SANDY SYMBIONTS: HOW TREE IDENTITY SHAPES
ECTOMYCORRHIZAL FUNGUS IN SAND PINE

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ABSTRACT

Sandy Symbionts: How Tree Identity Shapes Ectomycorrhizal Fungus in Sand Pine

The purpose of my thesis was to investigate the structure and composition of the ectomycorrhizal (EMC) fungal communities of sand pine (*Pinus clausa* var. *immuginata*). Root tips were sampled from four trees located within Eglin Air Force Base. Colonization was shown to differ significantly based on tree identity. Abundance, species diversity, and the ratio of ECM fungi to pathogenic fungi did not differ significantly based on tree identity. A total of 35 ECM operational taxonomic units (OTUs) were shown to colonize the four trees. Helotiales4, Lactarius2, and *Cenococcum geophilum* were found to be the most abundant OTUs, while Lactarius1 was found to be the least abundant. The results of my research suggest that difference in the ECM communities of sand pine is determined by variation in ECM fungal activity at the microscale of individual trees.

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Introduction

Nutrient cycling is a complex process critical to the health of an ecosystem. Certain nutrients, such as carbon, nitrogen, and phosphorus, are most commonly found in forms unusable by most terrestrial organisms. Producers, such as plants, are necessary for utilizing these untapped nutrient sources and converting them into forms useful for consumption by consumers, like animals. However, plants themselves can adapt in ways that increase their ability to maintain soil fertility and nutrient cycling (Hobbie, 2015).

In order to compensate for their inability to gather certain key nutrients, such as nitrogen and phosphorus, many plants have developed relationships with ECM fungi. In exchange for photosynthetically produced carbohydrates, ECM fungi supply their hosts with nitrogen, phosphorus, and other trace elements (Smith & Read, 2008). These fungi form symbiotic relationships with many of the trees that dominate the world's boreal, temperate, and tropical forests (Karst, Randall, Gehring, 2014). Certain plant species, such as pine trees (family Pinaceae), require ECM for normal growth and survival (Horton & Bruns, 2001). Yet despite the ubiquitous nature of ECM fungi, relatively little is actually known about the structure and composition of ECM communities.

ECM communities have shown impressive levels of species diversity in natural settings (Horton & Bruns, 2001). Previous studies have shown that the abundance of ECM species is inversely related to their rarity (Horton & Bruns, 2001). ECM fungi commonly show variation in their distribution at both microscales, such as individual tree

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colonized, and at large scales, such as entire geographical regions (Horton & Bruns, 2001). Research has also shown that soil cores taken centimeters apart can vary dramatically in terms of species presence and abundance (Horton & Bruns, 2001). Certain ECM species, such as *Tomentella sublilacina* and *Tylospora fibrillosa*, have been found to be present and numerically abundant across different regional pine forests (Horton & Bruns, 2001). However, the structure and composition of ECM communities may vary based on environmental conditions. For example, ECM fungi tend to show lower rates of colonization in soils disturbed by deforestation than in natural forest soil (Ding *et al.*, 2011). Other changes in environmental conditions, such as drought and fire, have also been shown to alter the composition of some ECM communities, but the degree to which the community is altered often depends on the severity of the stress felt by the host tree (Karst, Randall, Gehring, 2014).

Pines account for a major portion of forests found throughout the northern hemisphere, and have flourished throughout the southern hemisphere since their introduction (Karst, Randall, Gehring, 2014). Pines are commonly used as a source of timber for construction and wood based products, but they are also useful in restoring and maintaining habitats (Reverchon *et al.*, 2012). Despite their widespread species range, not all pines share identical ECM fungal communities. Coastal and montane pine forests in California are dominated by *T. sublilacina* in terms of presence and abundance, while pine forests in northern Europe show dominance by *T. fibrillosa* (Horton & Bruns, 2001). The number of species that compose a pine's ECM fungal community can vary dramatically based on host species and age (Horton & Bruns, 2001). Both adults and

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seedlings of *Pinus montezumae* contain a diverse ECM fungal community composed of members from the Russulaceae, Atheliaceae, Sebacinaceae, and Thelephoraceae families, but only a few species from these families colonized both adults and seedlings (Reverchon *et al.*, 2012). *Abies* and *Tsuga*, two genera of pine species found in Japan, show predominant colonization by members from the Cortinariaceae and Boletaceae families (Ishida, Nara, Hogetsu, 2006). The ECM fungal species *Thelephora terrestris* and *Rhizopogon vulgaris* have been shown to colonize *Pinus contorta* var. *latifola* seedlings in greenhouse conditions (Karst, Jones, Turkington, 2008). ECM communities can also vary in pines based on how closely together the host trees are located. Individual members of the species *Pinus cembroides*, which typically show isolation between trees, were found to have distinct ECM communities dominated by different ECM species (Horton & Bruns, 2001). ECM communities in pine can also vary based on environmental disturbances such as drought or fire, but the degree of impact varies between pine species (Karst, Randall, Gehring, 2014). Deforestation has also been shown to reduce the rate of colonization by ECM fungi (Ding *et al.*, 2011).

