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Changes in ectomycorrhizal community structure on two containerized oak hosts across an experimental hydrologic gradient

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Abstract Shifts in ectomycorrhizal (ECM) community structure were examined across an experimental hydrologic gradient on containerized seedlings of two oak species, *Quercus montana* and *Quercus palustris*, inoculated from a homogenate of roots from mature oak trees. At the end of one growing season, seedlings were harvested, roots were sorted by morphotype, and proportional colonization of each type was determined. DNA was subsequently extracted from individual root tips for polymerase chain reaction, restriction fragment length polymorphism, and rDNA sequencing of the ITS1/5.8S/ITS2 region to determine identities of fungal morphotypes. Twelve distinct molecular types were identified. Analysis of similarity showed that ECM fungal assemblages shifted significantly in composition across the soil moisture gradient. Taxa within the genus *Tuber* and the family *Thelephoraceae* were largely responsible for the changes in fungal assemblages. There were also significant differences in ECM community assemblages between the two oak host species. These results demonstrate that the structure of ECM fungal communities depends on both the abiotic and biotic environments and can shift with changes in soil moisture as well as host plant, even within the same genus.

Keywords Ectomycorrhizae · Community assembly · Soil moisture gradient · *Quercus montana* · *Q. palustris* · Molecular typing · ITS region

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Introduction

The interactions between the abiotic environment, mycorrhizal community assemblages, and host plants are currently not well-understood. These interactions have important consequences for plant performance in ecological settings as well as whole ecosystem processes (Klironomos et al. 2000; Maherali and Klironomos 2007), and they are critical for understanding the mechanisms that maintain both plant and fungal diversity. Factors controlling spatial variation in mycorrhizal assemblages (including both ectomycorrhizal fungi [ECM] and arbuscular mycorrhizal fungi [AM]) are, therefore, an active area of research. Like plants, mycorrhizal distributions are influenced by both abiotic and biotic factors (Bruns 1995), and heterogeneity of fungal communities are likely to be maintained by both soil heterogeneity and host plant characteristics (Abbott and Robson 1981;

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Klironomos et al. 1993; Klironomos 1995; Dickie et al. 2002b). Previous studies have also shown that individual mycorrhizal species can have different effects on their hosts, influence plant performance, and can alter allocation of resources (Dickie et al. 2002a; Klironomos 2003; van der Heijden et al. 2003). Therefore, abiotic factors that influence fungal communities can have feedbacks to plant performance.

Specifically with respect to ectomycorrhizal fungi, shifts in soil conditions or other abiotic factors, including soil temperature (Domisch et al. 2002), nutrient addition (Avis et al. 2003), elevated CO_2 (Godbold and Berntson 1997), and drought (Shi et al. 2002), have been shown to alter ECM community structure. In ecological settings, oaks are functionally obligately associated with a diversity of ECM fungi (included in the Ascomycota and Basidiomycota) and benefit from the symbiosis in terms of growth, seedling establishment, and survival (e.g., Avis et al. 2003; Smith et al. 2007a, b; Morris et al. 2008a).

While a large number of ECM taxa (~250) are specific at the family or genus level (Molina et al. 1992; Ishida et al. 2007), it is not known how commonly specificity occurs below the genus level. Individual fungi can function differently on different host plants and the same fungus can even form different types of symbioses (i.e., ectomycorrhizae, arbutoid, or orchid mycorrhizae) with different degrees of penetration into and between root cells depending on the host (Taylor and Bruns 1999; Villarreal-Ruiz et al. 2004). Thus, while ectomycorrhizal fungi may not require specific hosts, individual hosts may express preferences for certain fungal species over others when given the "choice." There is increasing evidence that strongly host-preferring fungi may dominate ECM communities (Tedersoo et al. 2008). Van der Heijden and Kuyper (2001) showed that genetic variation within a host plant species led to intraspecific differences in ECM and AM fungal colonization, indicating that recognition signals and specific gene for gene interactions may be involved that allow host plant selectivity. Such preferences may be dependent on other factors, including soil conditions. Within diverse communities of hosts and ECM fungi, selectivity may occur such that certain host–fungal associations are more likely to occur than others (Dickie 2007). A recent study found contrasting ECM communities on the roots of sympatric oaks in California, demonstrating that host plant species can be important in ECM assemblages even within the same genus (Morris et al. 2008b).

Attempts to study spatial variation in ECM fungal assemblages have been complicated by the difficulty in identifying ECM fungi. Advances in molecular techniques have allowed consistent identification of ectomycorrhizal taxa and have facilitated research on these issues in ECM-dominated systems (Gardes et al. 1991, 1996; Bruns et al. 1998, 2001; reviewed in Horton and Bruns 2001). Oak ECM fungi, in particular, have proven difficult to work with because oaks have very small fine roots compared to other ECM host species. However, a series of studies using molecular methods have demonstrated the feasibility of these methods for oak–ectomycorrhizal associations (e.g., Cooke et al. 1999; Pinkas et al. 2000; Nechwatal et al. 2001; Giomaro et al. 2002; Avis et al. 2003; Kennedy et al. 2003; Dickie and Fitzjohn 2007). In this study, we used molecular approaches to test whether ECM community structure shifts in response to an abiotic factor, soil moisture, and a biotic factor, host species. Specifically, the purpose of the study was to determine (1) whether ectomycorrhizal assemblages change in response to experimental changes in soil moisture regime and (2) whether oak hosts from two different phylogenetic lineages show differences in selectivity of ECM fungi. We used an experimental approach in which seedlings were grown in outdoor microcosms in order to standardize the inoculum and control water availability to each individual host plant.

