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1 **Host preference of ectomycorrhizal fungi in mixed pine-oak woodlands**

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8 ABSTRACT

9 Many ectomycorrhizal fungi (EMF) are generalists, but most plant genera that form
10 ectomycorrhizas have at least some fungal partners that are specific to that host genus. Because
11 shared mycorrhizal fungi mediate plant community interactions, host preference has implications
12 for plant succession and competition. We studied the EMF of oaks (*Quercus* spp.) and pines
13 (*Pinus* spp.) in a north Florida, USA, forest, focusing on symbionts shared with longleaf pine (*P.*
14 *palustris*). Longleaf pine is an important species in the southeast U.S., both for timber plantations
15 and for restoring savanna and woodland habitat. However, we found no research on the
16 composition of naturally-occurring EMF on longleaf pine roots. A lower proportion of EMF
17 operational taxonomic units (OTUs) were found colonizing both oaks and pines than expected,
18 providing evidence of host preference within the community. Although most EMF were detected
19 only on either oaks or pines, the OTUs found on both tended to be frequently-occurring and
20 abundant *Cenococcum* OTUs were found to be significantly associated with oaks, an unexpected
21 finding as this genus is widespread, with a broad host range. These results suggest that host
22 preference of EMF may structure EMF communities and therefore influence ecosystem effects of
23 mycorrhizal networks.

24 Keywords: host specificity; host preference; ectomycorrhizal fungi; mycorrhizal networks; *Pinus*
25 *palustris*

26

27 INTRODUCTION

28 Specificity in species' interactions is important in understanding community ecology,
29 coevolution, and even predicting extinction risk (Molina et al. 1992, Bruns et al. 2002,
30 Thompson 2009, Devictor et al. 2010). Ectomycorrhizal fungi (EMF), which are typically
31 beneficial root symbionts of trees, show a range of specificity in which plant hosts they colonize.
32 Host specificity refers to the breadth of plants with which a fungus can form mycorrhizas
33 (Molina and Horton 2015). Although many EMF, often referred to as generalists, can colonize a
34 broad range of hosts, some EMF show specificity to ectomycorrhizal host genera (Molina et al.
35 1992, Toju et al. 2013). Members of the Pinaceae, in particular, have many family- and genus-
36 restricted EMF partners (Molina et al. 1992, Bruns et al. 2002). EMF also exhibit host
37 preference, which refers to mycorrhizae forming between plant and fungal species more or less
38 frequently than expected by chance in an experimental setting, or more frequently on one host
39 than a different neighboring host species in field studies, despite a lack of limitations on
40 compatibility among symbionts (Molina and Horton 2015).

41 Host associations of EMF are especially compelling because interspecies mycorrhizal interaction
42 is a mediator of plant community ecology. Shared EMF allow the potential formation of
43 common mycorrhizal networks among plant species (Kennedy et al. 2003, Twieg et al. 2007,
44 Molina and Horton 2015), which can significantly alter the outcomes of plant-plant interactions
45 by transferring water, nutrients, hormones, and allelochemicals between plants (Newmann 1988,
46 Simard et al. 2012, Horton 2015). For example, EMF networks associated with canopy *Pinus*
47 *radiata* increased drought tolerance of conspecific seedlings and offset the negative effect of root
48 competition on the seedlings, likely by transferring water from adults to seedlings (Booth and
49 Hoeksema 2010). Stressed Douglas-fir (*Pseudotsuga menziesii*) transferred photosynthetic

carbon to ponderosa pine (*Pinus ponderosae*) via mycorrhizal networks (Song et al. 2015). The availability of compatible fungi can also drive plant succession and guild formation (Molina et al. 1992). For example, *Arctostaphylos* chaparral has been shown to provide EMF inoculum that drives *Pseudotsuga* succession in California central coast chaparral (Horton et al. 1999). In contrast, selective pressure on ruderal EMF such as some *Rhizopogon* spp. may explain why many of them are specialized to early successional trees – the need to locate and colonize a host when only a few seedlings are available may conflict with the ability to colonize a broad host range (Bruns et al. 2002). Understanding whether specialization is a benefit or a detriment to the symbiotic partners will help drive applied decisions about types of inoculum to use in nurseries as well as resolve theoretical questions about the evolution of specificity.

Although host specificity in some EMF taxa is documented, research is increasingly turning to host preference to examine possible effects of EMF host associations on plant communities. Molina and Horton 2015 define host preference as occurring when “consistent patterns of nonrandom assemblages between plant and fungal species are observed more or less frequently than expected by chance, despite an absence of compatibility limitations between the symbionts.” As a result of host preference, variation in host plant composition can drive dissimilarity in EMF communities (Ishida et al. 2007). For example, the frequency of most of the common fungi in an Australian wet sclerophyll forest was found to partially depend on host plant species (Tedersoo et al. 2008). Smith et al. (2009) found that EMF communities were structured by host in a mixed *Quercus* and *Pinus* stand, even when the fungi known to show host specificity were excluded. From a plant-focused perspective, host preferences by EMF could contribute to plant-soil feedbacks, which can promote or discourage plant species coexistence, depending on

whether EMF that prefer particular plants are relatively better or worse at promoting growth of those plants (Bever 2003, Bever et al. 2010).

Longleaf pine, *P. palustris*, is important commercially and ecologically in the southeast United States. Longleaf pine is resistant to many diseases that affect other pines grown commercially in the area (Otrosina et al. 1999) and can grow on poor soils that often make the most common commercial species, *P. taeda* (loblolly pine), weakened and more susceptible to disease (Eckhardt et al. 2010, Coyle et al. 2015). Longleaf pine is also more resistant to windfall than loblolly, an increasing concern as hurricane frequency and severity increase near the Gulf of Mexico (Gresham et al. 1991, Johnsen et al. 2009). Finally, longleaf pine is a keystone species in longleaf pine savannas, a critically endangered habitat that supports extremely high species diversity (Frost 1993, Mitchell et al. 2006).