Plants also form relationships with pathogenic fungal species. Interactions between plant species and their pathogens are thought to be regulated by many of the same variables that influence ECM communities, such as environmental conditions, stand age, and host tree species. Certain pathogenic fungi, such as *Phytophthora cinnamomi*, are commonly found infecting young trees in artificially planted stands of *Pinus clausa* but are not present in natural stands of the same species (Barnard *et al.*, 1995). Other pathogen species, such as *Inonotus circinatus* and *Phaeolus schweinitzii,* have been

observed infecting mature *P. clausa* (Barnard *et al.*, 1995). The structure of a stand also influences the pathogenic fungi community. A high diversity in the local tree population reduces the richness and infestation of pathogenic fungi that infect the leaves of *Quercus petraea* and *Tilia cordata* (Hantsch *et al.*, 2014). Pathogenic fungi also show a preference for specific host species. Although *Cronartium fusiforme* has been shown to infect a wide variety of southern pine species, slash pine (*Pinus elliottii*) and loblolly pine (*Pinus taeda*) are far more susceptible to infection than longleaf pine (*Pinus palustrius*) or shortleaf pine (*Pinus echinata*), but shortleaf pine is susceptible to a similar pathogenic fungi, *Cronartium quercuum* (Phelps & Czabator 1978).

     P. clausa [(Chapm. ex Engelm.) Vasey ex Sarg.], commonly known as sand pine, occurs throughout Florida and represents a unique system for studying the relationship between pines and their belowground fungi. Sand pine typically inhabits sandy and nutrient-poor habitats (Myers, 1990), and shows extensive ECM communities (Dumbroff, 1968). There are two varieties of sand pine; var. *clausa* occupies the Florida peninsula and var. *immuginata* is found predominantly in the Florida panhandle (Johnson *et al.*, 1997; Parker *et al.*, 2001b).

     Understanding the structure and composition of belowground fungi that associate with sand pine is particularly important because of its role in the Florida scrubland ecosystems (Myers 1990). These two varieties show no significant difference in belowground pathogenic fungal communities (Barnard *et. al.*, 1985), but there is a lack of knowledge regarding what impact variety has on ECM communities. Both of these varieties have experienced significant reduction in habitat range due to anthropogenic

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activities which have removed much of the coastal ecosystems for various purposes such as commercialization and timber (Christman & Judd, 1990).

In order to expand the understanding of the belowground fungal community for sand pine, I sampled four sand pine trees of var. *immuginata* located on Eglin Air Force Base in order to investigate three main questions. First, what is the composition of the ECM fungal community? Second, does the ECM fungal community vary between trees? Third, how does the pathogenic fungal community compare to the ECM community?

Methods

The fungal samples were extracted from 16 soil cores taken from Eglin Air Force

Base on May 28, 2014. Eglin Air Force Base is located in the western portion of the

Florida panhandle, and like much of coastal Florida, the soil present in each core was

mostly sand. The trees themselves are located on what was previously a bombing range.

Four soil cores (2.5 mm diameter, 15 mm deep) were taken from each tree, one in each

cardinal direction. Although data from only four trees is present, they are labeled 11, 12,

13, and 14 because this data represents a portion of a much larger study performed by Dr.

Rúa. The GPS coordinates and diameters of the trees are found in Table 1. Soil cores

Table 1: Tree Location and Diameter

Tree ID	Latitude	Longitude	Tree Diameter (cm)
11	N 30º29.510'	W 086°23.615'	79
12	N 30°29.500'	W 086°23.619'	73
13	N 30°29.500'	W 086°23.611'	98
14	N 30°29.502'	W 086°23.629'	68

were individually stored and kept on ice in insulating coolers until they were brought

back to the lab, and then stored at 4ºC to be processed within 21 days of collection.

Each core was individually sieved and hand rinsed with tap water to separate any

soil and other small particulates from the root tips, which were then placed in a Petri dish

with a small layer of water to facilitate separation. The root tips were then assessed for

fungal colonization by Dr. Rúa using a dissecting microscope. Any fungal matter found was morphotyped based on its shape, texture, color, and branching patterns as well as emanating hyphae or rhizomorphs. Two root tips of each observed morphotype were removed for identification via Polymerase Chain Reaction (PCR) and Sanger sequencing. DNA extraction was performed under the hood by Dr. Rúa on each fungal sample immediately following morphotyping. To extract DNA, each sample was placed in a separate well of a 96-well plate and the sample locations within the plate were recorded. Ten uL of Sigma Extraction Buffer was added to each sample, after which the plate was covered using a sterile silicon sealing mat and briefly centrifuged to thoroughly expose the sample to the buffer. The plate was then heated using the Techne Genius Thermocycler. The plate was heated first at 65°C for 10 minutes, then 95°C for 10 minutes. The plate was then removed from the thermocycler, and 30uL of Sigma Neutralization Solution was added to each well. Each well then received 160uL of PCR-grade water to dilute the extract to 20% concentration. The plate was then covered using adhesive aluminum, labeled, and stored at -20°C for approximately two months.

PCR was performed on the samples in late August 2014. All of the following steps for PCR were performed in a Biosafety II cabinet. Each well received: 2.2uL of PCR-grade water, 4uL of 2X RedTaq Premix (Apex, Inc.), and 04.uL of each primer (10 μM stock concentration). The primers used for this study were ITS1F and ITS4, which amplify a fungal-specific 800 base pair (bp) internal transcribed spacer (ITS) region (Gardes & Bruns, 1993). One uL of DNA extract was then added directly to each well. The plate was then sealed using a sterile silicon sealing mat and briefly vortexed and

centrifuged before being placed in the Applied Biosystems Thermocycler. Thermocycling

was performed following the steps listed in Table 2.