Materials and methods

Ectomycorrhizal inoculation

Ectomycorrhizal infected roots were collected 5–10 cm depth from mature oak trees in two 1-m^2 plots from two separate sites within the Smithsonian Environmental Research Center (SERC) forest in Edgewater Maryland. The forest encompasses 2,800 ac (1,133.16 h) on the western shore of the Chesapeake Bay, and the canopy is comprised largely of hardwoods from 22 genera including nine *Quercus* species. The first site was located beneath a mature tree of *Quercus montana* (=*Q. prinus*; chestnut oak) and the second was beneath a mature tree of *Quercus palustris* (swamp oak), each in monospecific stands. Roots were excavated to check for connectivity to the mother tree and permitted visible confirmation of ectomycorrhizal presence. Root segments were then collected with minimal agitation so that surrounding soil remained in place. Segments were cut into ~1cm pieces combined from both sites and manually homogenized. The soil–root–ectomycorrhizae homogenate ("soil inoculum") was kept moist and used to inoculate experimental oak seedlings on the same day. Inoculum thus consisted of fungi coming off of mature tree roots that we added to seedling containers (see below), as well as possible airborne spores. The specific composition of the inoculum was not identified but was the same for all treatments.

Cultivation of plants

Seedlings of *Q. montana* and *Q. palustris* were grown from seed in 36-cm tall germination pots with (total volume of

983 cm^3) in a 1:1 mixture of thoroughly homogenized potting soil and silica sand. Seeds were collected locally from multiple maternal trees within a 2-ha area of the Big Tree Plot. After 1 year, seedlings were replanted in 15-cm diameter pots, 30 cm deep. Seedlings showed no visible sign of ECM infection at the time of transplanting. In the bottom third of the pot, 10 g of the soil inoculum was incorporated into the soil. The seedlings were then placed in an outdoor rainout shelter in April for 60 days and watered regularly to establish mycorrhizal infection. Following this period, ten plants of each species were placed under each of three water treatments (60 plants total). Plants under the wet treatment were watered twice daily with 900 mL of water each time; plants in the medium treatment were watered three times a week with 900 mL of water each time; and plants in the dry treatment were watered once weekly with 900 mL of water. Plants were grown for 2 months under these water regimes beneath an outdoor rainout shelter that prevented any additional rainfall. Treatments corresponded to percent soil moisture values (mass H_2O per mass soil) as follows: dry, 8.9% (±0.09); med, 15.2% (±0.09); and wet, 25.0% (±0.08). Soil moisture values were based on gravimetric measurements in all pots 1 month after initiation of the treatments. These treatments correspond to natural hydrologic gradients within the SERC forest that vary from floodplain to upland sites (J. Cavender-Bares, unpublished). Plants were harvested, and ectomycorrhizal colonization was examined within a 2-week period. Not all of the initially planted seedlings survived the duration of the experiment, particularly in the dry treatment.

Root tip collection and mycorrhizal morphotypes

Whole intact root systems of each plant were removed from the soil and washed in a mild surfactant to remove soil particles. The root systems were separated into the top third, the middle third, and the bottom third. A representative sample of six 3.5-cm root pieces was taken from each third of the root system. These root samples were placed in a glass Petri dish with water and examined at ×10 magnification under a dissecting scope. Ectomycorrhizal morphotypes were distinguished based on color, surface appearance, hyphal abundance, and branching pattern. Effort was made to overestimate differences in morphotypes during this screening process. The number of root tips infected with each morphotype of ECM fungi was recorded along with the number of uninfected root tips for each sample. An average of 724 (±38) tips were examined per plant. Two root tips of each morphotype were taken from each plant for DNA isolation and subsequent molecular identification. Once morphotypes were matched with genotypes, this data was used to calculate proportional colonization of each fungal genotype.

Molecular identification of ECM fungi

DNA extraction and PCR A drop of distilled, deionized water was added to a microcentrifuge tube with the fresh root tip, which was lacerated with a bead beater using a 3-mm sterile glass bead. DNA was extracted using a standard protocol (DNeasy Plant and Fungal Mini Kit, Qiagen, Valencia, CA, USA). The internally transcribed spacer regions of the rDNA were amplified using the primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). Polymerase chain reaction (PCR) was performed in a PTC-100 thermal cycler (MJ Research, Waltham, MA, USA) in conditions previously described (Gardes and Bruns 1993).

