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Ectomycorrhizal diversity and community structure in stands of *Quercus oleoides* in the seasonally dry tropical forests of Costa Rica

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Abstract

Most conservation efforts in seasonally dry tropical forests have overlooked less obvious targets for conservation, such as mycorrhizal fungi, that are critical to plant growth and ecosystem structure. We documented the diversity of ectomycorrhizal (EMF) and arbuscular mycorrhizal (AMF) fungal communities in *Quercus oleoides* (Fagaceae) in Guanacaste province, Costa Rica. Soil cores and sporocarps were collected from regenerating *Q. oleoides* plots differing in stand age (early vs late regeneration) during the wet season. Sequencing of the nuclear ribosomal ITS region in EMF root tips and sporocarps identified 37 taxa in the Basidiomycota; EMF Ascomycota were uncommon. The EMF community was dominated by one species (*Thelephora* sp. 1; 70% of soil cores), more than half of all EMF species were found only once in an individual soil core, and there were few conspecific taxa. Most EMF taxa were also restricted to either Early or Late plots. Levels of EMF species richness and diversity, and AMF root colonization were similar between plots. Our results highlight the need for comprehensive spatiotemporal samplings of EMF communities in *Q. oleoides* to identify and prioritize rare EMF for conservation, and document their genetic and functional diversity.

1. Introduction

Seasonally dry tropical forests (SDTF) account for nearly half of the world's tropical and subtropical forests but remain one of the most endangered habitats (Murphy and Lugo 1986, Janzen 1988). High rates of forest clearing for pasture, frequent fires, and pressure from population growth have all contributed to severe fragmentation and degradation of SDTF (Trejo and Dirzo 2000). Most conservation efforts to date have been directed toward specific plant taxa or areas that contain high levels of plant endemism (Pennington *et al* 2009). Although valuable, this approach overlooks less obvious targets for conservation, such as mycorrhizal fungi, that are critical to plant establishment and function and ecosystem

structure. In this study, we document mycorrhizal diversity in an evergreen oak, *Quercus oleoides* (Fagaceae) dominated forest, in the Área de Conservación Guanacaste, Costa Rica.

Quercus oleoides forests support multiple trophic levels of biodiversity and provide key ecosystem services (Boucher 1981, Cavender-Bares *et al* 2011). Once found as discontinuous stands across Guanacaste province, *Q. oleoides* is now restricted to high-density stands (Janzen 1988). Nevertheless, steps are being made towards the conservation and restoration of the SDTF through management practices such as fire suppression. Studies have also demonstrated that both abiotic factors (e.g., soil fertility; Powers and Pérez-Aviles 2013) and biotic factors, such as fine root abundance, are important in SDTF regeneration (Hertel

et al 2003). By comparison, the potential role(s) of mycorrhizal fungi in shaping the SDTF, especially in regenerating *Quercus* forests, are less well known (Boucher 1981, Tedersoo *et al* 2007, Morris *et al* 2008, Klemens *et al* 2011, Smith *et al* 2011) even though *Quercus* are highly dependent on their mycorrhizal mutualists for resource acquisition and allocation (Egerton-Warburton and Allen 2001).

Within the SDTF, *Q. oleoides* is unique in that it hosts EMF (Basidiomycota, Ascomycota) in a forest dominated by understory taxa hosting AMF mutualisms (Glomeromycota; Janos 1983). EMF diversity and community structure in Neotropical forests is poorly characterized relative to those in temperate or boreal forests. Studies to date have shown that tropical forests harbor species-rich EMF communities (Muel-ler and Halling 1995, Tedersoo *et al* 2007, Morris *et al* 2008, Peay *et al* 2009, Smith *et al* 2011, but see Tedersoo *et al* 2010a, 2010b), and that EMF networks can enhance seedling establishment and promote monodominance (McGuire 2007). Variations in soil fertility (Peay *et al* 2015, Waring *et al* 2016a, 2016b), the size and phenology of the host plants and their distribution on the landscape can also influence EMF diversity (Morris *et al* 2008, Tedersoo *et al* 2010a, 2010b), as well as management activities such as fire suppression or restoration plantings that affect EMF host tree availability (Klemens *et al* 2011). For instance, trees in smaller patch size fragments might host fewer EMF taxa owing to reductions in inoculum potential and limits to root colonization (Peay *et al* 2007).

Quercus has also been shown to host both EMF and AMF (Egerton-Warburton and Allen 2001, Querejeta *et al* 2009), the balance of which can differ with plant phenology, environment or season. The presence of AMF in *Q. oleoides* has yet to be documented. However, studies have shown that seedlings and saplings may be colonized by AMF and EMF whereas mature trees are primarily associated with EMF (Egerton-Warburton and Allen 2001, Hertel *et al* 2003). AMF are also expected to be more prevalent in dry than wet soils (Querejeta *et al* 2009).