Table 2: PCR Steps and Conditions

Step	Temperature (^oC)	Duration	Number of Cycles
Initial Denaturation	94	3 minutes	1
Denaturation	94	45 seconds	35
Annealing	52	45 seconds	
Extension	72	72 seconds	
Final Extension	72	10 minutes	1

Samples were run on a 1% agarose gel with SYBR® Safe DNA gel stain

(Molecular Probes, Eugene, OR, USA) to determine which were successfully amplified

using PCR. Three uL of a ladder was then loaded into the first and last well of the gel,

followed by 2.5uL of PCR product into each individual well. The gel was exposed to

approximately 200 volts for 18 minutes. At the end of this time period, the gel was

analyzed using Trans UV, shown in Figure 1. Any samples that did not amplify

successfully were recorded and subjected to another round of PCR at a later date, while

samples that were amplified successfully were subjected to a 'clean up' process using

Exo-SAP (USB Corporation Cleveland, OH, USA). A mastermix was prepared under the

hood on an ice block using the following formula: 4uL of cold, PCR-grade water, 0.5uL

of ExoI (2U/uL), and 0.5uL SAP (1U/uL) per well. After mixing the appropriate amount,

5uL of mastermix was added to each well on a new plate followed by 5uL of PCR-

product. The plate was then sealed using a sterile silicon rubber mat, briefly vortexed and centrifuged, and placed in the Eppendorf Thermocycler for: 30 minutes at 37°C, 20 minutes at 80°C, and at least 5 minutes at 4°C. The plate was resealed using adhesive aluminum, labeled, and stored at -20°C for approximately one week.

Sequencing was performed using the forward primer ITS-5 (Gardes & Bruns, 1993) and the Big Dye Terminator Sequencing Kit (v3.1, Invitrogen Corp.). Each reaction required: 0.4uL of Big Dye Reaction Premix, 1.8uL of 5X Sequencing Buffer, 0.5uL of ITS5 primer (10uM), and 6.3uL of PCR-grade water. 9uL of mastermix and 1uL of cleaned PCR product was added to each well. After sealing with a sterile silicon rubber mat, briefly vortexing and centrifuging, the plate was placed in the Applied Biosystems Thermocycler and subjected to the steps listed in Table 3. Once the sequencing reaction finished, the plate was uncovered and placed in the vacufuge for 30 minutes at 45°C. After the reactions were dried, the plates were sealed and shipped overnight to Arizona State University's DNA Lab in Tempe, Arizona, where the Big Dye reactions were purified and read using an Applied Biosystems 3730 capillary genetic analyzer.

Table 3: Sequencing Reaction Steps

Step	Temperature (^{o}C)	Time	Number of Cycles
Initial Denaturation	96	1 minute	1
Denaturation	95	20 seconds	45
Annealing	52	20 seconds	
Extension	60	4 minutes	
Hold	4	10 minutes	1

Once the sequencing reaction finished, the plate was uncovered and placed in the vacufuge for 30 minutes at 45°C. After the reactions were dried, the plates were sealed and shipped overnight to Arizona State University's DNA Lab in Tempe, Arizona, where the Big Dye reactions were purified and read using an Applied Biosystems 3730 capillary genetic analyzer.

Once the raw sequences returned from ASU, they were visually processed using version 8.0.4 of the Geneious software (Biomatter Ltd). Sequences were visually analyzed for ambiguities within the nucleotide sequence, which were then replaced with the most probable nucleotides if possible. DNA sequences that had an ambiguity less than 3% and a length of at least 200 nucleotides were then subjected to operational taxonomic unit (OTU) assembly (at 97% similarity) using CAP3 software (Huang, 1999) on the University of Alaska, Fairbanks (UAF) informatics server, 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) database to obtain best matches

for taxonomic affiliation of OTUs. This process matched each morphotype with its most

similar identified organism. However, not all OTUs could be matched to an individual

species. OTUs that matched at 97% or greater were named appropriate to the species

level, 95-97% were named appropriate to the genus level, 92-95% were named

appropriate to the family level, and 90-92% were named appropriate to the order level.

After each morphotype was identified to its appropriate taxonomic level, they were then

classified based on whether or not the fungus was mycorrhizal or pathogenic.

   I used a number of metric to examine fungal community composition and identity.

      R (3.1.2) was used to analyze the raw data and determine if there were any

significant relationships present (R Core Team, 2015). To determine whether variation in

colonization was a function of tree identity, colonization was subjected to a linear model

using the *lm* function from the *nlme* package (Pinheiro *et. al.*, 2015). To determine

whether variation in ECM abundance was a function of tree identity, ECM abundance

was subjected to a generalized linear model with the poisson distribution created using

the *glm* function from the *stats* package (R Core Team, 2015). To assess the sources of

variation in the mycorrhizal community matrix due to tree identity, I used permutational

manova based on 1000 permutations and the Bray-Curtis method for calculating

dissimilarity indices via the *adonis* function in the *vegan* package (Oksanen *et. al.,* 2015).

Non-metric multidimensional scaling (NMDS) was used to visualize these results.

Indicator species analysis was performed to determine if any ECM species (OTUs) were

significantly associated with a tree using the *multipatt* function from the *indicspecies* package and 999 permutations (De Caceres & Legendre, 2009). Species richness was computed for each tree using the *diversity* function of the *vegan* package (Oksanen *et. al.,* 2015), and then subjected to a linear model using the *lm* function to determine whether variation in species richness is a function of tree identity. Binary analysis was used to determine if the proportion of ECM fungi and pathogenic fungi differed between each tree using the *glm* function from the *stats* package and the family binomial (De Caceres & Legendre, 2009).

