possible that the four soil cores obtained from each tree failed to adequately detect shifts in the presence of certain fungal species due to the soil texture. Patchy fungal dispersion and limited soil cores may cause certain ECM species to be under, or even not, represented in the samples and may also be a reason that no indicator species was detected. Therefore, future studies seeking to link environmental characteristics with changes in ECM community composition should include more soil cores.

While soil characteristics did not shape ECM community composition, they did shape fungal diversity. Previous studies have demonstrated that soil horizon has a

significant effect on fungal diversity as a result of varying levels of available organic matter (Buée *et al.*, 2007). Based on this knowledge, we hypothesized that percent sand, percent silt and percent clay would each alter fungal diversity with percent sand decreasing diversity and percent silt and percent clay increasing fungal diversity. Although percent clay surprisingly did not impact diversity, the data showed that percent silt and percent sand did influence the diversity of the fungal community. Percent silt has a strong, positive correlation with diversity, likely as a result of the fine soil texture retaining higher nutrient concentrations (Clemmensen *et al*., 2006). Percent sand had the opposite affect and displayed a negative relationship with community diversity. Soil with a greater proportion of sand is more porous and thus retains lower levels of water and nutrients (Pachepsky *et al*., 2001). Therefore, the dry, sandy soil is a more harsh environment than soil with higher percentages of silt and clay, and fewer species of ECM fungi can adapt and survive. These results concerning the relationship between percent silt and percent sand with regard to the diversity of the fungal community reflect our earlier soil texture correlations which identified a strong, negative correlation between silt and sand.

Previous research also supports the idea that reductions in soil moisture decreases fungal richness (Tedersoo *et al*., 2012). Therefore, we hypothesized that an increase in soil water would allow for greater fungal diversity. Instead we found that there was no significant effect on fungal diversity due to soil water. This may be because soil texture had a more significant impact on fungal diversity than soil water. It is also possible that

all of the soil samples were relatively low water concentrations and thus a significant different due to soil moisture was not detected.

In pines, soil water content is an important predictor for shaping ECM fungal abundance. For example, soil moisture altered fungal abundance in *Pinus edulis*, such that plants with prolonged exposure (roughly a century) to drought incurred a 50% decrease in ECM abundance while plants experiencing temporary drought underwent a two-fold greater ECM colonization then those plants grown under ambient conditions (Swaty *et al*., 2004). Because ECM abundance will increase in an attempt to access greater quantities of water and compensate for drought, we correctly hypothesized that each of the soil characteristics, would have a significant positive impact on the abundance of fungal root tips in our system since it is likely water limited (Mann and Gleick, 2015). Indeed the abundance of fungal tips increased in soil samples with a higher percent sand, likely because sand has a lower water retention so increasing the number of colonized tips probably improves the symbiont's access to water (Walczak, Rovdan & Witkowska-Walczak, 2002). Furthermore, elevated levels of percent silt and clay significantly increased ECM fungal abundance, possibly because fine soil particles have greater nutrient capture and retention rates and thus provided a more nutrient rich environment which supported greater fungal abundance (Silver *et al*., 2000).

Unfortunately, each of these soil characteristics lost significance when ECM fungal abundance was studied as a function of root length (colonization). Similar to ECM fungi, pine root growth is positively correlated with the water and nutrient content of the local soil environment (Klos *et al.*, 2009). Consequently, although the fungal abundance

increased with each of the soil texture measurements, the root growth increased as well, and over all contributed more significantly to the fungal abundance than the soil textures. Thus, soil water is critical to ECM success in slightly drier environments, even if there are not associated changes in ECM fungal diversity.

I also found unique results with respect to the relationship soil characteristics have with one another. I found both percent clay and percent silt displayed a negative correlation with the percent sand found in the soil, possibly indicating differences in nutrient storage capabilities of the soil. This is supported by a previous study on soil texture which revealed that when the percent silt of one soil sample is equivalent to the percent clay of another, the two samples will have similar properties especially with regard to their nutrient storage (Hook and Burke, 2000). Numerous studies have also shown a negative correlation between the percent sand and soil water content of a soil core (Pachepsky et al., 2001). Thus, we expected a strong, negative correlation to exist between the percent sand and water of our samples, however, this correlation was not found. A possible reason this relationship was not detected is daily fluctuation in soil water content. As these cores were collected on one day, it is possible the water content of even the sandy soil on the day of collection was higher than its annual average.

My thesis indicates that soil texture plays a critical role in altering the abundance and diversity of the ECM fungal community in *P. radiata*. While these findings are important, more studies of the effects of the soil environment on fungi in *P. radiata,* and in other pines, are necessary to provide a more complete understanding of how soil variables shape the ECM fungal community. These microbial associations are frequently

overlooked due to their below ground location and unassuming physical appearance, but their role in enhancing the durability and productivity of forest ecosystems is critical. As ECM fungi are vital to the ability of ecosystems to withstand environmental changes a clear understanding of what impacts these fungal communities is critical, especially when seeking to understand the full impact of global climate change on terrestrial ecosystems.

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