

Competitive interactions among three ectomycorrhizal fungi and their relation to host plant performance

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Summary

1. Competition strongly influences many species assemblages, but its role in mycorrhizal fungal interactions is not well understood. We examined interactions among three ectomycorrhizal (ECM) species to determine if the structure of competition could be characterized by either competitive networks (where no clear hierarchy exists in the outcome of competition between various species pairs) or competitive hierarchies (where one species out competes all other species).

2. Using a bioassay experiment, we inoculated *Pinus muricata* seedlings with three *Rhizopogon* species (*R. occidentalis*, *R. salebrosus*, and *R. vulgaris*) in single-, two-, and three-species treatments. After 7 months, we assessed the relative abundance of each species in each treatment using real-time PCR of internal transcribed spacer rDNA.

3. We found that *R. occidentalis* was strongly inhibited by *R. vulgaris* and *R. salebrosus* in all competition treatments. In contrast, *R. vulgaris* and *R. salebrosus* had similar ECM biomasses in the two-species treatment, but *R. vulgaris* had significantly higher biomass than *R. salebrosus* in the three-species treatment.

4. In the single-species treatments, seedlings colonized by the competitive dominants had higher shoot biomass and total leaf nitrogen, but also higher percentage ECM biomass. In the multi-species treatments, seedlings had either equivalent or somewhat lower shoot biomass and total leaf nitrogen than their respective single-species treatments.

5. Synthesis. Our results indicate that ECM competition does not appear to be characterized by strict networks or hierarchies. Instead, the outcome is dependent on the conditions of the local environment in which it occurs. There also does not seem to be a clear relationship between ECM competitive ability and plant performance, but competition does appear to negatively affect the ability of ECM fungi to provide benefits to their hosts.

Key-words: ectomycorrhizas, fungi, interspecific competition, *Pinus muricata*, real-time PCR, *Rhizopogon*, symbiosis

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Introduction

Identifying the factors that maintain the high richness of certain species assemblages has long been a central interest in ecology (Hutchison 1961; Connell 1978; Huston 1994; Hubbell 2001). Many studies on this

topic have focused on plant and animal assemblages, but the high richness of microbial organisms in numerous habitats is being increasingly recognized (Whitham *et al.* 1998; Hawksworth 2001). One group of microbial organisms that plays a key role in the ecological function of many terrestrial ecosystems is mycorrhizal fungi (Smith & Read 1997). The increase in research using molecular identification methods to characterize species assemblages has consistently demonstrated that high numbers of mycorrhizal species co-occur at very small spatial scales (Horton & Bruns 2001;

Vandenkoornhuysen *et al.* 2003). Although the ecological importance of these fungi is well documented (Van der Heijden & Sanders 2002), the factors maintaining their assemblage richness are less clear.

Similar to other species, niche partitioning has often been evoked to explain the high richness of mycorrhizal fungal assemblages (Bruns 1995). Recent studies examining ectomycorrhizal (ECM) fungal assemblage structure have identified a number of environmental factors along which niche partitioning can occur. They include many soil parameters such as depth (Dickie *et al.* 2002), nutrient status (Lilleskov *et al.* 2002; Avis *et al.* 2003), temperature and moisture conditions (Swaty *et al.* 2004; Izzo *et al.* 2005; Koide *et al.* 2007), and litter type (Conn & Dighton 2000). Other factors known to promote ECM species richness include physical disturbances such as fire (Taylor & Bruns 1999), dispersal limitation (Deacon & Fleming 1992; Peay *et al.* 2007), and host age and species (Visser 1995; Ishida *et al.* 2007). While identifying axes of niche differentiation is important, it is also necessary to understand how ECM species directly interact with one another to determine differences between fundamental and realized niches.

Given the importance of competition in many plant and animal assemblages (Keddy 2001), this type of interaction may also play a significant role in the structuring of ECM assemblages (Koide *et al.* 2005). The potential role of competition was highlighted by Bruns (1995), who noted that competitive networks (i.e. species $A > B$, $B > C$, but $C > A$) could allow for high levels of species richness to be maintained at local spatial scales. Early studies, particularly in marine species assemblages, suggested that competitive networks may be an important phenomenon (Buss & Jackson 1979; Russ 1982), but many subsequent studies have shown that competitive hierarchies (i.e. species $A > B$, $B > C$ and $A > C$) are more common (Keddy 2001). Recently, however, multiple studies examining microbial organisms have found that competitive networks can drive assemblage dynamics (Huisman *et al.* 2001; Kerr *et al.* 2002), which has re-stimulated interest in how this phenomenon may contribute to local-scale species coexistence.

In this study, we assessed the interactions among three ECM species to examine the structure of ECM competition. We focused on a group of closely related species in the genus *Rhizopogon*, which we have previously shown to have highly asymmetric pair-wise species interactions (Kennedy & Bruns 2005; Kennedy *et al.* 2007). In our study system at Point Reyes National Seashore, CA, USA, *Rhizopogon occidentalis*, *R. salebrosus* and *R. vulgaris* are among the most commonly encountered species in areas where *Pinus muricata* has re-established following the 1995 Mt. Vision fire (Peay *et al.* 2007). High densities of spores of all three species are co-dispersed by small mammals into young *P. muricata* forests (T. Bruns, unpublished data), making them likely to be frequent natural competitors of uncolonized *P. muricata* seedlings.