*Gel Electrophoresis of PCR Products*

Trans UV analysis following gel electrophoresis showed consistent amplification by PCR (Figure 1).
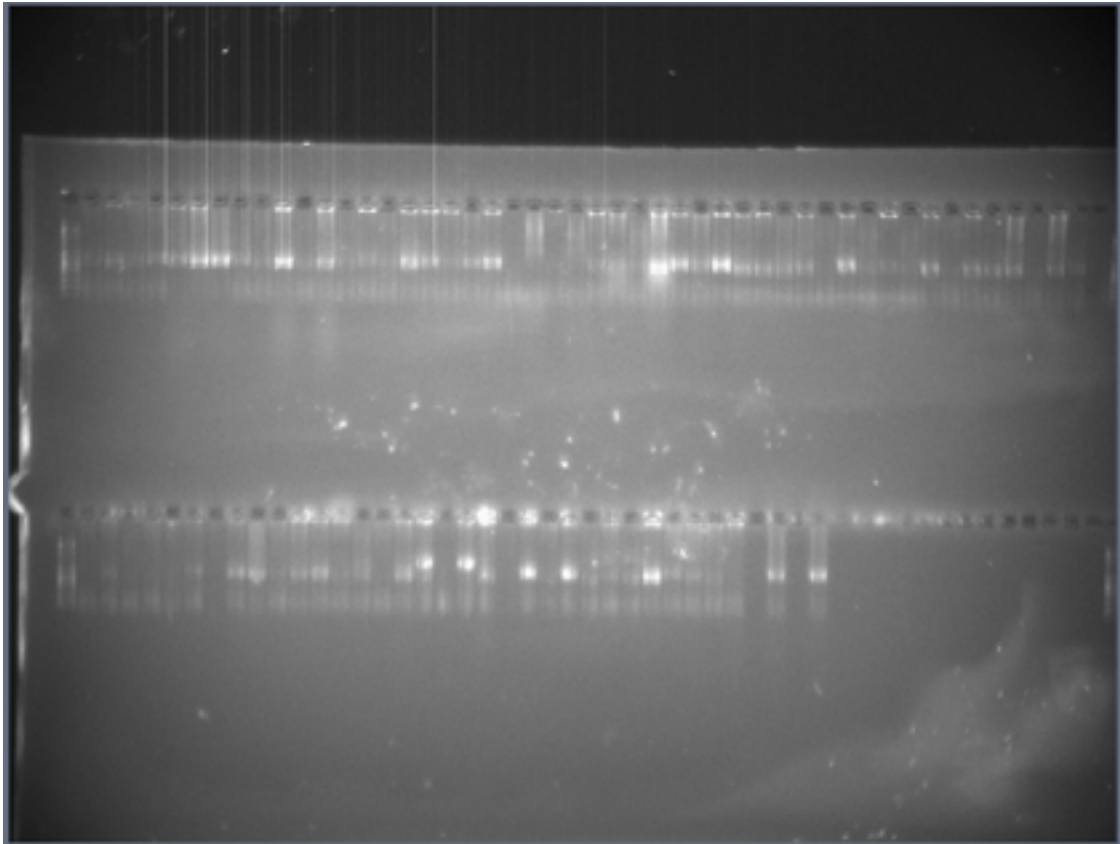
Figure 1: Trans UV Analysis of Gel Electrophoresis. A ladder was added to the first and last column of each row to measure sequence size (bp) by distance traveled.