While research has examined the amount of EMF mycelium found in longleaf pine stands (Runion et al. 1997, Hendricks et al. 2006, Sims et al. 2007, McCormack et al. 2010), we were unable to find surveys of the EMF taxa present beyond observation of a single *Thelephora terrestris* sporocarp. *Pisolithus tinctorius* has been trialed as a possible inoculum on longleaf, with varying results (Kais et al. 1981, Cram et al. 1999). EMF are expected to be important to longleaf pine success, as their typical habitat is sandy, fire-maintained communities, where acquisition of water is important and the minimal organic layer may make nutrient acquisition difficult (Hendricks et al. 2006).

This research examines host preference of EMF in oaks and pines, with a focus on *P. palustris*. Specifically, we set out to answer the question of whether EMF with broad host range or narrow host range are more prevalent in longleaf-dominant pine-oak forests by sampling the roots of

longleaf pine trees and paired nearby oak or pine trees. We hypothesized that EMF with broad host range would be more commonly detected and constitute a higher proportion of colonized root tips. We further hypothesized that due to the dominant nature of multi-host EMF, the proportion of taxa colonizing oaks, pines, or both would be consistent with an assumption of no host specificity.

METHODS

Site description

Samples were collected from Eglin Air Force Base near Niceville, FL, USA (30.5247, -86.4921). The area includes pine plantations as well as areas with more varied vegetation. Sites within the base were selected in consultation with base staff to include all pine species occurring locally and a variety of oak species. Unfortunately, the difficulty in finding appropriate trees for sampling meant that sites chosen often contained a variety of soil types, making soil texture and soil organic matter more useful measures than site. Soils ranged from very sandy and well-drained to saturated soils with high organic matter content to soils with high clay content. The predominant soil type in the area is Lakeland sand (NRCS Soil Survey Staff 2016). All sampling sites were located within approximately 30 km of each other. *P. palustris* is common at the study location, as is *P. taeda*. *Quercus laevis* is the most frequent oak species, although others are also common, including *Q. geminata* and *Q. incana*. Apparent oak hybrids were also common and were excluded from sampling, although hybridization in oaks is not always apparent from phenotype. Soil pH ranged from about 4-6, with 5 being a typical value.

Sample collection and processing

115 Sampling was conducted May 12-14, 2014. To maximize the likelihood of finding fungal species
116 shared by different hosts, samples were taken between pairs of mature trees. Because of the site
117 management's interest in increasing the use of *P. palustris* for plantations, each pair of trees was
118 composed of a *P. palustris* and another tree. Other tree species included the other pine species
119 present at Eglin (*P. clausa* (n = 8), *P. elliotii* (n = 8), and *P. taeda* (n = 8)) and a variety of red
120 and white oaks chosen due to on-site abundance (red oaks: *Q. arkansana* (n = 5), *Q.*
121 *hemisphaerica* (n = 5), *Q. incana* (n = 6), and *Q. laevis* (n = 7); white oaks: *Q. geminata* (n = 7)
122 and *Q. margaretta* (n = 6)). Between each pair of trees, four 7 cm diameter by 15 cm deep cores
123 were taken in the root zone of each tree and compounded. Sixty pairs of trees were sampled, for
124 a total of 120 samples. Sixty samples were from *P. palustris*, 24 from other *Pinus* species, and 36
125 from *Quercus* species. When possible, trees were selected such that their root zones as estimated
126 by canopy dripline were within two to three meters of each other, a typical distance for EMF
127 spatial autocorrelation (Lilleskov et al. 2004). However, finding appropriate tree species pairs led
128 to sampling trees with trunks up to ten meters apart. Where they could be reached, leaves were
129 also collected from sampled trees to provide a reference for analysis of plant DNA in roots.
130 Additional pine needles sampled from trees in other locations along the Gulf Coast were also
131 used to create reference sequences. Soil was kept in coolers in the field to prevent samples
132 heating in the sun and refrigerated at the end of each sampling day. Upon return to the lab,
133 samples that could not be processed within two weeks of harvest were frozen at 0 °C until
134 processing. Soil was sieved using a 2 mm mesh, debris was removed, and roots were washed
135 with tap water and placed in a Petri dish. Samples with large quantities of roots were
136 subsampled. Colonized root tips were classified into morphotypes based on color, surface
137 texture, and branching pattern under a dissecting scope, and the number of root tips

corresponding to each morphotype was counted. Three tips from each morphotype in each sample were saved for molecular identification. Sieved soil from each sample was saved for soil texture and soil organic matter assays. Soil texture was measured using a LaMotte soil texture test (LaMotte Company, Chestertown, MD, USA). Soil organic matter content was measured using a loss-on-ignition method. Soil was dried to a steady weight at 100 °C, then a subsample was placed in a tin of known weight, weighed, heated in a muffle furnace for two hours at 360 °C, and reweighed when cool enough to handle (Davies 1974).

DNA was extracted from all sampled root tips on the day the soil sample was processed. Components of Sigma Extract-N-Amp extraction kits (Sigma-Aldrich, St. Louis, MO, USA) were used as described by Rúa et al. (2015) with the exception that extracts were diluted with 160 µL PCR-grade water and were stored at -20 °C. To facilitate Sanger sequencing of EMF species sampled, the Internal Transcribed Spacer (ITS) region of fungal nuclear DNA was amplified using forward primer ITS1-F and reverse primer ITS4 (Gardes and Bruns 1993). Amplification reactions for each sample contained 2.2 µL PCR-grade water, 4 µL of 2X RedTaq Premix (Apex Bioresearch Products, Inc., San Diego, CA, USA), 0.4 µL of each primer at 10 µM concentration, and 1 µL of DNA extract. Reactions occurred in sterile 96-well PCR plates sealed with a sterile silicone sealing mat, briefly vortexed and centrifuged, and amplified as follows: initial denaturation at 94 °C for 3 min; 40 cycles of denaturation for 45 s at 94 °C, annealing for 45 s at 53 °C, extension for 72 s at 72 °C; and a final extension for 10 min at 72 °C.