Our goal was to document EMF diversity and AMF status in *Q. oleoides* as a first step in aligning EMF conservation with that of plants. We characterized EMF species richness and community structure in *Q. oleoides* in two naturally regenerated secondary forest plots (9 versus 25 years of restoration, i.e., fire suppression); determined AMF abundance in these plots; and compared the EMF community in *Q. oleoides* against similar studies in *Quercus* woodlands and forests. Using these data, we tested four hypotheses: (1) EMF diversity in *Q. oleoides* increases with regeneration time; (2) age differentiated plots host distinct suites of EMF fungi; (3) AMF root colonization is higher during early regeneration; and (4) there is a high turnover among EMF communities owing to differences in climate and environment.

2. Materials and methods

2.1. Site description

We worked in the Sector Santa Rosa of the Área de Conservación Guanacaste in northwestern Costa Rica (10.84°N, 85.62°W). Mean annual temperatures are ~25 °C, and mean annual precipitation at Santa Rosa is 1765 mm, most of which falls during a distinct wet season from May to December (Becknell and Powers 2014). Soils in the area are heterogeneous, and the infertile plateaus developed from volcanic ignimbrites support savanna-associated species including *Q. oleoides*, *Byrsonima crassifolia*, and *Curatella americana* (Ulate 2001). We examined EMF in *Q. oleoides* in a pair of plots, each 20 m × 50 m, that had been established in a previous study (Powers *et al* 2009). One plot represented an early regeneration stand of *Q. oleoides* (9 years old, hereafter referred to as ‘Early’) while the other plot represented later regeneration (25 years old, ‘Late’). Stand age estimates were made previously by Powers *et al* (2009) using multiple methods (e.g. satellite imagery) to determine the number of years a patch of land occurred as forest. These two stands each contained at least five *Q. oleoides* trees of similar size (dbh) and age, and occurred at similar elevations (213–218 m above sea level).

2.2. Soil sampling

Soil cores and sporocarps were collected in July 2011 (wet season), when EMF activity and diversity is expected to be high. Within each plot, five *Q. oleoides* representative of stand maturity were sampled. For each tree, eight soil cores (each 7.5 cm wide, 15 cm deep) were collected at the dripline: the four cardinal positions and their intermediaries. This resulted in a total of 40 soil cores from each plot. Soil cores were stored at 2 °C–3 °C and sorted within three days of collection. Each core was washed in water to remove adhering soil and roots examined under a dissecting microscope. EMF roots were identified by the presence of a fungal mantle, turgidity and the absence of root hairs. EMF roots were separated from each core using forceps, rinsed in a tub of tap water, pooled together and placed directly into cetyltrimethylammonium bromide (CTAB) buffer (Gardes and Bruns 1993). The abundance of EMF root tips in each core was visually estimated as low, medium or high but no attempt to morphotype roots was made at the time. Sporocarps of terrestrial epigeous fungi were also collected in each plot and samples of tissue placed directly into CTAB. Root and sporocarp samples were kept refrigerated (except during transportation), shipped back to the Chicago Botanic Garden, and then stored at –20 °C until DNA extraction.

2.3. DNA sequencing and analysis

EMF root tips in each sample were sorted into morphological types (morphotype) based on mantle

color, texture, and emanating hyphae. Root tips of each morphotype were frozen at -80°C and lyophilized, after which DNA was extracted using Qiagen DNeasy Plant Mini Kit (Qiagen USA, Valencia, California) following manufacturer's instructions. Polymerase chain reaction (PCR) amplification of the internal transcribed spacer region using primers ITS1F (Gardes and Bruns 1993) and ITS4 (White *et al* 1990) was attempted for all samples using the protocol of Wilson *et al* (2007). Successful amplifications, i.e., single amplicons, were cleaned and cycle sequenced using BigDye v3.1 on an Applied Biosystems 3730 DNA sequencer (Applied Biosystems, Foster City CA). Samples with multiple amplicons or mixed sequences (some sporocarps, root tips) were cloned using an Invitrogen TA Cloning Kit with plasmid vector 2.1 (Invitrogen, Carlsbad, California). The PCR product was ligated using T4 ligase into the plasmid vector, and used to transform DH5- strain of *Escherichia coli*. Bacterial cells were plated onto LB Agar + kanamycin + Xgal and incubated for 18–22 h at 37°C . Up to 30–40 transformed colonies sampled per plate were PCR amplified with primers M13F and M13R (Invitrogen) and evaluated by restriction fragment length polymorphism (RFLP) analysis using restriction enzymes Alu I and Hinf I (Promega Corp., Madison, Wisconsin). Replicates of each distinctive RFLP pattern were selected for cycle sequencing using the primers M13F and M13R.