ECM Community Composition

The ECM community contained a small number of highly abundant OTUs, a large number of moderately abundant OTUs, and a small number of rare OTUs (Figure 2). The three most prevalent OTUs were Helotiales4 with 324 colonized root tips, Lactarius2 with 270, and *Cenococcum geophilum* with 243. The least abundant OTU was Lactarius1, with only 3 colonized root tips. The total OTU abundance did not differ significantly by tree identity (dF=1,55, p=0.347).
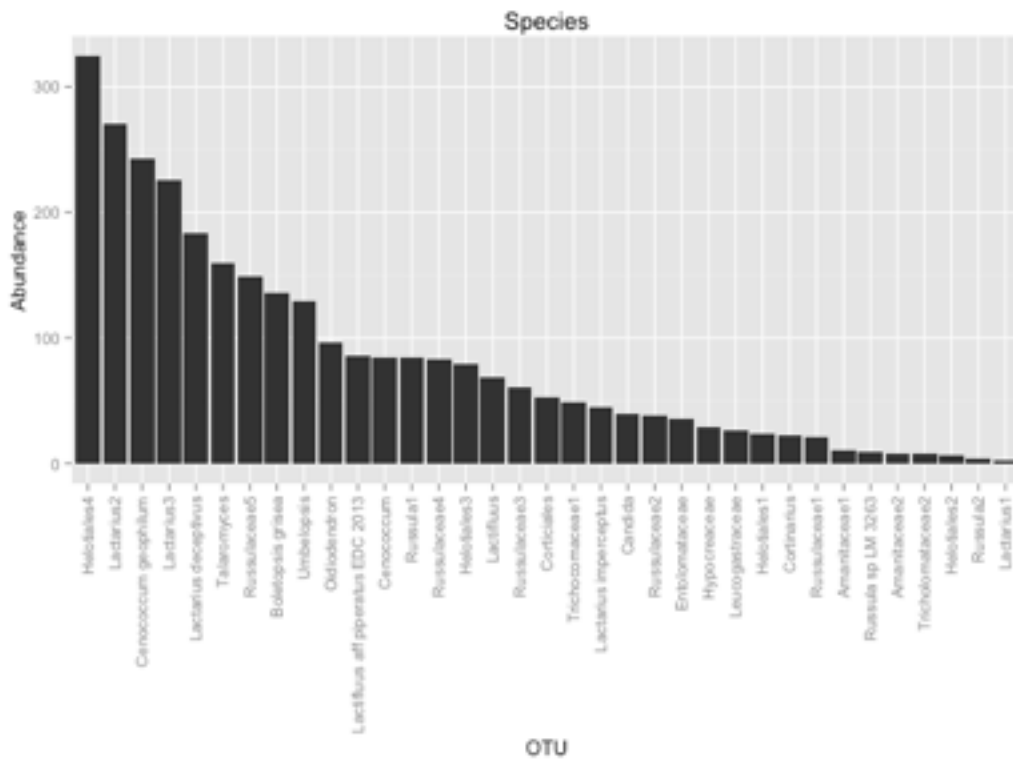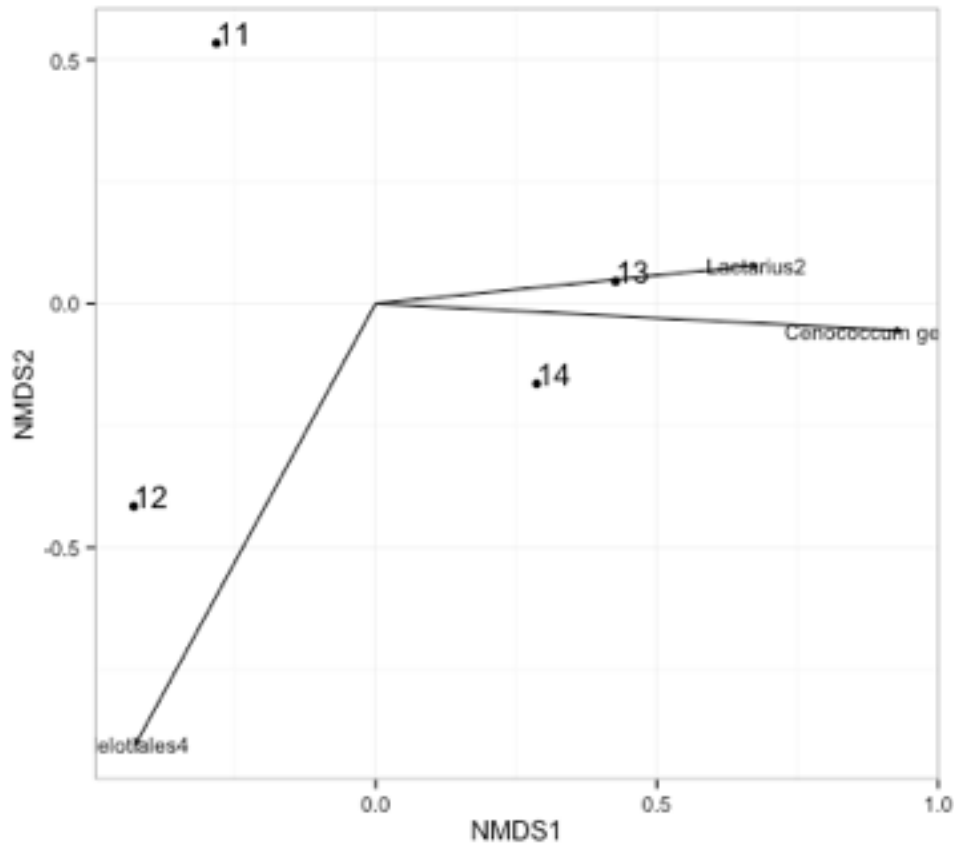

Figure 2: Relative Abundance by OTU. Abundance is quantified as the total number of root tips, shown on the y-axis, colonized by each OTU, shown on the x-axis.