Using a bioassay experiment to manipulate ECM species composition, we inoculated *R. occidentalis*, *R. salebrosus* and *R. vulgaris* onto *P. muricata* seedlings in all possible combinations and assessed the outcome of competition using real-time polymerase chain reaction (PCR).

Methods

EXPERIMENTAL DESIGN

The experiment consisted of three different ECM competitive treatments. The single-species treatment had each species inoculated individually, the two-species treatment had all pair-wise inoculations of the three species, and the three-species treatment had all three species inoculated together. All treatments had 10 replicates ($n = 70$). In addition, there were 10 replicates that received no ECM inoculum. To avoid potential contamination from other ECM species (a common phenomenon encountered in greenhouse and field experiments), the experiment was conducted in a growth chamber previously determined to be free of ECM inoculum. The spore inoculum used in the experiment was collected from sporocarps of each *Rhizopogon* species within the *P. muricata* forests at Pt. Reyes. In January 2005, collections were made several metres apart and typed by ITS-RFLP analysis to verify morphological species identifications (Kennedy & Bruns 2005). For each species, eight to 12 sporocarps (each from separate collections) were macerated in distilled water and then filtered through cheese-cloth to make spore slurries. Spore densities within each species' slurry were assessed using a haemocytometer and stored at 4 °C until used.

Soil used for the experiment came from a mixed scrub-grassland site (38 11.807' N, 122 57.736' W) within Pt. Reyes and was collected in summer 2005. The soil at that site is classified as a Kehoe variant coarse sandy loam, which is a deep, well-drained soil derived from Quartz-diorite bedrock moraine. That site was chosen because it had been previously tested and found to contain no *Rhizopogon* inoculum; however, very low quantities of inoculum from other ECM species were present (T. Bruns, unpublished data). To eliminate the resident ECM inoculum, the soil was mixed 1 : 1 (v/v) with coarse grain sand and autoclaved at 121 °C for 1 hour. The soil was then divided into the quantities necessary for each treatment and the spores of each species were added in at 10^6 spores mL⁻¹ per species. Soils were mixed using an additive design, where the spore density of each species was the same across treatments (this was accomplished by varying the amount of uninoculated soil added in with each treatment), but total densities were higher in the multi-species treatments than the single species treatments. In mid-August 2005, 100 mL of spore-containing soil was added to replicate 160 mL capacity cone-tainers lined with a small quantity of polyfill to prevent soil

loss (Steuwe and Sons, Corvallis, OR, USA). Four surface-sterilized *P. muricata* seeds were planted into each replicate and after 1 month all cone-tainers were thinned to one seedling per replicate. Seedlings were watered weekly to provide adequate soil moisture. The growth chamber was set at a light intensity of $350 \text{ mol m}^{-2} \text{ s}^{-1}$, a 16 : 8 h light : dark cycle, and temperature ranging from 18 to 20 °C.

SEEDLING HARVEST

In mid-March 2006, seven months after planting, seedlings in all treatments were harvested. At harvest, each seedling was removed from its cone-tainer and the soil was gently rinsed away from the root system. Under a 10× dissecting scope, all live ECM root tips were removed from the root system. The ECM root tips of each replicate were then flash frozen in liquid nitrogen, lyophilized for 48 h, and weighed. The stem, leaves and remaining non-ECM portion of seedling root system were individually oven-dried at 60 °C for 72 h and then weighed. Percentage ECM biomass for each seedling was calculated as $(\text{ECM root tip biomass}/(\text{ECM root tip biomass} + \text{non-ECM root biomass})) \times 100$. Foliar C and N were measured on ground needle tissue on a CE Elantech NC1200 elemental analyser (Lakewood, NJ, USA). Total leaf nitrogen was calculated as percentage leaf nitrogen \times leaf biomass.

REAL-TIME PCR

Since the ECM root tips of *Rhizopogon* species could not be readily distinguished morphologically, we used real-time PCR TaqMan assays to estimate the relative abundance of each species from bulked ECM root tip samples of each seedling. Greater details about this method are described in Kennedy *et al.* (2007). The primers and fluorescently labelled probes for each species were designed from internal transcribed spacer (ITS) rDNA sequences using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www_slow.cgi). For *R. vulgaris*, the forward and reverse primer and probe sequences were (forward: 5'-ATAATTCGGAGGGCTGTAG-3', reverse: 5'-TGAGTTGTGAAAACAGAAGAGC-3', probe:

5'-TexasRed-CTGGCCTTGAAACGAGGCA-BHQ2-3'). Primer and probe sequences for *R. occidentalis* and *R. salebrosus* were the same as those previously described (Kennedy *et al.* 2007). All the ECM root tips of each seedling were bulk extracted using an UltraClean Soil DNA kit following manufacturer instructions to obtain maximum DNA yields (MoBio Laboratories, Solana Beach, CA, USA).