ITS1F and ITS4 sequences (M13F, M13R sequences from clones) were edited and assembled in CodonCode Aligner 3.5.7 (CodonCode Corp., Dedham, Massachusetts; www.codoncode.com/). Contig assembly parameters were set to assemble sequences at a threshold of 97% similarity or greater. Contig assembly was applied to all sequences derived from root tips. The resulting contigs were then identified as individual fungal species recovered on *Q. oleoides* root tips and the plasmid vector regions were removed from each root tip contig. Queries of sporocarp and root tip ITS sequences against GenBank (www.ncbi.nlm.nih.gov/genbank/) and UNITE (<http://unite.ut.ee/>) databases were performed using BLAST. Query matches from Genbank and UNITE were downloaded as FASTA files and added to the database for phylogenetic analysis as per Smith *et al* (2007). BLAST matches that yielded $\geq 97\%$ similarity to GenBank or UNITE sequences were used to provide species names to sequences. Query results $< 97\%$ similarity were only used to provide taxonomic identification above species rank. For phylogenetic analysis, individual root tip and sporocarp sequences were assembled into an ITS sequence matrix using Mesquite v. 3.04 (Maddison and Maddison 2015). Initial automatic alignment was performed in MUSCLE (Edgar 2004) followed by additional manual alignment in Mesquite.

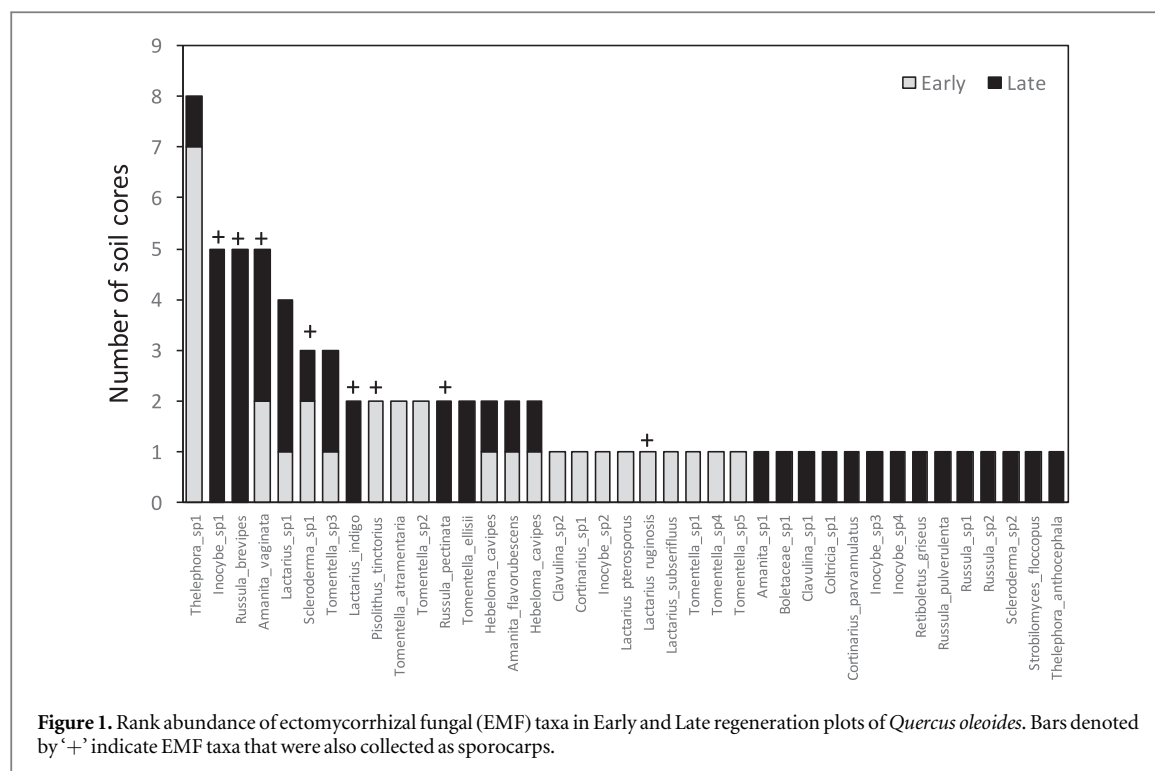
2.4. AMF quantification

Dried fine roots were cleared and stained with Trypan blue (Koske and Gemma 1989). Stained roots were mounted on glass slides in polyvinyl-lactic acid glycerol, and examined and analyzed using light microscopy.