RFLP analysis and DNA sequencing Each PCR sample was then digested for restriction fragment length polymorphism (RFLP) using *Hin*fI and *Alu*I restriction enzymes (New England Biolabs, Beverly, MA, USA) as per manufacturer's suggested conditions. Agarose gel electrophoresis was used to size the fragments generated from the RFLP. Fragment sizes were calculated using the Gelreader software (NCBI). Samples with similar morphotypes were run side by side to increase the likelihood of identifying small differences in band sizes. RFLP types that appeared similar (±5% fragment length) were grouped together. We sequenced representatives of each unique RFLP type for identification and to clarify the validity of the RFLP typing. Multiple representatives were sequenced for RFLP types that were frequent. To avoid overgrouping samples within a RFLP type, we sequenced two samples from each type that had a continuum of sizes.

Single-pass sequencing of the ITS1/5.8S/ITS2 region of the rDNA was performed on an ABI3100 Genetic Analyzer and analyzed with the Sequence Analysis 3.4.1 software (Applied Biosystems, Foster city, CA, USA). Internal transcribed spacer (ITS) sequences were initially grouped based on similar BLAST (Altschul et al. 1990) affinities, aligned using the software package ClustalX (Jeanmougin et al. 1998), and then manually adjusted. Pairwise sequence distances were calculated in phylogenetic analysis using parsimony (Swofford 2001) with adjustment so that gaps were not included. ECM taxon names were designated based upon the taxonomic level supported from the BLAST results. For example, a RFLP type sequence whose BLAST score sequence grouped closely with a wide range of ascomycetes would be named Ascomycota. Numbering identified RFLP types as unique roughly at the species level. DNA sequences from this study have been submitted to the GenBank (accession numbers: FJ008030–FJ008041).

While exact taxonomic identification was not critical for this study, we attempted to evaluate the affinities of each of the sequence types to further our understanding of oak–

Table 1 DNA sequence analysis results of the ITS1/5.8S/ITS2 region from RFLP types based on GenBank blast searches

Sequence type	Accession no.	Base pairs used[a]	Best match to vouchered specimen	Percent match/ no. of bp[b]	*E* value	Max ident (%)	Image	Estimate of taxonomic affinities	*Hinfl* and *AluI* fragment sizes
AGAR	FJ008036	629	DQ486700 *Entoloma sinuatum*	88/629	0.0	89	Fig. 1a	Agaricales	H: 400/335/260 A: 275/200/ 160/140
ASCO1	FJ008037	509	AF351582 *Trichophaea hybrida*	90/198	1E-64	90	Fig. 1e	Ascomycota	H: 300/220/110 A: 620
ASCO2	FJ008038	579	AF351582 *Trichophaea hybrida*	90/198	1E-64	90	Fig. 1c	Ascomycota	H: 220/130/110/ 90/80 A: 370/170/80
ASCO3	FJ008039	576	AY219841 *Wilcoxina mikolae*	89/198	3E-60	89	Fig. 1j	Ascomycota	H: 325/300 A: 360/170/90
ASCO4	FJ008040	578	AY219841 *Wilcoxina mikolae*	88/198	2E-58	88	Not shown	Ascomycota	H: 325/210/90 A: 450/170
CENO	FJ008033	494	AY394919 *Cenococcum geophilum* isolate	99/494	0.0	99	Fig. 1b	*Cenococcum*	H: 160/120/110/ 90/85 A: 380/190
THEL1	FJ008035	580	AF272912 *Tomentella botryoides*	98/552	0.0	99	Fig. 1i	*Tomentella*	H: 200/180/145 A: 460/90
THEL2	FJ008034	653	DQ974776 *Tomentella fuscocinerea*	93/633	0.0	93	Fig. 1f	*Tomentella*	H: 180/145 A: 460/190
THEL3	FJ008041	574	EU427330 *Thelephora terrestris*	93/574	0.0	93	Fig. 1g	Thelephora	H: 355/165/150 A: 460/160
TUB1	FJ008032	570	AJ278140 *Tuber maculatum*	87/568	0.0	88	Fig. 1k	Tuberaceae/ *Tuber*	H: 370/190/120 A: 560/100
TUB2	FJ008030	568	DQ402505 *Tuber borchii* isolate ECMm2	95/457	0.0	95	Fig. 1h	Tuberaceae/ *Tuber*	H: 370/170/100 A: 605/120
TUB3	FJ008031	640	AF003917 *Tuber dryophilum*	89/550	0.0	89	Fig. 1d	Tuberaceae/ *Tuber*	H: 370/175/100 A: 700

Taxonomic study IDs were assigned to each sequence type representing at least 4% DNA sequence distance. The length of the sequence used in the BLAST search and the taxon names for the best match to a vouchered specimen in the GenBank are given, along with the percentage match. RFLP fragment sizes are approximate and do not reflect fragments below 80-bp size or multiple fragments of the same length

[a] The number of base pairs used for the BLAST analysis

[b] The percent sequence similarity that the best match had across the number of base pairs where the match occurred

ectomycorrhizal fungal associates. The sequence types that we assigned to each morphotype were a reflection of our best estimate of these taxonomic affinities based on not only the strongest sequence match to a named vouchered specimen, but also to other vouchered specimens that had similarly strong matches.