Although abundance did not differ between trees, colonization was found to differ significantly between each tree ($F_{1,55}=4.093$, $p=0.0479$). Tree 11 showed the lowest amount of colonization while Trees 12 and 13 showed approximately equal amounts of colonization (Figure 3). Tree 14 had a colonization rate between Tree 11, and Trees 12 and 13.
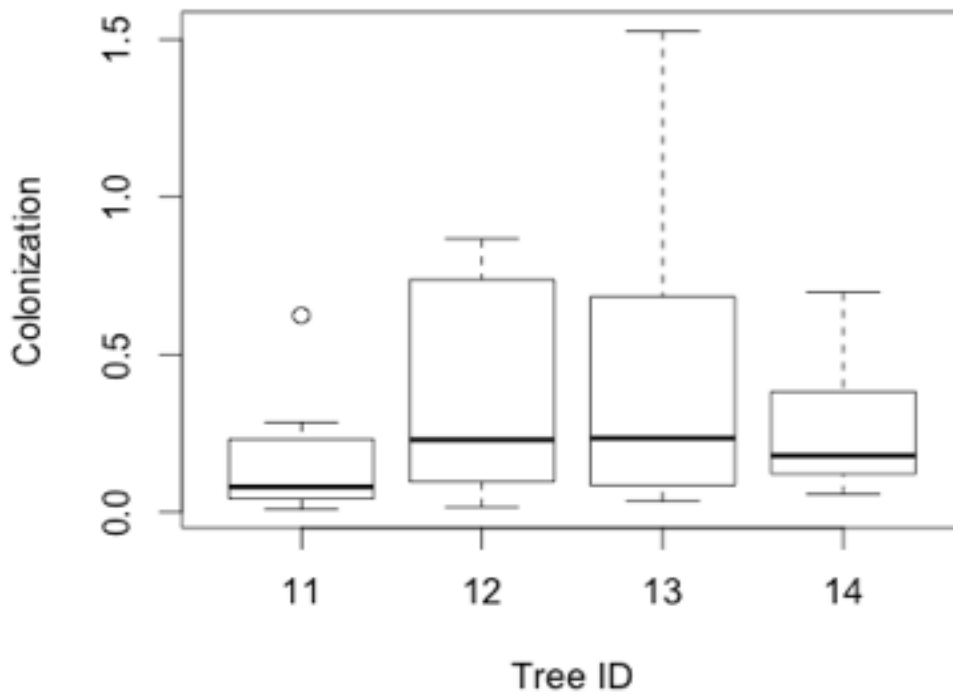
Figure 3: Colonization Rate by Tree. Tree ID is shown on the x-axis. Colonization, shown on the y-axis, factors out any influence that the size of the tree may have had on abundance by dividing the amount of root tips (abundance) by the total length of the root. This provides a ratio for the number of colonized root tips per millimeter of root length, which can be compared between each tree.

While Trees 13 and 14 shared the greatest degree of similarity in ECM community composition, there was found to be no significant difference in the overall composition of each tree's ECM community ($F_{1,2}$=1.3498, p=0.25, Figure 4). Analysis of indicator species also showed no variation in ECM community composition.


Figure 4: NMDS of OTU Abundance by Tree Identity. Each dot represents one of the four trees, with their location on the graph relating to the similarity of their ECM communities. The lines show where each of the three most abundant OTUs were found, with close proximity showing high abundance at the corresponding tree.

Species Richness

There was found to be no significant difference in the richness of the ECM fungal community based on tree identity according to both the Simpson Species Diversity Index ($F_{1,2}$=2.018, p=0.2913, Figure 5) and the Shannon Species Diversity Index ($F_{1,2}$=2.123, p=0.2824, Figure 6). Tree 13 showed a slightly higher value for both indices than the other trees, while Tree 11 showed a slightly lower value.
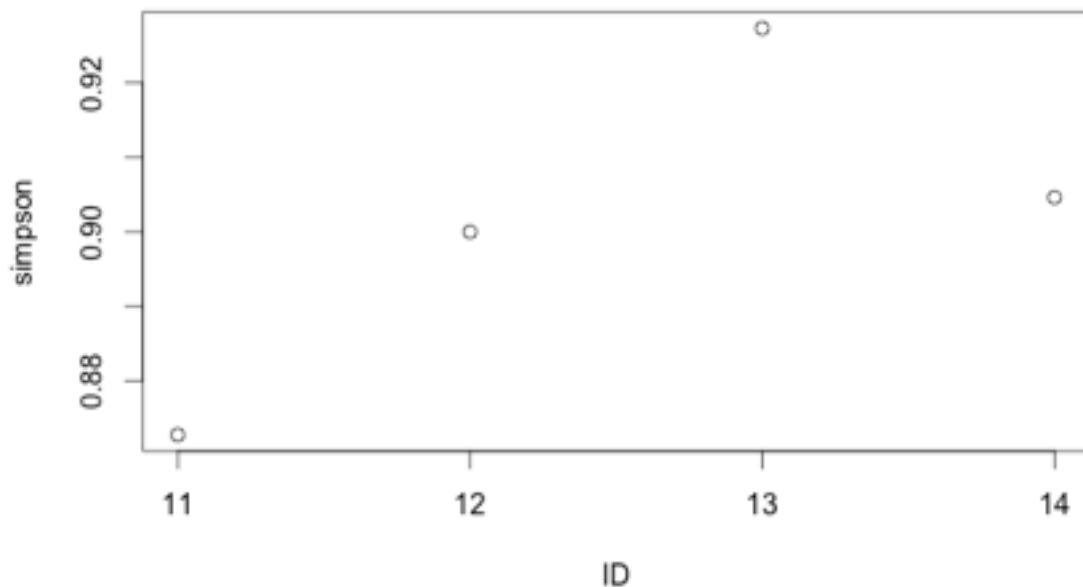
Figure 5: Simpson Species Diversity Score by Tree. The y-axis shows the range of Simpson scores found, but it does not represent a continuous axis. This produced the illusion that species diversity varied based on Tree Identity, shown on the x-axis.
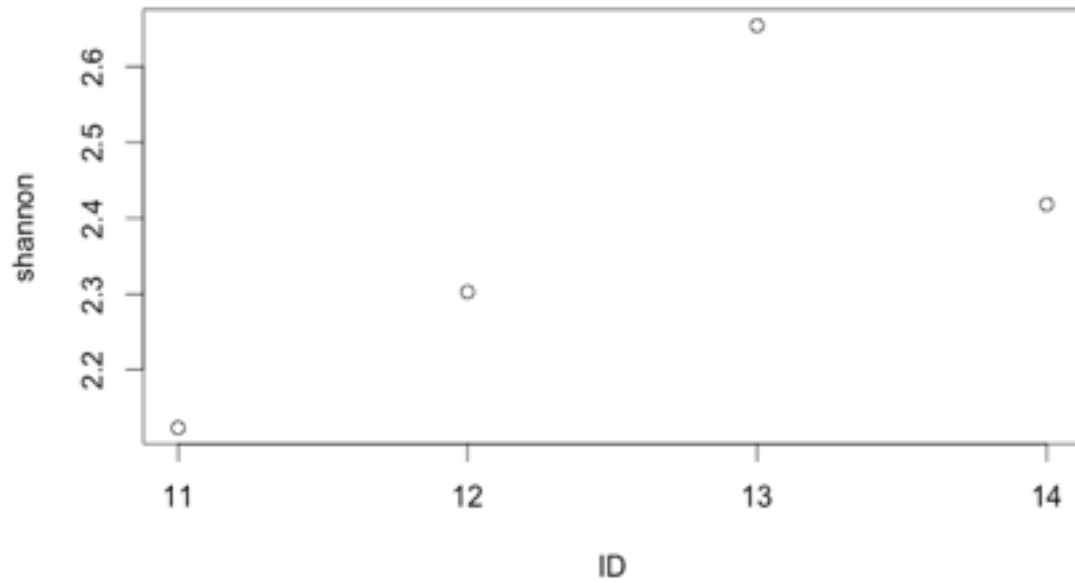
Figure 6: Shannon Species Diversity Score by Tree. The y-axis shows the range of Shannon scores found, but it does not represent a continuous axis. This produced the illusion that species diversity varied based on Tree Identity, shown on the x-axis.