2.5. Statistical and phylogenetic analyses

Minimum estimates of EMF species richness were generated for each core and plot using EstimateS (Colwell 2005). Each estimate was based on 500 randomizations of sample order without replacement. From the output, we calculated species richness (S, Chao 1), diversity (Fishers alpha), and evenness (Simpson's E). We compared within and between-plot EMF richness and diversity using *t*-tests. At the plot level, we also documented the number of singletons, i.e., taxa that occurred only once across all samples in the plot, as indicators of rarity. EMF community composition between Early and Late plots was visualized using non-metric multidimensional scaling (NMDS) based on the Bray-Curtis index of community dissimilarity. Significant differences in species composition between Early and Late plots were tested using analysis of similarity (ANOSIM) and statistical significance tested against 9999 null permutations. Two NMDS and ANOSIM analyses were undertaken: soil cores alone, and cores and sporocarp combined. AMF root colonization data were transformed ($\ln(1 + x)$) and differences between plots analyzed using *t*-tests.

To compare EMF assemblages in *Q. oleoides* with those in other oak woodlands, EMF sequences from our *Q. oleoides* plots ($n = 139$; denoted CR) were combined with 205 EMF sequences from communities associated with *Q. douglasii* from California, USA (98 sequences; CA, Smith *et al* 2007), *Q. rubra* and *Q. prinus* from North Carolina USA (72 sequences; NC, Walker *et al* 2005), and *Q. crassifolia* from Mexico (36 sequences; MX; Morris *et al* 2008). Of the 344 sequences in the dataset, 50 root tip sequences represented fungi in the Ascomycota. The Ascomycota from Costa Rica were mostly represented by plant pathogenic fungi in the Pleosporales (*Alternaria*) and Hypocreales (*Myrothecium*) with few sequences attributable to EMF Ascomycota. As a result, further analyses were limited to fungi representing the Agaricomycetes (Basidiomycota). Phylogenetic analysis using maximum likelihood was performed in RAxML (Stamatakis 2006), as implemented through the CIPRES Web Portal (Miller *et al* 2010). Default priors were used, with adjustments 1000 bootstrap replicates. Bipartition (bootstrap) results were visualized using FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>). Trees were midpoint rooted and bootstrap values $> 70\%$ were used to assess clade support.



Phylogenetic turnover between communities was based on presence-absence diversity. Clades represented by >1 sequences were reduced to a single representative sequence, and SpacodiR was used to generate P_{ST} and Π_{ST} : values >0 correspond to phylogenetic clustering and <0 corresponds to overdispersal (Eastman *et al* 2011). Analyses were performed on models comparing the following: individual communities (CR versus CA versus MX versus NC), temperate (CA+NC) versus tropical habitats (CR+MX), wet (NC+MX) versus dry habitats (CR+CA), and young (CR+NC) versus old stands (CR+CA).

3. Results

3.1. Taxonomic representation

Of the 80 soil cores collected, 11 soil cores (all in the Early plot) contained no *Quercus* root tips and were eliminated from analysis. In the remaining soil cores ($n = 69$), those with the highest abundance of root tips were used for analyses. This yielded 23 cores, 11 from the Early plot and 12 from the Late plot, thereby providing at least two soil cores from each tree for DNA extractions along with sporocarp samples.

Using a 97% sequence similarity cutoff, we identified 139 fungal OTUs, comprising 90 sequences from root tips and 49 sequences from sporocarps that represent fungal lineages generally considered to be EMF. These OTUs corresponded to 37 EMF taxa, the majority of which were Basidiomycota. Nineteen EMF species were found on root tips, the most abundant being the Thelephorales (*Thelephora*, *Tomentella*) and Russulales (*Russula*, *Lactarius*; figure 1). Ectomycorrhizal ascomycetes were relatively rare on root tips

and the remaining species were from fungal lineages traditionally considered saprophytic (e.g. *Alternaria*) or endophytic (e.g. *Cladophialophora*). Sequences representing *Laccaria*, *Calostoma* and *Suillus* were also identified among root tip samples. Because of active research on these taxa in the laboratory, these sequences may have represented contamination and were removed from further analyses.

The abundance distribution of EMF was log-normal with one common species (*Thelephora* sp. 1; 70% of soil cores), and more than half of all EMF species found only once in an individual soil core (figure 1). In addition, there was little overlap in species between plots with many EMF taxa restricted to either Early or Late plots: *Pisolithus* was only detected in the Early plot whereas members of the Boletaceae (*Boletus*, *Retiboletus*, *Strobilomyces*), and *Coltricia* were only recovered in the Late plot. Species accumulation curves did not level off suggesting that further sampling is needed to accurately document EMF diversity (figure S1). Even so, the pattern of EMF accumulation for both Early and Late plots was similar.