PCR to identify the plant host amplified the chloroplast DNA locus bounded by psbA and trnH primers using a touchdown PCR program due to difficulties in finding an optimum annealing temperature (Sang et al. 1997). The thermocycling parameters started with 3 minutes at 94 °C, then 15 cycles were run, over which the annealing temperature was decreased from 55 °C to 51

°C in 0.2 °C increments, followed by 25 cycles with an annealing temp of 51 °C. The cycles were denatured for 40 s at 94 °C, 40 s at the annealing temp, and 45 s at 72 °C. The cycling was followed by a final extension of 10 min at 72 °C. Representative sequences have been accessioned to GenBank (MF945989 - MF945997). The psbA-trnH locus was selected because of its relatively high variability, particularly in oaks (Simeone et al. 2013) and common use as a plant barcoding locus (Hollingsworth et al. 2011). The trnL-trnF locus (Taberlet et al. 1991) was also tested and did not provide additional resolution in identifying plant species.

Success of PCR was evaluated on a 1% agarose gel cast with SYBR® Safe DNA gel stain (Molecular Probes, Eugene, OR, USA). Successful PCR reactions had excess primer and mononucleotides removed enzymatically, with each reaction containing 0.05 µL ExoI (New England Biolabs, Ipswich, MA, USA), 0.2 µL Antarctic Phosphatase (New England Biolabs, Ipswich, MA, USA), 4.75 µL PCR-grade water, and 5 µL of amplified DNA. Reactions were incubated at 37 °C for 30 min, then 80 °C for 20 min, followed by at least 5 min at 4 °C. Purified fungal DNA was sequenced using the forward primer ITS5 (White et al. 1990) and purified plant DNA was sequenced using the psbA forward primer. All sequencing used the Big Dye Terminator Sequencing Kit (v3.1, Invitrogen Corp., Grand Island, NY, USA), with each sequencing reaction containing 0.4 µL BigDye Reaction Premix, 1.8 µL BigDye 5X Sequencing Buffer, 0.5 µL primer at 10 µM concentration, 6.3 µL PCR-grade water, and 1 µL purified DNA. Sequencing reactions were incubated thus: initial denaturation at 96 °C for 1 min; 45 cycles of denaturation at 95 °C for 20 s, annealing at 52 °C for 20 s, and extension at 60 °C for 4 min. A ramp speed of no more than 1 °C/s was used. Reactions were dried and shipped overnight to the DNA Lab at Arizona State University, Tempe, AZ, where the Big Dye reactions were purified

and read on an Applied Bioscience 3730 capillary genetic analyzer (Applied Biosystems, Foster City, CA, USA).

The fungal sequences obtained were edited, assembled into operational taxonomic units (OTUs) at 97% similarity, and identified by comparison to sequences in public databases. The methods used were as described in Rúa et al. (2015), with the exception that matches >99% similarity were assigned a species epithet (or genus if no closely-matching sequence was identified to species), 95-99% similarity to closest match assigned to the same genus as the match, and 90-95% similarity to closest match assigned to the taxonomic family of the match. This enabled us to assign identities to many more sequences without overstating their similarity to the reference sequences. Fungal sequence lengths ranged from 200-828 bases. Plant DNA sequences were aligned and compared to sequences from collected leaves, and compared to sequences on the GenBank database using the BLAST utility. Because of the limited range of hosts, some short plant sequences were usable, and sequence lengths ranged from 49-721 bases.

Data analysis

Although the sampling strategy was planned to collect fine roots from particular host trees, the identities obtained through Sanger sequencing frequently did not match the intended host. Because the host identities did not support analyzing the samples separately, samples from the same pair were pooled for analysis.

The locus sampled could only resolve the plant hosts into three groups: red oaks (*Quercus* section *Lobatae*), white oaks (*Quercus* section *Quercus*), and pines (genus *Pinus*). Due to a low number of root tips identified as belonging to white oaks, only 3 fungal OTUs were identified on white oaks (*Russula* 7, *Cenococcum geophilum*, and Gloniaceae3). Therefore, red and white oaks

were pooled for analysis. Given these constraints in identifying hosts, we effectively sampled 84 pines and 36 oaks. Data were analyzed using R version 3.2.5 (R Core Team 2016). A list of fungal species associated with each identifiable plant group was compiled and compared to determine the amount of overlap within and among groups. A chi-squared goodness-of-fit test was conducted to determine if the occurrence of OTUs associated with oaks, pines, both, or an unidentified host was consistent with a null hypothesis of no specificity. The chi-square test was conducted with and without OTUs that occurred only once. As data were non-normal, Spearman's rank correlation was used to relate occurrence (presence/absence) and abundance (count of colonized root tips) of common taxa with soil organic matter and soil texture, using the `cor.test()` function. Proportion tests were run using `prop.test()` to test if the proportion of common OTUs, genera, and families found on pine varied significantly from the proportion of pine root tips to oak root tips, suggesting host preference.

RESULTS

292 EMF morphotypes were identified and sequences accessioned to GenBank (MF945998 - MF946289). These were categorized as 164 EMF OTUs, which represented 16,290 ectomycorrhizal root tips. See Table 1 for a list of detected OTUs, total number of occurrences, number of occurrences on oaks and pines, and number of root tips associated with each. The OTUs occurring in the most samples were *Russula* 7 (11 samples), *Cenococcum geophilum* (in 9 samples), *Russula* 2 (7 samples), *Hebeloma* 1 (5 samples), and *Lactifluus piperatus* (5 samples). At least one oak or pine host was identified for 120 of the fungal OTUs. Of OTUs with an identified host, 85 OTUs were found only with pines, 25 OTUs were found only with oaks, and 10 OTUs were detected on both hosts. A chi-squared test found that this distribution of OTUs is

significantly different from the expected distribution ($\chi^2_1 = 14.239$, $p < 0.001$), with most of the chi-squared value coming from fewer OTUs than expected found on both oak and pines ($\chi^2 = 5.114$), and more OTUs than expected found on oaks but not pines ($\chi^2 = 6.940$). When the analysis was restricted to the 40 OTUs that occurred in at least two samples, 25 OTUs were found only with pines, 4 OTUs were found only with oaks, and 9 OTUs were detected on both hosts. Because of the low number of expected observations in some categories, Yates' continuity correction was applied. This distribution was not significantly different from the expected distribution ($\chi^2_1 = 2.147$, $p = 0.143$).