Pathogen Abundance

Only Tree 11 had a larger abundance of pathogenic fungi than ECM, however, there was no significant difference between the abundance of ECM fungi and the abundance of pathogenic fungi based on tree identity (dF=87, z= 0.849, p= 0.396, Figure 7).
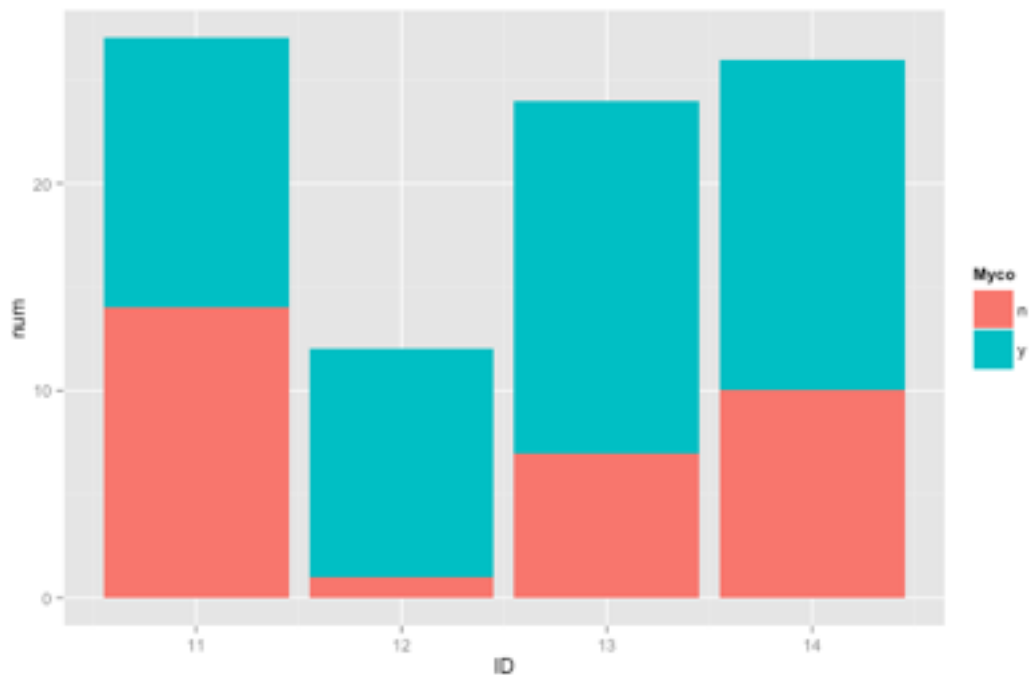
Figure 7: Binary Analysis Between ECM and Pathogens. The y-axis refers to the number of colonized root tips while x-axis illustrates tree ID. The proportion of ECM per tree is shown in teal, while the pathogenic fungi are shown in salmon. Total bar height represents total number of colonized tips per tree.

Discussion

For my thesis I investigated the belowground fungal community found near sand pine. Specifically, I investigated the composition of the ECM community and how that community changed between individual trees. I also investigated how the pathogenic fungal community compared to the ECM community. I found that while tree identity significantly influences the rate of colonization by ECM fungi, tree identity produced no significant difference in the abundance of colonized root tips, species diversity, or the relative abundance of pathogenic fungi in the overall fungal community.