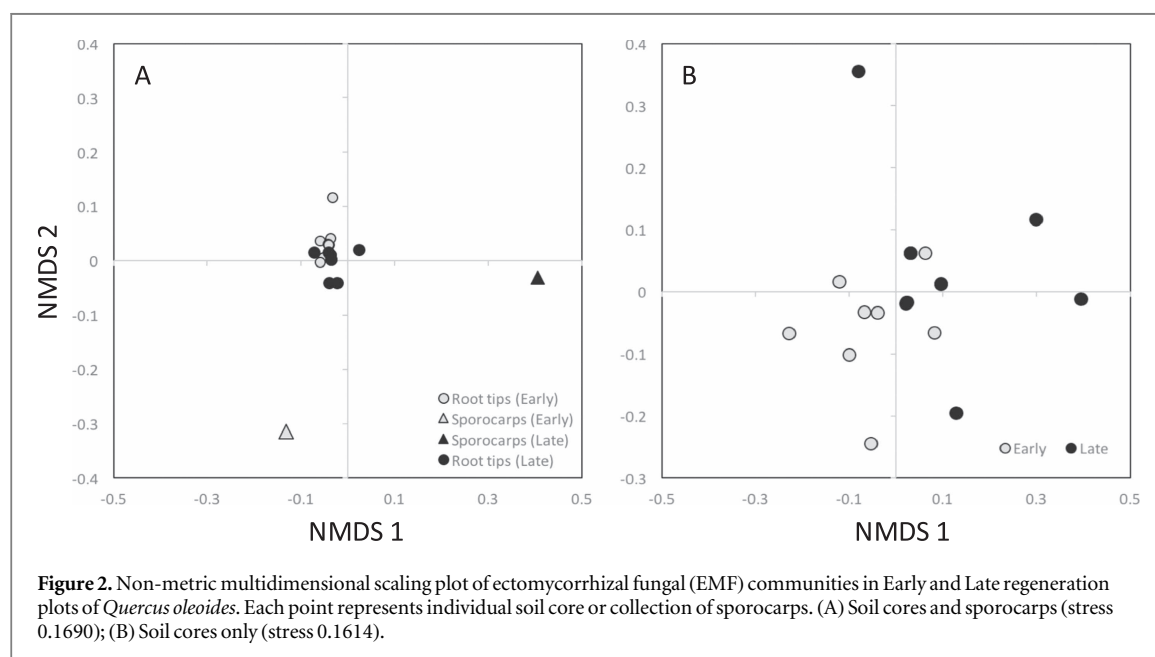
Twenty-five species of epigeous EMF fruiting bodies were identified. These represented 10 different genera: *Russula* (5 species), *Inocybe* (4), *Amanita* (3), *Lactarius* (3), *Clavulina* (2), *Scleroderma* (2), and one species each of *Cortinarius*, *Pisolithus*, *Strobilomyces*, *Thelephora*, *Retiboletus* and an unidentified Boletaceae. Eight of these had matches to root tip EMF taxa (figure 1): *Amanita* aff. *vaginata*, *Scleroderma* sp. 1, *Inocybe* sp. 1, *Lactarius* aff. *ruginosus*, *Lactarius* aff. *indigo*, *Pisolithus* *tinctorius*, *Russula* aff. *brevipes*, and *Russula* aff. *pectinata*. Two sporocarp samples were identified as *Cantharellus* (Late regeneration) but were

Table 1. Metrics of species richness and diversity in soil core and plot level in the Early and Late *Quercus oleoides* stands.

Level	Metric ^a	Early	Late	Significance (<i>P</i>)
Plot	Species richness	18	25	0.403
	Singletons	11	18	—
	Diversity	22.8	28.5	0.680
	Evenness	0.766	0.826	0.397
Core	Species richness	3.2 (0.5)	3.0 (0.5)	0.597
	Richness (Chao1)	5.85 (1.8)	5.55 (1.4)	0.101
	Diversity	3.39 (0.9)	2.27 (0.9)	0.038 ^b
	Evenness	0.926 (0.03)	0.935 (0.10)	0.751

^a Species richness is the total number of species present; Diversity is given as Fisher's alpha, Evenness is expressed as Simpson's E.

^b Values differ significantly at $P < 0.05$.



omitted from analyses due to poor sequence alignment using ITS markers.

3.2. EMF species richness and community structure

In total, 18 and 25 species of EMF, respectively, were found on Early and Late plots (table 1) with more singletons (rare taxa) detected in the Late than Early plot. With the exception of soil core diversity (Fisher's alpha), we found that EMF species richness, diversity and evenness did not differ significantly between plots. Soil core EMF diversity was significantly higher in Early than in Late plots.