However, the OTUs shared by oaks and pines included dominant fungi that were the most commonly detected OTUs and substantial proportions of the total number of root tips, including *Russula* 2 (4.2% of root tips), *Cenococcum geophilum* (3.4% of total root tips), *Russula* 7 (3.0% of root tips), *Hebeloma* 1 (2.1% of root tips), and *Lactifluus piperatus* (1.2% of root tips).

Common EMF found only on pines were *Russula* 1 (detected in 4 cores, 1.6% of root tips), *Lactarius imperceptus* (detected in 4 cores, 1.6% of root tips), *Lactarius* 3 (detected in 3 cores, 3.1% of root tips), *Rhizopogonaceae* 1 (detected in 3 cores, 2.1% of root tips), *Rhizopogon* 3 (detected in 2 cores, 2.6% of root tips), and *Amanitaceae* 1 (detected in 2 cores, 2.1% of root tips). A few other taxa also represented similarly high percentages of total root tips but host identity could not be determined: *Hygrophorus* 1 (3.1% of root tips) and *Lactarius corrugis* (2.8% of root tips).

Comparing taxa at the family level, Russulaceae dominated the EMF community, colonizing 41% of identified root tips and occurring in 49 out of 60 samples. Gloniaceae, the family that includes *C. geophilum*, was detected in 21 samples, representing 7.4% of total root tips.

249 Rhizopogonaceae was found in only 11 samples, but colonized the second-highest number of
250 root tips, 8.5%.

251 *Russula* 2 was negatively correlated with the percent silt in the soil (occurrence $\rho = -0.303$, $p =$
252 0.19 ; abundance $\rho = -0.310$, $p = 0.016$). Russulaceae occurrence was negatively correlated with
253 soil organic matter, although the relationship with Russulaceae abundance was not significant
254 (occurrence $\rho = -0.289$, $p = 0.026$; abundance $\rho = -0.155$, $p = 0.236$). Russulaceae occurrence
255 was also positively correlated with percentage sand in the soil, and the relationship between
256 abundance and sand showed a similar trend (occurrence $\rho = 0.316$, $p = 0.014$; abundance $\rho =$
257 0.221 , $p = 0.089$). No other common OTUs or families were significantly correlated with soil
258 organic matter or soil texture.

259 Of OTU occurrences with both host plant and EMF identified, 145 were from pines and 42 were
260 from oaks. Proportion tests were run on EMF taxa with the null hypothesis of 78% of OTU
261 occurrences belonging to pines and 22% belonging to oaks. No common OTUs were
262 significantly associated with one host or the other. When aggregated to genus level, *Cenoccocum*
263 was significantly associated with oaks (5/10 occurrences, $p = 0.037$). The effect was stronger at
264 the family level, with 9/15 occurrences of Gloniaceae occurring on oaks ($p < 0.001$). No other
265 taxa were significant. Rhizopogonaceae, which is a family known to have host specificity for
266 Pinaceae, did occur exclusively on pines, but with 13 occurrences and heavy sampling of pines
267 the effect was only near-significant ($p = 0.052$).

268 DISCUSSION

269 Finding *Rhizopogon* and Rhizopogonaceae species only on pines is consistent with what is
270 already known about host specificity in EMF (Molina et al. 1992, Horton and Bruns 1998). The

distribution of OTUs among oaks and pines also demonstrates that host specificity affects EMF community structure. However, most commonly-occurring taxa and abundant taxa were found on both oak and pine, and this dominance of multihost fungi is typical of EMF communities (Kennedy et al. 2003, Richard et al. 2005, Roy et al. 2008, Toju et al. 2013). One possible explanation for this is that the need for both partners to quickly form a symbiosis to successfully compete for resources drives the lack of specificity found in dominant EMF (den Bakker et al. 2004). Alternately, the diverse community of compatible EMF may swamp the host plant's ability to preferentially reward the most beneficial fungus and start the process of evolving specificity (Thompson 2009). The EMF guild may also be coevolving relatively uniformly with their host plants in diffuse coevolution (Hoeksema 2010).

The results of the chi-squared tests suggest an interaction between whether EMF associate with oaks and whether they associate with pines. There were fewer taxa than expected associating with both oaks and pines, suggesting that host preference is common among fungi. Also, despite the presence of pine-specific fungi in the Rhizopogonaceae and no known oak-specific fungi detected, there were more OTUs than expected found on oaks but not pines, further evidence that host preference is important in these forests. While taxa found in only one sample cannot be said to exhibit host preference per se, including these taxa can still provide useful information about host preference in the community as a whole. Excluding fungi found in only one sample is also a substantial loss in power, and the non-significance of this test may be due to this limited sample size.

The patchiness of mycorrhizal occurrence leading to uncommon OTUs representing a large number of root tips is also consistent with typical EMF community structure (Horton and Bruns 2001). Typifying this pattern was the family Rhizopogonaceae, which was found in only 11 out

of 60 samples, but was the second most abundant family in terms of roots tips colonized. There is also evidence that environment plays a role in defining niche for EMF, as Russulaceae occurred more often in sandier soils. Both of these observations have implications for our understanding of species coexistence in communities of EMF. For example, a patch occupancy model has been hypothesized to explain coexistence among EMF species (Hoeksema and Kummel 2003), but it assumes that EMF are randomly distributed among all available host root tips. Patchy colonization and distinct soil niches for particular species clearly violate this assumption.

The finding that *Cenococcum* was significantly associated with oak was surprising, as it is an ubiquitous genus with a broad host range. In a previous study at Eglin, *Cenococcum geophilum* was the most common EMF in longleaf pine plantations (Busby, unpublished data). These conflicting findings demonstrate that the patchiness of ectomycorrhizal root tip occurrence also produces difficulty in achieving adequate sampling and in interpreting results. The inability to show significant host preference of Rhizopogonaceae despite the family being fairly common and host specific suggests that more intensive sampling may have uncovered additional patterns.

The dominance of generalist EMF also suggests that common mycorrhizal networks (CMNs) may form between pines and oaks, despite the likelihood that some fungi exhibit host preference. Environmental factors could also affect the degree of sharing, with physical disturbance, such as burrowing or rooting animals leading to disconnection of potentially multihost fungi. Factors affecting the amount of ectomycorrhizal biomass, such as water and nutrient availability and distribution, could also affect genet size of EMF and thus their ability to colonize multiple hosts.