Statistical analysis

We used analysis of similarity (ANOSIM) devised by Clarke (1993) using the computer program PRIMER 5.1 to determine whether ECM fungal assemblages differed between host species and between soil moisture treatments. The advantage of the ANOSIM test is that it does not assume any underlying distribution to the data; instead, it is a nonparametric test, based only on the rank order of the matrix values. The parameters were set to analyze similarity between samples using the Bray–Curtis index. Data were standardized and square root transformed prior to analysis. The SIMPER procedure was used to determine which taxa where most important in determining similarity among and between treatment groups. Analysis of variance (ANOVA) was used to test for effects of host and soil moisture treatments on ECM species richness and percent colonization of roots (arcsine transformed). Adequacy of the models was assessed by inspecting residual plots.

Fig. 1 Images show 11 of the morphotypes identified on roots of *Q. palustris* or *Q. montana* that were associated with the sequence types in Table 1: **a** AGAR, **b** CENO, **c** ASCO2, **d** TUB3, **e** ASCO1, **f** THEL2, **g** THEL3, **h** TUB2, **i** THEL1, **j** ASCO3, **k** TUB1. ECM are shown on *Q. palustris* roots for **a**, **b**, **c**, **e**, **f**, **h**, **i**, **j**, **k** and on *Q. montana* roots for **d** and **g**. *White bars* indicate 300 μm. Details of taxon assignments are given in Table 1 ▶


a
300 µm
b
c
d
e
f
g
h
i
j
k

Table 2 Relative abundance, measured as mean percent colonization (PC) of root tips±SE, for ECM fungal taxa across three soil moisture treatments (dry, medium, and wet) on two oak hosts, *Q. palustris* and *Q. montana*

Fungal taxon	*Q. palustris*									*Q. montana*								
	Dry			Med			Wet			Dry			Med			Wet		
	PC	SE	*P*	PC	SE	*P*	PC	SE	*P*	PC	SE	*P*	PC	SE	*P*	PC	SE	*P*
AGAR	0	0	0	0	0	0	3.47	±1.50	0.63	0	0	0	0	0	0	0	0	0
ASCO1	0	0	0	7.63	±5.82	0.63	0	0	0	0	0	0	0	0	0	0	0	0
ASCO2	0	0	0	0	0	0	0	0	0	0	0	0	2.96	±2.96	0.14	0	0	0
ASCO3	0.12	±0.12	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ASCO4	0.12	±0.12	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CENO	1.7	±1.70	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
THEL1	0.5	±0.37	0.4	0	0	0	0	0	0	0	0	0	0	0	0	0.18	±0.18	0.17
THEL2	0	0	0	8.41	±3.96	0.63	0	0	0	2.03	±1.06	0.67	4.21	±2.44	0.71	0	0	0
THEL3	0	0	0	0	0	0	0	0	0	0	0	0	2.8	±2.70	0.29	0	0	0
TUB1	6.87	±1.20	1	16.7	±6.29	1	9.3	±2.4	0.75	0.25	±0.25	0.33	1.06	±0.73	0.29	5.38	±4.22	0.5
TUB2	18.6	±8.96	1	7.99	±3.63	0.88	34.7	±8.13	1	17.7	±13.55	0.67	12.31	±4.97	0.71	20.13	±7.94	0.83
TUB3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.23	±2.23	0.17
N			5			8			8			3			7			6
Percent colonization[a]	39.2	±11.51		50.4	±8.20		54.1	±5.86		40.3	±11.16		37.49	±6.57		47.57	±6.79	

Taxon names are explained in Table 1

P proportion of seedlings on which fungal taxon occurs within each oak host and treatment, *N* final seedling samples sizes

[a] Mean percentage of total ECM colonized root tips±SE

Results

Fungi detected on seedling roots

The conservative progression from morphotypes to RFLP types to sequence types allowed us to refine our identifications and, ultimately, all comparisons were based on the final sequence types. For example, we identified a mean of 4.3 ECM morphotypes per plant compared to a final of 2.4 sequence types per plant. For over 91% of the infected tips, we were able to match morphotypes to sequences unambiguously. For <9% of tips, we were not able to identify tips unambiguously, and these data were not included in the analysis. A total of 24 unique RFLP types were identified based on the patterns obtained with two different restriction enzymes. DNA sequence analysis of these RFLP types revealed that many of the band differences leading to their uniqueness ended up being attributable to variation in gel electrophoresis. Therefore, only 12 fungal sequence types were identified overall (Table 1; Fig. 1).

BLAST similarities to samples contained in the GenBank allowed us to estimate approximate taxonomic affiliations for most of the sequence types. The most frequently occurring types were affiliated with the family Tuberaceae (TUB1, TUB2, and TUB3) and the Thelephoraceae (THEL1, THEL2, and THEL3). The Tuberaceae samples primarily matched the well-represented *Tuber* species in the GenBank. The Thelephoraceae samples showed strong affinities to the genera *Tomentella* (THEL1 and THEL2) and *Thelephora* (THEL3). Four types were associated with Ascomycota (ASCO1, ASCO2, ASCO3, and ASCO4), although a more definitive grouping was not obvious based on equal matches to a wide range of ascomycetes. Other taxa detected included *Cenococcum geophilum* (CENO) and one type that had affinities within the Agaricales (AGAR).