NMDS showed that: (1) EMF assemblages in soil cores differed significantly from sporocarp assemblages (figure 2(A); ANOSIM, $r = 0.114$, $P = 0.006$); and (2) EMF sporocarp communities in Early and Late plots are quite distinct from one another. Axis 1 (68.9% variation) separated soil core communities from Late plot sporocarp assemblages, while Axis 2 (22.6% variation) discriminated Early plot sporocarp assemblages from soil core communities. Analyses of

soil cores alone (figure 2(B)) demonstrated that EMF composition in root tips was largely similar between plots (ANOSIM, $r = -0.175$, $P = 0.811$). Even so, the increasing dispersion of soil cores in Late plots in NMDS space suggests an increase in EMF heterogeneity with time.

3.3. Inter-regional phylogenetic relationships

Maximum likelihood phylogenetic analyses comparing EMF communities in *Q. oleoides* with those in four *Quercus* species is shown in figure 3. This inter-regional comparison showed that members of the Thelephoraceae and Russulaceae dominated all EMF communities, and the phylogenetic positions of *Q. oleoides* EMF were intermingled with EMF from other oak forests. As a result, analyses of beta diversity in *Quercus* showed that there were no significant differences ($P > 0.05$) in the phylogenetic statistics (P_{ST} , Π_{ST}) based on host, latitudinal position, seasonal moisture variability, or stand age (table 2).

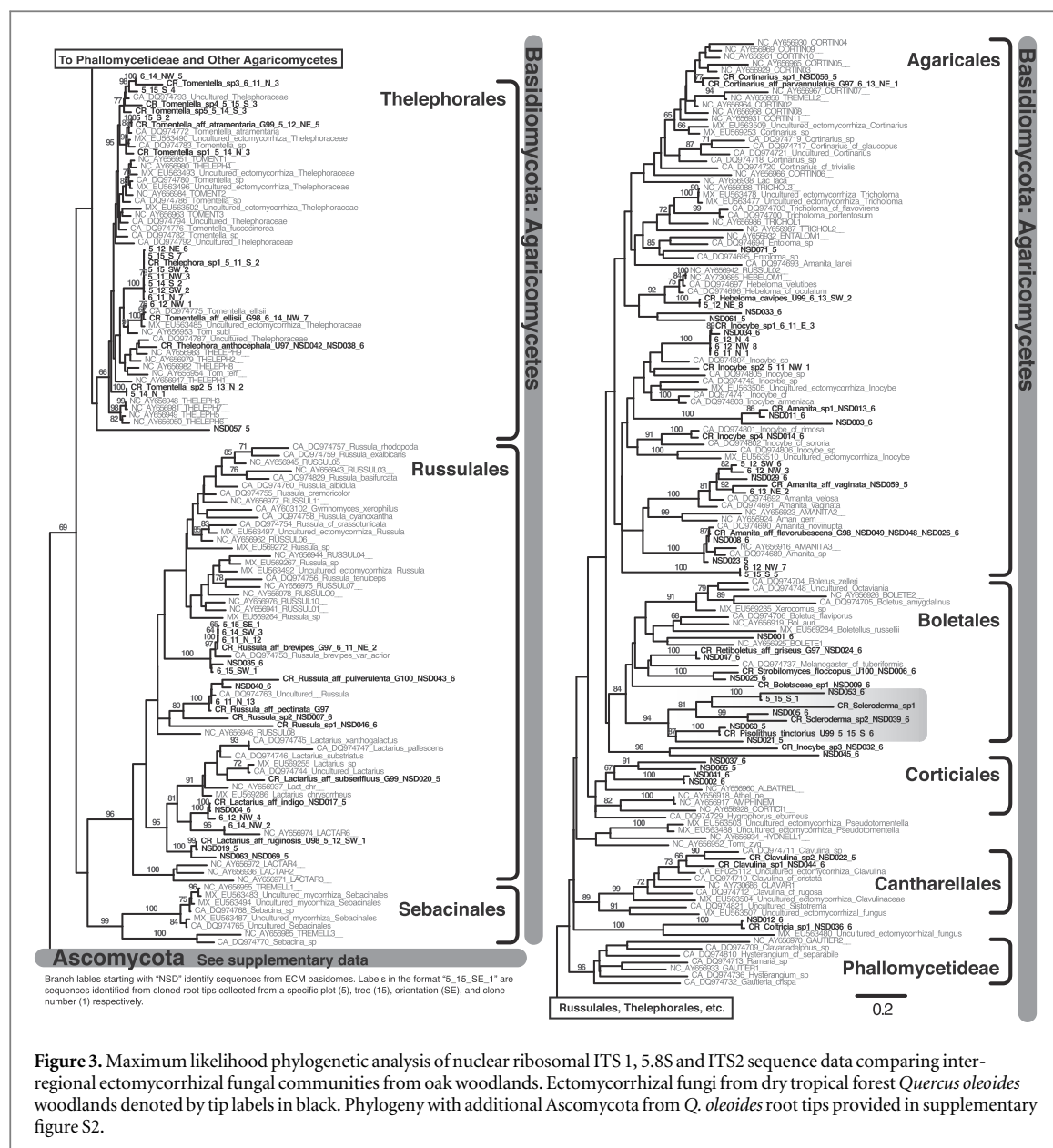
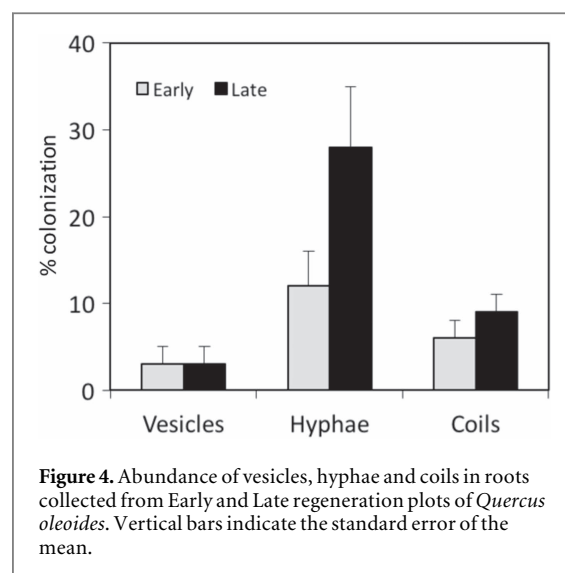