Lack of plant DNA barcodes with resolution at fine taxonomic levels is a barrier to investigating host specificity of plant symbionts at the plant species level (Shaw et al. 2005). The plant taxa used for this study were particularly difficult to resolve because oaks have both narrowly-defined species and hybridize extensively, and pines also hybridize, leading to difficulties in using chloroplast loci (Hollingsworth et al. 2011, Piredda et al. 2011, Simeone et al. 2013). Improving the available loci for identifying plants from environmental samples will make identification of root tips to plant species more accessible to projects with large numbers of samples.

Overall, however, the decreasing cost of sequencing is facilitating studies of specificity and coevolution. While ectomycorrhizal root tips are still important as functional units of symbiosis, high-throughput sequencing of soil allows for detection of many more organisms than other sampling methods, enhancing ability to detect specificity (Öpik et al. 2009). Advances in sequencing also make it easier to detect cryptic species and construct phylogenies to investigate patterns of specialization (Roy et al. 2008, Rochet et al. 2011).

Understanding preference in mycorrhizal associations is a promising path to increased understanding of symbiosis, coevolution, and plant community ecology. This study was limited in its applicability by the lack of appropriately spaced trees and heterogenous soil types – future studies with stands of only two tree species and more consistent soils could help to tease apart host-species-level patterns and effects of environment on host preference. While many mycorrhizal fungi have an apparently broad host range, host preference may structure EMF communities and should be included when considering the possible ecosystem effects of mycorrhizal networks.

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Fungal OTU	Total occurrences	Oaks	Pines
<i>Russula</i> 7	11	3	4
<i>Cenococcum geophilum</i>	9	3	4
<i>Russula</i> 2	7	1	5
<i>Hebeloma</i> 1	5	1	3
<i>Lactifluus piperatus</i>	5	1	3
<i>Lactarius corrugis</i>	4	-	-
<i>Lactarius imperceptus</i>	4	-	3
<i>Russula</i> 1	4	-	2
<i>Amanita brunnescens</i>	3	-	2
<i>Cortinarius quarcticus</i>	3	-	2
<i>Lactarius</i> 3	3	-	2
<i>Rhizopogon</i> 1	3	-	3
Rhizopogonaceae 1	3	-	2
Russulaceae 2	3	-	1
Amanitaceae 1	2	-	1
<i>Amanita recutita</i>	2	1	2
Bankeraceae 1	2	-	1
<i>Cortinarius</i> 2	2	-	-
Gloniaceae 1	2	1	-
Gloniaceae 2	2	1	1
Gomphaceae 1	2	-	1
<i>Laccaria trichodermophora</i>	2	-	2
<i>Lactarius</i> 1	2	-	2
<i>Lactarius</i> 2	2	1	1
<i>Lactarius</i> 4	2	-	2
<i>Pulveroboletus</i> 1	2	-	2
<i>Rhizopogon</i> 3	2	-	2
<i>Russula</i> 10	2	-	1
<i>Russula</i> 17	2	-	1
<i>Russula</i> 4	2	1	1
<i>Russula</i> 8	2	1	-
Russulaceae 1	2	-	1
Russulaceae 17	2	1	-
Russulaceae 3	2	1	-
Russulaceae 4	2	-	2
Suillaceae 2	2	-	1
<i>Suillus decipiens</i>	2	-	1
<i>Tomentellopsis zygodesmoides</i>	2	-	2
<i>Tricholoma flavovirens</i>	2	-	2
<i>Tuber</i> 1	2	-	1
<i>Amanita</i> 1	1	-	1
<i>Amanita</i> 2	1	-	-
<i>Amanita</i> 3	1	-	1
<i>Amanita</i> 4	1	-	1
<i>Amanita</i> 5	1	-	1
<i>Amanita</i> 6	1	-	1

<i>Amanita</i> 7	1	-	-
<i>Amanita</i> 8	1	-	1
<i>Amanita</i> 9	1	-	-
Amanitaceae 2	1	-	1
Amanitaceae 3	1	-	-
Amanitaceae 4	1	-	-
Boletaceae 1	1	1	-
Cantharellaceae 1	1	-	1
Cantharellaceae 2	1	-	1
<i>Cenococcum</i> 1	1	1	-
<i>Cenococcum</i> 2	1	-	-
<i>Cenococcum</i> 3	1	1	-
<i>Cenococcum</i> 4	1	-	-
<i>Cenococcum</i> 5	1	-	-
<i>Cenococcum</i> 6	1	-	-
<i>Cenococcum</i> 7	1	-	-
<i>Clavulina</i> 1	1	-	1
<i>Clavulina</i> 2	1	1	-
Clavulinaceae 1	1	-	1
Cortinariaceae 1	1	-	-
Cortinariaceae 2	1	-	-
Cortinariaceae 3	1	-	1
<i>Cortinarius</i> 1	1	-	1
<i>Cortinarius</i> 10	1	-	1
<i>Cortinarius</i> 3	1	-	-
<i>Cortinarius</i> 4	1	-	-
<i>Cortinarius</i> 5	1	-	1
<i>Cortinarius</i> 6	1	1	-
<i>Cortinarius</i> 7	1	-	-
<i>Cortinarius</i> 8	1	1	-
<i>Cortinarius</i> 9	1	-	1
Gloniaceae 5	1	1	-
Gloniaceae 3	1	1	-
Gloniaceae 4	1	1	-
Hydnaceae 1	1	1	1
<i>Hydnellum caeruleum</i>	1	1	-
<i>Hydnum</i> 1	1	-	1
<i>Hydnum</i> 2	1	1	-
<i>Hygrophorus</i> 1	1	-	-
Inocybaceae 1	1	-	1
<i>Laccaria</i> 1	1	-	-
<i>Lactarius</i> 10	1	-	1
<i>Lactarius</i> 11	1	-	-
<i>Lactarius</i> 12	1	-	1
<i>Lactarius</i> 13	1	-	-
<i>Lactarius</i> 5	1	-	1
<i>Lactarius</i> 6	1	1	-