Evidence for host selectivity in ECM assemblages ANOSIM showed significant differentiation in ECM fungal assemblages between hosts for both relative abundance and presence/absence data (Tables 2 and 3). Differences in relative abundance of two *Tuber* types (TUB1 and TUB2), a Thelephoraceae (THEL2), and an Ascomycota species (ASCO1) contributed to the host differentiation. For the presence/absence data, the increased frequency of occurrence of TUB1 on *Q. palustris* and of THEL2 on *Q.*

Table 3 ANOSIM results for host selectivity and soil moisture based on relative abundance and presence/absence of ECM fungal types

	Sample statistic (global *R*)	*P* value
Host		
Relative abundance	0.269*	0.004*
Presence/absence	0.35*	0.002*
Soil moisture		
Relative abundance	0.382*	0.001*
Presence/absence	0.241*	0.001*

*$P<0.05$

Table 4 Pairwise tests for differentiation between treatments for relative abundance and presence/absence data

Groups	*R* statistic	*P* value
Relative abundance		
Dry–med	0.065	0.279
Dry–wet	0.188**	0.06**
Med–wet	0.372*	0.002*
Presence/absence		
Dry–med	0.311*	0.007*
Dry–wet	0.324*	0.005*
Med–wet	0.469*	0.001*

*$P<0.05$, significant contrasts; **$P<0.10$, marginally significant contrasts

montana were largely responsible for the host effect. Furthermore, CENO, AGAR, ASCO3, and ASCO4 occurred only on *Q. palustris*, while ASCO2 occurred only on *Q. montana*, although frequencies of these taxa were low.

ECM assemblages shifted with soil moisture ECM fungal assemblages also differed significantly across soil moisture treatments (Table 3). For relative abundance data, pairwise comparisons show that medium and wet treatments differed significantly ($P=0.002$; Table 4), dry and wet treatments were marginally different ($P=0.06$), and medium and dry treatments were not significantly different. Pairwise comparisons of presence/absence of ECM taxa demonstrated more clearly that hydrologic conditions altered ECM communities associated with the two oak species (Table 4). In this analysis, all treatments differed significantly in ECM composition ($P=0.001$ to $P=0.007$). Variation in the relative abundance of TUB2 contributed strongly to the soil moisture effect for the relative abundance data; the increased presence of THEL2 in the medium moisture treatment relative to the dry and wet treatments contributed strongly to the soil moisture effect for the presence/absence data. THEL2 and two of the Ascomycota taxa (ASCO1 and ACO2) occurred only in the medium treatment, *C. geophilum* and the two Ascomycota types (ASCO3 and ASCO4) occurred only in the dry treatment, whereas TUB3 and AGAR occurred only in the wet treatment. *C. geophilum* may include cryptic species (Douhan and Rizzo 2005; Douhan et al. 2007), but at least some of the species identified as *C. geophilum* are known to survive under extremely dry conditions (−5.5 MPa) and can survive desiccation (Pigott 1982).

Species richness and percent colonization ANOVA showed that species richness of ECM species did not differ significantly across water treatments ($F_{2,31}=2.12$, $P=0.137$), but there was a significant effect of host on species richness ($F_{1,31}=13.23$, $P=0.001$). *Q. palustris* had a higher average number of ECM species per individual host plant than *Q. montana* (2.8±0.15 and 1.87±0.24, respectively). The interaction between host species and water treatment was not significant. There was no difference between soil moisture treatments ($F_{2,31}=0.95$, $P=0.399$) or between host species ($F_{1,31}=1.1$, $P=0.303$) in percent colonization of root tips and no interaction.

Discussion

This study presents two important findings for the structure of ECM communities. First, we found that the composition of ECM assemblages shifted significantly across a hydrologic gradient on two oak host species. These results suggest that the ECM communities respond either directly to water availability in the environment and/or to host signals of water stress and reduced growth rates. ECM species have been observed to show differential sensitivity to drought (e.g., Coleman et al. 1989; Shi et al. 2002), soil type, soil depth, and other abiotic variables (e.g., Dickie et al. 2002b; Moser et al. 2005; Korkama et al. 2006) but have also recently been shown to vary strongly with plant growth rates (Korkama et al. 2006). It remains to be explored whether shifts in ECM communities over variation in water availability serve to (1) buffer (or minimize) the effects of variation in water availability on the host, (2) exacerbate the effects of water availability gradients, or (3) have no effect. There is evidence in pine systems, for example, suggesting that water transfer from ECM hyphae to roots differs both among ECM types and among host species (Plamboeck et al. 2007). To the extent that ECM species differ in their ability to ameliorate water stress, shifting fungal composition with soil moisture could have important consequences for drought responses of plants (Kennedy and Peay 2007).