Table 2. Phylogenetic beta diversity analysis of inter-regional *Quercus* EMF communities.

Model	P_{ST}	P value	Π_{ST}	P value
Host <i>Quercus</i> species	0.021 49	0.730	0.001 39	0.224
Tropical versus temperate	0.010 33	0.460	0.001 33	0.486
Wet versus dry	0.013 17	0.164	0.004 15	0.155
Early versus late hosts	0.012 47	0.370	0.002 40	0.358

3.4. AMF root colonization

AMF hyphae, coils and vesicles were detected in *Q. oleoides* roots (figure 4). There was no significant difference in abundance of vesicles ($P = 0.919$) between Early and Late plots. Coils and hyphae were more abundant in roots from Late than Early plot but these differences were not significant (hyphae $P = 0.069$; coils $P = 0.084$).



4. Discussion

This is the first study to document both the diversity of EMF and the presence of AMF in *Q. oleoides* in the SDTF, a biome in which little is known of its mycorrhizal diversity. We found that *Q. oleoides* hosted distinct assemblages of EMF, EMF species richness and diversity were largely similar in stands of differing ages, and fine roots hosted both EMF and AMF. Additionally, comparisons of EMF communities in *Q. oleoides* versus those in *Quercus* species in a xeric woodland (Smith *et al* 2007), tropical cloud forest (Morris *et al* 2008), and temperate montane forest (Walker *et al* 2005) showed that *Q. oleoides* hosts a unique EMF community with few conspecific taxa and a marked absence of Ascomycota.

We documented 37 EMF taxa from *Q. oleoides* dominated forests. While our study may have been limited to a single sampling period and small spatial extent, this level of richness is in general agreement with previous studies of EMF communities in *Q. crassifolia* in montane cloud forests ($n = 42$; Morris *et al* 2008) and another study of *Q. oleoides* stands in this region (Waring *et al* 2016a, 2016b). Similar to temperate *Quercus* forests and tropical angiosperm forests, the EMF community in *Q. oleoides* was dominated by members of the Russulaceae and Thelephoraceae (Walker *et al* 2005, Smith *et al* 2007, Tedersoo *et al* 2007, Peay *et al* 2009). However, members of the Sebaciales and Pezizales, which are common constituents of temperate *Quercus* EMF communities, were not detected in our study. This outcome may reflect EMF community responses to the soil physico-chemical environment (Waring *et al* 2016a, 2016b), a level of EMF-host plant specificity, or a combination of both factors (Tedersoo *et al* 2007). Nevertheless, our results show that EMF were common and diverse in *Q. oleoides*, in terms of both species and known EMF lineages.

Contrary to expectations, most measures of species richness and diversity did not differ significantly between plots (Hypothesis 1). Instead, we detected more rare species (singletons) in the Late plot. In addition, we found greater core-to-core variation in EMF in Late than Early plots (NMDS); similar results have been noted in other forests (Peter *et al* 2001). Evidence suggests that young stands tend to be dominated by small, intermingled EMF genets while mature stands contain larger-sized genets that increase the number of EMF species combinations and spatial variability (Peter *et al* 2001). It is possible that similar processes operate in *Q. oleoides* stands but without further sampling, we cannot say with certainty whether stand age or other factors (biotic, abiotic) accounted for our results.