<i>Lactarius</i> 7	1	-	-
<i>Lactarius</i> 8	1	1	-
<i>Lactarius</i> 9	1	-	1
<i>Lactarius subserifluus</i>	1	-	1
<i>Lactifluus</i> 1	1	-	-
<i>Lactifluus</i> 2	1	1	-
<i>Lactifluus</i> 3	1	-	-
<i>Lactifluus</i> 4	1	-	1
<i>Lactifluus</i> 5	1	-	-
<i>Lactifluus</i> 6	1	-	-
<i>Lactifluus</i> 7	1	-	-
<i>Ramaria</i> 1	1	-	1
<i>Rhizopogon</i> 2	1	-	1
<i>Rhizopogon</i> 4	1	-	1
<i>Rhizopogon</i> 5	1	-	-
Rhizopogonaceae 2	1	-	1
Rhizopogonaceae 3	1	-	-
<i>Russula</i> 11	1	-	1
<i>Russula</i> 12	1	-	1
<i>Russula</i> 13	1	-	1
<i>Russula</i> 14	1	-	-
<i>Russula</i> 15	1	-	-
<i>Russula</i> 16	1	-	-
<i>Russula</i> 18	1	-	1
<i>Russula</i> 3	1	1	-
<i>Russula</i> 5	1	-	1
<i>Russula</i> 6	1	-	-
<i>Russula</i> 9	1	-	1
Russulaceae 10	1	-	-
Russulaceae 11	1	-	1
Russulaceae 12	1	-	1
Russulaceae 13	1	-	1
Russulaceae 14	1	-	1
Russulaceae 15	1	-	-
Russulaceae 16	1	-	-
Russulaceae 6	1	-	1
Russulaceae 7	1	-	1
Russulaceae 8	1	-	-
Russulaceae 9	1	-	1
<i>Russula cyanoxantha</i>	1	-	1
<i>Russula flavisiccans</i>	1	-	1
<i>Sarcodon scabrosus</i>	1	1	-
<i>Scleroderma polyrhizum</i>	1	-	1
<i>Sistotrema</i> 1	1	-	-
Suillaceae 1	1	1	-
Suillaceae 3	1	-	1
<i>Suillus</i> 1	1	-	-

<i>Thelephoraceae</i> 1	1	-	1
<i>Thelephoraceae</i> 2	1	-	1
<i>Thelephoraceae</i> 3	1	-	-
<i>Thelephoraceae</i> 4	1	-	1
<i>Tomentella</i> 1	1	-	1
<i>Tomentella</i> 2	1	1	-
<i>Tomentella</i> 3	1	-	1
<i>Tomentella</i> 4	1	-	1
<i>Tomentella</i> 5	1	1	-
<i>Tomentella</i> 6	1	-	1
<i>Tomentella</i> 7	1	-	1
<i>Tomentella</i> 8	1	1	-
<i>Tomentella</i> 9	1	1	-
<i>Tomentellopsis</i> 1	1	-	1
<i>Tricholoma</i> 1	1	-	-
<i>Tricholoma</i> 2	1	-	1
<i>Tricholoma</i> 3	1	-	1
<i>Tricholoma</i> 4	1	-	1
<i>Tricholoma</i> 5	1	-	-
<i>Tricholoma</i> 6	1	-	1
<i>Tricholoma</i> 7	1	-	-
<i>Tricholoma</i> 8	1	-	-
<i>Tricholoma</i> 9	1	-	1
<i>Tylospora</i> 1	1	-	1

Total occurrences is the number of samples in which the OTU was found. Occurrences on oak may not sum to total occurrences due to hosts that were neither oak nor pine, inability to identify an OTU on different hosts within the same sample.

Total root tips	BLAST match
486	UDB014194
557	KC967410
680	AB507025
352	GU328547
202	KF220050
454	JQ753822
257	JQ272401
263	FJ803979
76	KC855217
154	UDB000748
497	AJ633589
206	AJ810040
347	DQ351512
123	AJ633583
346	UDB015627
88	JX844736
90	UDB015699
104	JQ991693
91	JN943886
39	JQ711879
199	FJ196943
53	KC152146
70	KF220050
113	AY456344
98	AJ633589
192	UDB011961
431	AJ810034
91	JQ396469
262	JX457011
82	FJ196947
73	KF810121
109	AY281091
186	UDB014058
124	KF220092
106	JQ753908
231	L54088
153	AF166508
48	UDB011640
130	JF899574
176	GQ379737
26	KC424527
39	JX029931
36	KF359589
9	HE820439
21	FM999626
55	KC855218

Draft

89 JX029931
32 KC855224
41 KC855217
58 KJ638264
22 EU819463
316 KJ638264
55 DQ273368
17 AB445116
27 AB211251
54 AY818585
128 JN943886
36 JN943890
68 JX316439
19 EF619647
32 EF619647
32 KJ701295
46 JN247429
23 FM999678
4 AY456373
18 GQ159913
46 UDB011758
209 GQ159913
195 FJ157077
89 UDB018654
87 FJ157077
104 UDB018664
15 GU328603
114 JN197989
36 KJ705113
9 KJ705138
28 JX029949
77 KF879454
43 AY818585
24 JN943889
102 UDB012035
118 EU622335
38 KC686877
29 HE820661
512 EU292531
125 HQ604561
91 JX030197
7 AJ633589
107 AJ633589
46 AY456347
27 AY456347
87 AJ633589
81 AJ633589

69	UDB000836
43	KF937340
29	AY456344
16	EU819482
56	KF220048
43	KF220050
56	KF220015
31	KF220017
56	KF220050
48	KF220050
94	JQ753830
62	HM234140
212	JX017263
18	JX017263
121	JX017263
8	DQ822821
49	AB507025
36	FJ196947
16	FJ196947
39	KM576559
46	JX457011
92	DQ778004
64	UDB016029
102	AB507025
18	JQ396496
39	AB507025
293	UDB014194
86	HE820652
8	HE820682
25	AB218078
32	AB507025
39	AB507025
36	GU328540
41	FR852027
93	AB769910
18	KJ769279
103	KF220050
116	JQ272401
54	AJ633583
66	EU598196
6	EU598162
68	KC571778
63	EU718123
67	FR838002
144	L54088
26	L54088
114	AF166510