Second, we found evidence for ECM community shifts across hosts. Earlier studies have suggested that closely related host species, such as trees from the same genus or family, support similar ECM communities (Horton and Bruns 1998; Cullings et al. 2000). A recent study of sympatric oaks from different subgenera, however, shows evidence that ECM community assemblages vary with host species (Morris et al. 2008b). Therefore, some degree of divergence in specificity among oaks of different subgenera may be expected. In this study, while the most frequent ECM species (those in the genus *Tuber* and a Thelephoroid species) were found on both *Quercus* hosts, the ECM communities on the two hosts differed in their abundance and frequency. Our results thus suggest that the two oak species, which represent different subgenera, do partition the ECM symbiotic environment, similar to the Morris et al. (2008b) study. Divergence times between the two oak lineages based on minimum fossil ages are estimated at

40 mya (Daghlian and Crepet 1983; Manos et al. 1999), and the species are intersterile (Nixon 1997). Partitioning of symbionts may increase the local diversity of ECM taxa. It may also facilitate the resource partitioning and co-occurrence of these distantly related oak lineages, which are known to co-occur more often than oak species within the same lineage (Mohler 1990; Cavender-Bares et al. 2004a, b).

The diversity (defined as species richness) of the ECM assemblages in this study was low compared to field studies (Dickie 2007). Limited diversity may be expected in short-term microcosm experiments where colonization is restricted largely to ECM species in the inoculum. Other soil fungal groups in the SERC forest have had similar diversity to other systems (e.g., AM fungi, C.E. Lovelock, unpublished data; orchid fungi, McCormick et al. 2004). *Acer rubrum*, a species that is known to limit ECM colonization of oaks (Dickie et al. 2002a), is very common in the SERC forest (Lovelock and Miller 2002; Parker and Tibbs 2004), which could have limited ECM abundance in the inoculum. Despite low ECM diversity, we still detected an ECM community response to a gradient in water availability, perhaps suggesting that water availability may have an even stronger effect in other forest systems.

In conclusion, our data support the idea of strong abiotic and biotic controls on ECM communities in oak forests. Whether water availability affects ECM fungal assembly directly or indirectly through variation in plant growth rates is yet to be determined. However, because of strong effects of ECM species on plant performance and competitive interactions (Pande et al. 2007), we anticipate that ECM species could amplify or ameliorate suboptimal environmental conditions, thereby influencing tree community composition.

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References

Abbott LK, Robson AD (1981) Infectivity and effectiveness of five endomycorrhizal fungi—competition with indigenous fungi in field soils. Aust J Agric Res 32:621–630. doi:10.1071/AR9810621

Altschul SF, Gish W, Miller W, Myers EW, Lipman D (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Avis PG, McLaughlin DJ, Dentinger BC, Reich PB (2003) Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. New Phytol 160:239–253. doi:10.1046/j.1469-8137.2003.00865.x

Bruns TD (1995) Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. Plant Soil 170:63–73. doi:10.1007/BF02183055

Bruns TD, Szaro TM, Gardes M, Cullings KW, Pan JJ, Taylor DL, Horton TR, Kretzer A, Garbelotto M, Li Y (1998) A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. Mol Ecol 7:257–272. doi:10.1046/j.1365-294X.1998.00337.x

Bruns TD, Lilleskov EA, Bidartondo MI, Horton TR (2001) Patterns in ectomycorrhizal community structure in pinaceous ecosystems. Proceedings of the Joint Meeting of the American Phytopathological Society, the Mycological Society of America, and the Society of Nematologists, pp S163–S164

Cavender-Bares J, Ackerly DD, Baum DA, Bazzaz FA (2004a) Phylogenetic overdispersion in Floridian oak communities. Am Nat 163:823–843. doi:10.1086/386375

Cavender-Bares J, Kitajima K, Bazzaz FA (2004b) Multiple trait associations in relation to habitat differentiation among 17 Floridian oak species. Ecol Monogr 74:635–662. doi:10.1890/03-4007

Clarke K (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol 18:117–143

Coleman M, Bledsoe C, Lopushinsky W (1989) Pure culture response of ectomycorrhizal fungi to imposed water stress. Can J Bot 67:29–39. doi:10.1139/b89-005

Cooke DEL, Jung T, Williams NA, Schubert R, Bahnweg G, Osswald W, Duncan JM (1999) Molecular evidence supports *Phytophthora quercina* as a distinct species. Mycol Res 103:799–804. doi:10.1017/S0953756299008606

Cullings K, Vogler D, Parker V, Finley S (2000) Ectomycorrhizal specificity patterns in a mixed *Pinus contorta* and *Picea engelmannii* forest in Yellowstone National Park. Appl Environ Microbiol 66:4988–4991. doi:10.1128/AEM.66.11.4988-4991.2000

Daghlian CP, Crepet WL (1983) Oak catkins, leaves and fruits from the Oligocene Catahoula formation and their evolutionary significance. Am J Bot 70:639–649. doi:10.2307/2443119

Dickie IA (2007) Host preference, niches and fungal diversity. New Phytol 174:230–233. doi:10.1111/j.1469-8137.2007.02055.x