The relationship between pines and their belowground fungal community is a field of ever expanding research (Karst, Randall, Gehring, 2014), and the results from my thesis have helped to contribute to this body of knowledge. In my study, the three most common OTUs were Helotiales4 with 324 colonized root tips, Lactarius2 with 270 colonized root tips, and *Cenococcum geophilum* with 243 colonized root tips; however, these three OTUs did not necessarily colonize the same trees. Interestingly, another OTU from the same genus, Lactarius1, was the least common OTU with only 3 colonized tips. Members of the Lactarius genus and the species *C. geophilum* have both been found as prominent members the ECM community of other pines such as *P. montezumae* (Reverchon *et al.*, 2012) and genera *Abies* and *Tsuga* (Ishida, Nara, Hogetsu, 2006). In *P. montezuma*, *C. geophilum* only colonized the roots of seedlings, but the results from my research have shown that it can also colonize adult sand pine. Other studies have shown

that *C. geophilum* is a key member of the ECM community of *Pinus armandii* in southern

China (Ding *et. al.*, 2011). *C. geophilum* has been observed in almost all studies

regarding the ECM community of pines (Horton & Bruns, 2001), possibly as a result of

its ability to withstand drought (Karst, Randall, Gehring, 2014). The ECM fungal species

T. terrestris, which commonly colonizes seedlings of *Pinus sylvestris* (Menkis & Vasaitis,

2010) as well as seedlings of *P. contorta* var. *latifola* (Karst, Jones, Turkington, 2008),

was not found to be a significant member of the ECM communities of my trees. Finally,

the most abundant OTU in my study, Helotiales4, was not found to be a significant

member of the ECM communities of other pine species (Ishida, Nara, Hogetsu, 2006;

Reverchon, 2012; Karst, Jones, Turkington, 2008; Ding *et. al.* 2011), but this may

because I could only resolve its identity to order.

Despite the differences in abundances of these three common species, no

significant difference was found in the abundance or diversity between each tree's ECM

community. This is most likely a result of low environmental variation and a small

sample size. Similar results are shown in studies using similar sample sizes (Ishida, Nara,

Hogetsu, 2006), which sampled five trees from each of eight host species, and in the

process found that ECM communities differed significantly between codominant tree

species. Increasing sample size in terms of number of samples collected from a particular

tree and increasing the sample area around each tree has been shown to increase the

number of distinct ECM present (Horton & Bruns 2001). Several studies have shown that

the most abundant ECM fungi by biomass are often found in one or two soil cores, and

soil cores taken a few centimeters apart may radically alter results in terms of species

presence and abundance (Horton & Bruns 2001). These results are supported by other

studies showing that the most abundant ECM fungal species are often found in small,

concentrated patches and may vary temporally (Horton & Bruns 2001). For example, the

ECM fungal composition and abundance of soil cores taken one year apart were

significantly different from those of soil cores taken at the same time the following year,

although the two cores were taken 25 cm apart (Horton & Bruns 2001). Thus, increasing

both the number of soil cores and the sample region allows researchers to determine

patterns in the widespread distribution of ECM fungal species (Horton & Bruns 2001).

Furthermore, geographic isolation can also stimulate tree identity differences in the ECM

fungal community. In stands composed of isolated *P. cembroides*, individual trees were

found to have an ECM community dominated by a single ECM species, which may be a

result of limited spore dispersal or by the scattered nature of *P. cembroides* distribution

(Horton & Bruns 2001). The results from the previously mentioned study (Horton &

Bruns 2001) and others like it (Ding *et al.*, 2011; Karst, Randall, Gehring, 2014;

Reverchon *et al.*, 2012; Menkis & Vasaitis 2010) show that ECM communities can differ

significantly based on environmental conditions, geographic location, and host species.

Although I did not find any significant differences in the structure and richness of

the ECM communities based on host tree identity, the rate of colonization, which

provided a measure of ECM abundance as a function of tree size by accounting for root

length, was found to differ significantly based on tree identity. This result is seen in other

studies as well. In nursery studies of *P. sylvestris*, the rates of colonization by ECM fungi

can vary dramatically between different trees of the same species (Menkis & Vasaitis,

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2010). However, results from nursery studies in *P. contorta* var. *latifola* show no significant difference in the rates of colonization (Karst, Jones, Turkington, 2008). These differences in colonization rates, despite using a single host species, suggest variation in the rates of ECM fungal activity at the microscale (individual trees).

Pathogenic fungal species represented a surprisingly large portion of the total ECM community in my samples, but the relative number of tips colonized by pathogens did not differ significantly based on tree identity. Prior studies of both varieties of sand pine have shown that patterns of pathogenic fungal infection do not significantly vary between host variety, but instead based on stand type and age (Barnard *et al*., 1995). While my trees showed colonization by OTUs matched to pathogenic fungal genera such as *Fusarium* and *Penicillium*, past studies have shown that species such as *P. cinnamomi* and *I. circinatus* are also major pathogens of sand pine (Barnard *et al*., 1995). Based on results from a study in non-pine species, the amount of local tree diversity negatively impacts the richness of fungal foliage pathogens (Hantsch *et al*., 2014). As a comparison, seedling mortality due to fungal infection in *Sebastiana longicuspis*, a common tropical tree, was three times higher in low diversity plots than in high diversity plots (Bell, Freckleton, Lewis, 2006). The results of these two studies suggest that a large diversity in local tree species reduces the viability of many pathogenic fungi, as these species tend to specialize in infecting one particular species (Prell, 1996).

Although there was little variation in belowground fungi found between my four trees, this data is still valuable. The structures of belowground fungal communities have not been extensively studied in many tree species, such as sand pine. This data serves as a

starting for understanding sand pine fungal communities. Sand pine is an important

member of the natural Florida scrub habitat, and because of its critical role in restoring

Florida's damaged scrub habitats and ensuring nutrient cycling, understanding its total

fungal community-including both ECM and pathogens-is a necessary tool in determining

the success of habitat restoration.

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