Draft

47	KM402988
83	UDB002646
31	GQ219947
9	FR731298
80	FM999528
21	UDB018519
34	UDB018688
19	UDB018457
29	HM370471
39	DQ482015
56	GU907787
32	UDB018519
181	UDB018441
42	UDB018503
124	EU563482
15	UDB019449
26	HQ285404
62	AF309522
66	AF309522
4	KC152249
31	FJ596911
64	HQ285404
99	FJ596910
47	AY969614

aks and pines
ntify host, or

Species	Common name	Representative sequence
<i>Pinus elliottii</i>	Slash pine	TTGATCTATTTCAATAGGTGGATAAATACTCTGATGGATTG
<i>Pinus palustris</i>	Longleaf pine	CGCTGTTGATCTATTTCAATAGGTGGATAAATACTCTGATG
<i>Pinus taeda</i>	Loblolly pine	GCTGTTGATCTATTTCAATAGGTGGATAAATACTCTGATGG
<i>Pinus clausa</i>	Sand pine	AAAACCCAATTTATCTATCTACAGTCATTTCTTCCAAGAA
<i>Quercus geminata</i>	Sand live oak	TCTACAATGGATAAGACTTAGGTATTAGTNTATATGAGTI
<i>Quercus laevis</i>	Turkey oak	CTATCNCCCCTCCCTGNATGTAGTATAAATTACAAAAAT
<i>Quercus margaretta</i>	Sand post oak	TCCGNCCNATCCCCCACTCCCTGTATGTAGTATAAATTAC
<i>Quercus hemisphaerica</i>	Laurel oak	ATCCCCCACTCCCTGTATGTAGTATAAATTACAAAAATTT
<i>Quercus incana</i>	Bluejack oak	CNANTCCCTGTATGTAGTATAAATTACAAAAATTTCAATT
<i>Quercus arkansana</i>	Arkansas oak	TCCGCCCNATCNCNCCACTCCCTGTATGTAGTATAAATTAC

All sequences are from the psbA-trnH locus. Sequences derived from leaf samples, except for *Pinus clausa* , which

Draft

TTATCGTGTATTGCTTAATTGAAGCATACCAAGCCTTTCATTCATTTTATTGAAAGGCTTGGT
GATTGTTATCGTGTATTGCTTAATTGAAGCATACCAAGCCTTTCATTCATTTTATTGAAAGGC
ATTGTTATCGTGTATTGCTTAATTGAAGCATACCAAGCCTTTCATTCATTTTATTGAAAGGCT
TAAAAAATGAGCTAACGTTTGTTCTTATGT
ATTGAAAATAAATANGAAAGGAGCANTGNCTNCCNTCTTGATAAAACNNGAAGNTGATNA
TTCAATTAATTTCAATTTCTACCATTCCTCTTGTTTTGTTTCCCTTTCTTATAAGATAAAAGAC
AAAAATTTCAATTTCTACCATTCCTCTTGTTTTGTTTCCCTTTCTTATAAGATAAAAGACAAG
CAATTAATTTCAATTTCTACCATTCCTCTTGTTTTGTTTCCCTTTCTTATAAGATAAAAGACAA
AATTTCAATTTCTACCATTCCTCTTGTTTTGTTTCCCTTTCTTATAAGATAAAAGACAAGAGG
AAAAATTTCAATTAATTTCAATTTCTACCATTCCTCTTGTTTTGTTTCCCTTTCTTATAAGATA

h is GenBank sequence KC157368. We were unable to obtain *P. clausa* leaves due to height.

Draft

ATGCTTCAATTGTTTGGTGTTATTCTTCATACTTCTTCCCATTCCATCAATAATGGATATGTGC
TTGGTATGCTTCAATTGTTTGGTGTTATTCTTCATACTTCTTCCCATTCCATCAATAATGGATA
TGGTATGCTTCAATTGTTTGGTGTTATTCTTCATACTTCTTCCCATTCCATCAATAATGGATAT

TTGCTCCTTNCTTATTTATTAGCCATTTAATTNATTANNNNNNTGCAATTACNATNTNNNCNTC
AAGAGGCAGAAAATAAATGGAAAGTTTTATCTTTTTTATTTAAATAAATAAATAAAAACTTC
AGGCAGAAAATAAATTGAAAGCTTTATCTTTTTTATTTAAATAAATAAATAAAAACTTC
AGAGGCAGAAAATAAATGGAAAGTTTTATCTTTTTTATTTAAATAAATAAATAAAAACTTCAC
CAGAAAATAAATGGAAAGTTTTATCTTTTTTATTTAAATAAATAAATAAAAACTTCACCGAT/
AAAGACAAGAGGCAGAAAATAAATGGAAAGTTTTATCTTTTTTATTTAAATAAATAAATAA/

Draft

AGTTCCCCTGCATCCAGCAGGAATTGAACCCGCGAGTTCGCCAATTATGAGTTGGGCGCTTT
GTGTCAGTTCCCCTGCATCCAGCAGGAATTGAACCCGCGAGTTCGCCAATTATGAGTTGGGC
GTGTCAGTTCCGCTGCATCCAGCAGGAATTGAACCCGCGAGTTCGCCAATTATGAGTTGGGC

CGTTTCNNTTATTTTTTTACNNAANANAAAAANANTATNAGCTTTTGNTNNTTNNTCTCANNAT
CACCGATAAAAAACATATATNAAATAAAAAATTACTACATAAGTCAAATAGGATACTCAAATCC
CACCGATAAAAAACATATATAAAATAAAAAATTACTACATAAATCAAATAGGATACTCTTTTAC
CGATAAAAAACATATATAAAATAAAAAATTACTACATAAGTCAAATAGGATACTCAAATCCTT
AAAAACATATATAAAATAAAAAATTACTACATAAGTCAAATAGGATACTCAAATCCTTTTTCG
AACTTCACCGATAAAAAACATATATAAAATAAAAAATTACTACATAAGTCAAATAGGATACTC

Draft

'AACCATTCAGCCATGGATGCTGGATAAAGATCATCAACATATTCATTCTATAATATGAGTAT
'GCTTTAACCATTCAGCCATGGATGCTGGATAAAGATCATCAACATATTCATTCTATAATATG
'GCTTTAACCATTCAGCCATGGATGCTGGATAAAGATCATCAACATATTCATTCTATAATATGA