Dickie IA, Fitzjohn RG (2007) Using terminal restriction fragment length polymorphism (T-RFLP) to identify mycorrhizal fungi: a methods review. Mycorrhiza 17:259–270. doi:10.1007/s00572-007-0129-2

Dickie IA, Koide RT, Steiner KC (2002a) Influences of established trees on mycorrhizas, nutrition, and growth of *Quercus rubra* seedlings. Ecol Monogr 72:505–521

Dickie IA, Xu B, Koide RT (2002b) Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. New Phytol 156:527–535. doi:10.1046/j.1469-8137.2002.00535.x

Domisch T, Finer L, Lehto T, Smolander A (2002) Effect of soil temperature on nutrient allocation and mycorrhizas in Scots pine seedlings. Plant Soil 239:173–185. doi:10.1023/A:1015037127126

Douhan GW, Rizzo DM (2005) Phylogenetic divergence in a local population of the ectomycorrhizal fungus *Cenococcum geophilum*. New Phytol 166:263–271. doi:10.1111/j.1469-8137.2004.01305.x:1-9

Douhan GW, Martin DP, Rizzo DM (2007) Using the putative asexual fungus *Cenococcum geophilum* as a model to test how species concepts influence recombination analyses using sequence data from multiple loci. Curr Genet 52:191–201. doi:10.1007/s00294-007-0150-1

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Gardes M, Fortin J, White TJ, Bruns TD, Taylor JW (1991) Identification of indigenous and introduced symbiotic fungi in mycorrhizae by amplification of the internal transcribed spacer. Can J Bot 69:180–190

Gardes M, Bruns TD, Clapp JPE (1996) ITS-RFLP matching for identification of fungi. In: Clapp JPE (ed) Methods in molecular biology; species diagnostics protocols: PCR and other nucleic acid methods. Humana, Louisville, KY, pp 177–186

Giomaro G, Sisti D, Zambonelli A, Amicucci A, Cecchini M, Comandini O, Stocchi V (2002) Comparative study and molecular characterization of ectomycorrhizas in *Tilia americana* and *Quercus pubescens* with *Tuber brumale*. FEMS Microbiol Lett 216:9–14. doi:10.1111/j.1574-6968.2002.tb11407.x

Godbold DL, Berntson GM (1997) Elevated atmospheric CO_2 concentration changes ectomycorrhizal morphotype assemblages in *Betula papyrifera*. Tree Physiol 17:347–350

Horton TR, Bruns TD (1998) Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas fir (*Pseudotsuga menziesii)* and bishop pine (*Pinus muricata*). New Phytol 139:331–339. doi:10.1046/j.1469-8137.1998.00185.x

Horton TR, Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Mol Ecol 10:1855–1871. doi:10.1046/j.0962-1083.2001.01333.x

Ishida TA, Nara K, Hogetsu T (2007) Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer-broadleaf forests. New Phytol 174:430–440. doi:10.1111/j.1469-8137.2007.02016.x

Jeanmougin F, Thompson J, Gouy M, Higgins D, Gibson T (1998) Multiple sequence alignment with Clustal X. Trends Biochem Sci 23:403–405

Kennedy PG, Peay KG (2007) Different soil moisture conditions change the outcome of the ectomycorrhizal symbiosis between *Rhizopogon* species and *Pinus muricata*. Plant Soil 291:155–165. doi:10.1007/s11104-006-9183-3

Kennedy PG, Izzo AD, Bruns TD (2003) There is high potential for the formation of common mycorrhizal networks between understorey and canopy trees in a mixed evergreen forest. J Ecol 91:1071–1080. doi:10.1046/j.1365-2745.2003.00829.x

Klironomos JN (1995) Arbuscular mycorrhizae of *Acer saccharum* in different soil types. Can J Bot 73:1824–1830. doi:10.1139/b95-193

Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301. doi:10.1890/02-0413

Klironomos JN, Moutoglis P, Kendrick B, Widden P (1993) A comparison of spatial heterogeneity of vesicular–arbuscular mycorrhizal fungi in two maple forest soils. Can J Bot 71:1472–1480

Klironomos JN, McCune J, Hart M, Neville J (2000) The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. Ecol Lett 3:137–141. doi:10.1046/j.1461-0248.2000.00131.x

Korkama T, Pakkanen A, Pennanen T (2006) Ectomycorrhizal community structure varies among Norway spruce (*Picea abies*) clones. New Phytol 171:815–824. doi:10.1111/j.1469-8137.2006.01786.x

Lovelock CE, Miller R (2002) Heterogeneity in inoculum potential and effectiveness of arbuscular mycorrhizal fungi. Ecology 83:823–832

Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316:1746–1748. doi:10.1126/science.1143082

Manos PS, Doyle JJ, Nixon KC (1999) Phylogeny, biogeography, and processes of molecular differentiation in *Quercus* subgenus *Quercus* (Fagaceae). Mol Phylogenet Evol 12:333–349

McCormick MK, Whigham DF, O'Neill J (2004) Mycorrhizal diversity in photosynthetic terrestrial orchids. New Phytol 163:425–438. doi:10.1111/j.1469-8137.2004.01114.x