Age differentiated stands of *Q. oleoides* clearly hosted distinct suites of EMF fungi (Hypothesis 2). In fact, only five EMF taxa were common in soil cores between Early and Late plots. This transition suggests a degree

of succession within the EMF community. Both *Thelephora* and *Tomentella*, which were common in the Early plot, are considered pioneer EMF (Visser 1995). In comparison, species of *Russula* and *Lactarius* were encountered more often in the Late plot. These taxa have been categorized as later stage (successional) fungi (Last *et al* 1987, Visser 1995, Peay *et al* 2009, Wang *et al* 2012). Such shifts in community composition can provide insights into functional changes in *Q. oleoides*.

Both the Thelephoraceae and Russulaceae have been shown to be drought tolerant (Koide *et al* 2007) and are categorized as ‘contact type’ mycorrhizae that grow in close contact with the soil substrate (Agerer 2001). However, they vary in their capacity to acquire inorganic N (NO_3 , NH_4) and organic N (proteins, amino sugars). *Thelephora* species can utilize inorganic sources of N (Lilleskov *et al* 2002), which may benefit establishment of *Q. oleoides* during early stand development when soil inorganic N levels (as NO_3) are expected to be high (Sandoval-Pérez *et al* 2009). By contrast, *Russula* are considered to have a preference for organic N substrates. The prevalence of *Russula* along with the increase in the level of soil organic matter (% C) in the older plot (Powers *et al* 2009) would be consistent with this function. This result also suggests changes in EMF increase the capacity of *Q. oleoides* to access organic N sources as soils change with stand age, successional status, and host maturity (Cavender-Bares *et al* 2009; Peay *et al* 2009, Waring *et al* 2016a, 2016b).

Quercus oleoides roots were also colonized by AMF; this is the first study to document dual colonization in *Q. oleoides*. However, AMF colonization levels did not differ significantly between Early and Late plots (Hypothesis 3), a result consistent with another study in this region that was conducted across a wider range of site ages and forest types (Waring *et al* 2016b). However, AMF hyphal colonization was higher in roots from Late than Early plots. Possibly, this increase reflects a resource requirement in the mature trees that is better acquired by AMF such as P; *Q. oleoides* forests have lower soil P levels than other dry forest types (Powers *et al* 2009). Alternatively, AMF may be vital to the persistence of *Q. oleoides* during the dry season since AMF are implicated as an adaptation to prolonged drought in other *Quercus* (Querejeta *et al* 2009). Understanding the extent to which *Q. oleoides* seedlings and saplings are colonized by AMF and the functional consequences of these associations should be an important consideration for future conservation in the SDTF, especially in the establishment of *Q. oleoides* in AMF dominated pastures (Klemens *et al* 2011).

Finally, comparisons between *Q. oleoides* EMF communities and *Quercus* EMF communities in a cloud forest, xeric woodland, and temperate montane forest revealed no significant trend in phylogenetic turnover between these communities (Hypothesis 4).

This outcome is striking given that large woody plants like *Quercus* generally have climate-dominated niches (Hawkins *et al* 2011), host preference plays an important role in structuring *Quercus* EMF communities (Cavender-Bares *et al* 2009, Morris *et al* 2009) and there are environmental differences among sites. It is difficult to determine precisely what may be driving this pattern without further study. Evidence from plant communities (Chai *et al* 2016), suggests that a high degree of environmental variation (e.g. soil fertility) over very short distances can limit phylogenetic turnover. Instead, the unique EMF community in *Q. oleoides* reflects the presence of few conspecifics, under-represented taxa (*Cantharellus*) and the absence of Ascomycota.

Globally, efforts to conserve fungi lag well behind those of plants. The availability of long-term datasets is the strongest factor limiting EMF conservation, especially at large spatial and temporal scales. Our study, like many, only provided a snapshot of EMF diversity. Thus, the first essential step is to compile comprehensive EMF surveys (sporocarps, root tips) with which to identify and prioritize EMF for conservation, identify long-term associations, and identify sets of species that, in concert with environmental data, can indicate and predict ecosystem conditions.

Management practices include protecting habitat and providing future habitat through land management. The comparable levels of EMF diversity in *Q. oleoides* to other *Quercus* species suggests that regeneration practices to date (fire suppression) have been successful in conserving common EMF. However, the majority of EMF species were either uncommon or rare, and conserving these taxa may require inoculating *Q. oleoides* seedlings with specific EMF and oak plantings in pastures (Klemens *et al* 2011, Komonen *et al* 2015). Once established, these pastures could comprise reservoirs of EMF species with genes and species that can be exchanged with the *Q. oleoides* forest. Finally, the long-term persistence of EMF may be compromised by reductions in the abundance of its host. For example, the strong El Niño Southern Oscillation event of 2015 resulted in widespread mortality of *Q. oleoides* (Powers, personal observation). Thus, EMF should become special targets for conservation in this region, especially with the prediction of a future drier climate.

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