'ACNAANAAGGATTTNAANANCCTATTTGACTTATGTAGTAATTTTTATTTTATATATGTTTTT
'TTTTTCGTATCTTGAGTTAAAATAAAAAAAGCTTATATTATATTCTATTAAGTAAAAAAGTA/
'TTTTTCGTATCTTGAGTTAAAATAAAAAAAGCTTATATTATATTCTATTAAGTAAAA/
'TTTCGTATCTTGAGTTAAAATAAAAAAAGCTTATATTATATTCTATTAAGTAAAAAAGTAAA/
'TATCTTGAGTTAAAATAAAAAAAGCTTATATTATATTCTATTAAGTAAAAAAGTAAAAAGAAA
'AAATCCTTTTTTCGTATCTTGAGTTAAAATAAAAAAAGCTTATATTATATTCTATTAAGTAAAA/

Draft

AGACTCAGATCTAAATTTGGGTAGTTCGGGATTGGGACCCATTTACATTCTTTCTCTCATACT
AGTATAGACTCAGATCTAAATTTGGGTAGTTCGGGATTGGGACCCATTTACATTCTTTCTCTC
.GTATAGACTCAGATCTAAATTTGGGTAGTTCGGGATTGGGACCCATTTACATTCTTTCTCTCA

ATCGGTGAAGTTTTTATTTATTTATTTAAATAAAAAAGATAAAACTTTCCATTTATTTTCTGCC
AAAGAAAAGACGACTAAATAGTAATTACAATTATATAATAATTAAATGGCTAATAAATAAG/
AATATTCTATTAAGTAAAAAAGTAAAAGAAAAGACGACTAAATAGTAATTACAATTATATA
AGAAAAGACGACTAAATAGTAATTACAATTATATAATAATTAAATGGCTAATAAATAAGAA/
AGACGACTAAATAGTAATTACAATTATATAATAATTAAATGGCTAATAAATAAGAAAGGAG
AAGTAAAAGAAAAGACGACTAAATAGTAATTACAATTATATAATAATTAAATGGCTAATAA

Draft

TTGATTCATGTCAAATATTCTCAATAGACATTCAAATAACATTTTCATTGAATTAATTTGATAAT
ATACTTGATTCATGTCAAATATTCTCAATAGACATTCAAATAACATTTTCATTGAATTAATTTG
ATACTTGATTCATGTCAAATATTCTCAATAGACATTCAAATAACATTTTCATTGAATTAATTTGA

CTCTTGCTTTTTATCTTATAAGAAAGGGAAACAAAACAAGAGGAATGGTAGAAATTGAAATT
AAAGGAGCAATAATCAACTTCTTGTTTTATCAAGAGGGTTGACATTGCTCCTTTCTTATTTAT
ATAATTAAAGGGCTAATAAATAAGAAAGGAGCAATGTCAACCCTCTTGATAAAAACAAGAAC
AGGAGCAATAATCAACTTCTTGTTTTATCAAGAGGGTTGACATTGCTCCTTTCTTATTTATTTT
CAATAATCAACTTCTTGTTTTATCAAGAGGGTTGACATTGCTCCTTTCTTATTTATTTTCAATA
ATAAGAAAGGAGCAATAATCAACTTCTTGTTTTATCAAGAGGGTTGACATTGCTCCTTTCTT

Draft

TAAGCCATACAACCTTGTTTCGAGAGTTGAGAGTTAGTCATCGATCTTGCTTTGATCCCCTATCA
ATAATAAGCCATACAACCTTGTTTCGAGAGTTGAGAGTTAGTCATCGATCTTGCTTTGATCCCC
ATAATAAGCCATACAACCTTGTTTCGAGAGTTGAGAGTTAGTCATCGATCTTGCTTTGATCCCCT.

AATTGAAATTTTTGTAATTTATACTACATACAGGGAGTGGGGGATAGGGGCGGATGTAGCCA
TTCAATAACTCATATATACTAATAACCTAAGTCTTATCCATTTGTAGATGGCGCTTCGACAGA.
TTGATTATTGCTCCTTTCTTATTTATTTTCAATAACTCATATATACTAATAACCTAAGTCTTATC
CAATAACTCATATATACTAATAACCTAAGTCTTATCCATTTGTAGATGGCGCTTCGACAGAAG
ACTCATATATACTAATAACCTAAGTCTTATCCATTTGTAGATGGCGCTTCGACAGAAGCTAAG
ATTTATTTTCAATAACTCATATATACTAATAACCTAAGTCTTATCCATTTGTAGATGGCGCTTC

Draft

GAGGTCACCGACCCACATAAGAACAAACGTTAGCTCATTTTTTTATTCTTGGAAGAAATGACTC
TATCAGAGGTCACCGACCCACATAAGAACAAACGTTAGCTCATTTTTTTATTCTTGGAAGAAA'
ATCAGAGGTCACCGACCCACATAAGAACAAACGTTAGCTCATTTTTTTATTCTTGGAAGAAATC

AAGTGGATCAAGGCAGTGGATTGTGAATCACCCATGCGCGA
AGCTAAGTCTAGAGGGAAATTATGAGCATTACGTTCTGCATAACATAATGCTCTTTGGATTG
TCATTTGTAGATGGAGCTTCGACAGAAGCTAAGTCTAGAGGGAAATTATNAGCATTAC
CTAAGTCTAGAGGGAAATTATGAGCATTAC
CTCTAGAGGGAAATTATGAGCATTAC
GACAGAAGCTAAGTCTAGAGGGAAATTATGAGCATTACGTT

Draft

GTAGATAGATAAATTGGTTTTTTCCGGGGCGGACGTAGCCAAGTGGACCAAGGCAGTGGATT
TGACTGTAGATAGATAAATTGGTTTTTTCCGGGGCGGACGTAGCCAAGTGGACCAAGGCAGT
GACTGTAGATAGATAAATTGGTTTTTTCCGGGGCGGACGTAGCCNAGTGGACCAAGGCAGTC

3NNAATC

Draft

GTGAATCCACCATGCGCGA
GGATTGTGAATCC
GGATTGT

Draft