Mohler CL (1990) Co-occurrence of oak subgenera: implications for niche differentiation. Bull Torrey Bot Club 117:247–255. doi:10.2307/2996693

Molina R, Massicotte H, Trappe JM (1992) Specificity phenomena in mycorrhizal symbiosis: community-ecological consequences and practical implications. In: Allen MF (ed) Mycorrhizal functioning. Chapman and Hall, London, UK, pp 357–423

Morris M, Perez-Perez M, Smith M, Bledsoe C (2008a) Multiple species of ectomycorrhizal fungi are frequently detected on individual oak root tips in a tropical cloud forest. Mycorrhiza 18:375–383. doi:10.1007/s00572-008-0186-1

Morris MH, Smith ME, Rizzo DM, Rejmánek M, Bledsoe CS (2008b) Contrasting ectomycorrhizal fungal communities on the roots of co-occurring oaks (*Quercus* spp.) in a California woodland. New Phytol 178:167–176. doi:10.1111/j.1469-8137.2007.02348.x

Moser AM, Petersen CA, D'Allura JA, Southworth D (2005) Comparison of ectomycorrhizas of *Quercus garryana* (Fagaceae) on serpentine and nonserpentine soils in southwestern Oregon. Am J Bot 92:224–230. doi:10.3732/ajb.92.2.224

Nechwatal J, Schlenzig A, Jung T, Cooke DEL, Duncan JM, Osswald WF (2001) A combination of baiting and PCR techniques for the detection of *Phytophthora quercina* and *P. citricola* in soil samples from oak stands. For Pathol 31:85–97. doi:10.1046/j.1439-0329.2001.00232.x

Nixon KC (1997) *Quercus* Linnaeus. In: F o N A E Committee Flora of North America North of Mexico. Oxford University Press, New York, pp 431–506

Pande V, Palni UT, Singh SP (2007) Effect of ectomycorrhizal fungal species on the competitive outcome of two major forest species. Curr Sci 92:80–84

Parker GG, Tibbs DJ (2004) Structural phenology of the leaf community in the canopy of a *Liriodendron tulipifera* L. forest in Maryland, USA. For Sci 50:387–397

Plamboeck AH, Dawson TE, Egerton-Warburton LM, North M, Bruns TD, Querejeta JI (2007) Water transfer via ectomycorrhizal fungal hyphae to conifer seedlings. Mycorrhiza 17:439–447

Pigott C (1982) Survival of mycorrhiza formed by *Cenococcum geophilum* Fr. in dry soils. New Phytol 92:513–517. doi:10.1111/j.1469-8137.1982.tb03409.x

Pinkas Y, Maimon M, Shabi E, Elisha S, Shmulewich Y, Freeman S (2000) Inoculation, isolation and identification of *Tuber melanosporum* from old and new oak hosts in Israel. Mycol Res 104:472–477. doi:10.1017/S0953756299001458

Shi LB, Guttenberger M, Kottke I, Hampp R (2002) The effect of drought on mycorrhizas of beech (*Fagus sylvatica* L.): changes in community structure, and the content of carbohydrates and nitrogen storage bodies of the fungi. Mycorrhiza 12:303–311. doi:10.1007/s00572-002-0197-2

Smith M, Douhan G, Rizzo D (2007a) Ectomycorrhizal community structure in a xeric *Quercus* woodland based on rDNA sequence analysis of sporocarps and pooled roots. New Phytol 174:847–863. doi:10.1111/j.1469-8137.2007.02040.x

Smith M, Douhan G, Rizzo D (2007b) Intra-specific and intra-sporocarp ITS variation of ectomycorrhizal fungi as assessed by rDNA sequencing of sporocarps and pooled ectomycorrhizal roots from a *Quercus* woodland. Mycorrhiza 18:15–22. doi:10.1007/s00572-007-0148-z

Swofford DL (2001) PAUP: phylogenetic analysis using parsimony. Sinauer, Sunderland, MA

Taylor DL, Bruns TD (1999) Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. Mol Ecol 8:1837–1850. doi:10.1046/j.1365-294x. 1999.00773.x

Tedersoo L, Jairus T, Horton BM, Abarenkov K, Suvi T, Saar I, Koljalg U (2008) Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. New Phytol 180:479–490. doi:10.1111/j.1469-8137.2008.02561.x

van der Heijden EW, Kuyper TW (2001) Does origin of mycorrhizal fungus or mycorrhizal plant influence effectiveness of the mycorrhizal symbiosis? Plant Soil 230:161–174. doi:10.1023/A:1010377320729

van der Heijden MGA, Wiemken A, Sanders IR (2003) Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant. New Phytol 157:569–578. doi:10.1046/j.1469-8137.2003.00688.x

Villarreal-Ruiz L, Anderson IC, Alexander IJ (2004) Interaction between an isolate from the *Hymenoscyphus ericae* aggregate and roots of *Pinus* and *Vaccinium*. New Phytol 164:183–192. doi:10.1111/j.1469-8137.2004.01167.x

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis N, Gelfand D, Sninsky J, White T (eds) PCR - Protocols and Applications - A Laboratory Manual. Academic Press, New York, pp 315–322