Real-time PCR reactions were run on an ABI Prism 7300 detection system (Applied Biosystems, Foster City, CA, USA). Reactions with DNA from *R. occidentalis* and *R. salebrosus* or *R. salebrosus* and *R. vulgaris* were multiplexed (i.e. primers and probes of both species were present in a single reaction), while those with *R.*

occidentalis and *R. vulgaris* were conducted in simplex (i.e. primers and probes of only one species were present). Preliminary tests revealed no differences in the fluorescence values for *R. occidentalis* and *R. vulgaris* in simplex and multiplex reactions and we observed no fluorescence of non-target template in all single-species treatment samples, indicating no cross reactivity between the primers and probes in the multiplex reactions. Each 15 µL reaction contained 1 × TaqMan IQ Supermix (Bio-Rad, Hercules, CA, USA) and probes of each species at 200 nM. The primer concentrations for *R. occidentalis* and *R. salebrosus* were 400 nM, and 800 nM for *R. vulgaris*. Higher concentrations of *R. vulgaris* did not increase C_t values, but rather improved the overall shape of the reaction curves. One µL of genomic DNA of unknown concentration was added to the reaction (1 µL for each species was added in the standard multiplex reactions so total DNA volume was 2 µL). Total reaction volumes were adjusted by adding the necessary amount of water. PCR cycling conditions and plate set-up were identical to those of Kennedy *et al.* (2007). Data were analysed with the baseline set from cycles 3–15 and the threshold manually placed in the exponential phase of the reaction. Due to differences in fluorophore intensity, the thresholds were manually set at 10 000 in the log Δ fluorescence per cycle plot for *R. occidentalis*, 4000 for *R. salebrosus*, and 500 for *R. vulgaris*.

To estimate species abundances using real-time PCR, the C_t values of the reactions need to be converted into units of ECM biomass (C_t is the cycle number at which the fluorescence value exceeds the background fluorescence and crosses the threshold). This can be accomplished by developing independent relationships between C_t values and known quantities of ECM biomass for each species. In this study, we used ECM root tips from identically grown *P. muricata* seedlings for generating standards. For each species, we weighed out 20 mg of lyophilized bulked root tips. We diluted the DNA extracts at 10-fold intervals (10^{-1} to 10^{-6}) and obtained a C_t value for each dilution. The relationships between C_t value and ECM biomass were then used to convert the C_t values of the unknown samples into relative estimates of ECM biomass. All of these relationships had very high r^2 values (> 0.99) and slopes within the expected ranges of good reaction efficiencies (-3.10 to -3.57). We found that there were generally good correlations between the actual ECM root tip biomass and predicted ECM root tip biomass in the single-species treatments (Fig. 1). However, the predicted biomass was always lower than the actual biomass and the slopes of the relationships differed between the three species (Fig. 1). To account for these differences in the multi-species treatments, we divided the predicted biomasses of each species (given by the standard curve) by the slopes of the predicted-actual biomass relationships in the single-species treatments. We refer to this final value as inferred ECM biomass as in Kennedy *et al.* (2007).

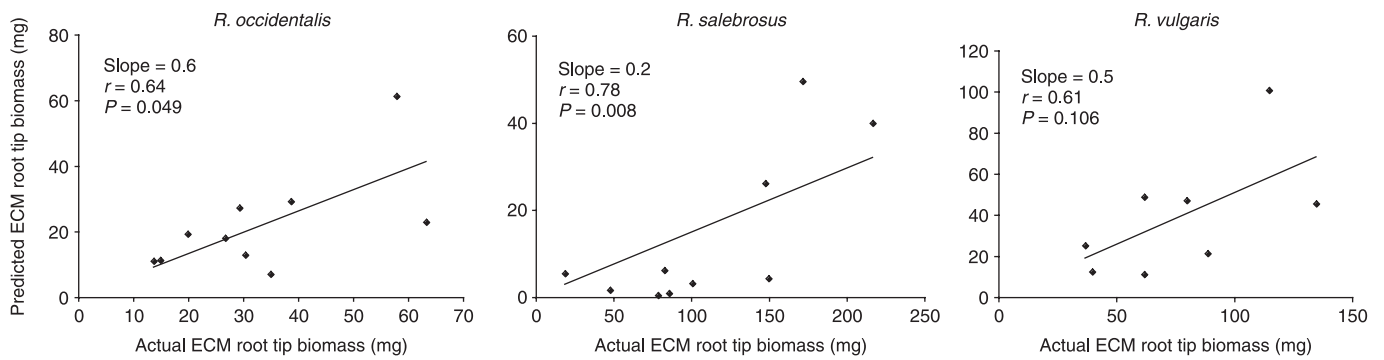


Fig. 1. Predicted vs. actual ectomycorrhizal (ECM) root tip biomass in the single species treatments. The predicted ECM root tip biomass is determined by real-time PCR. r represents Pearson's correlation coefficient. P -values are based on regressions of predicted vs. actual ECM root tip biomass.

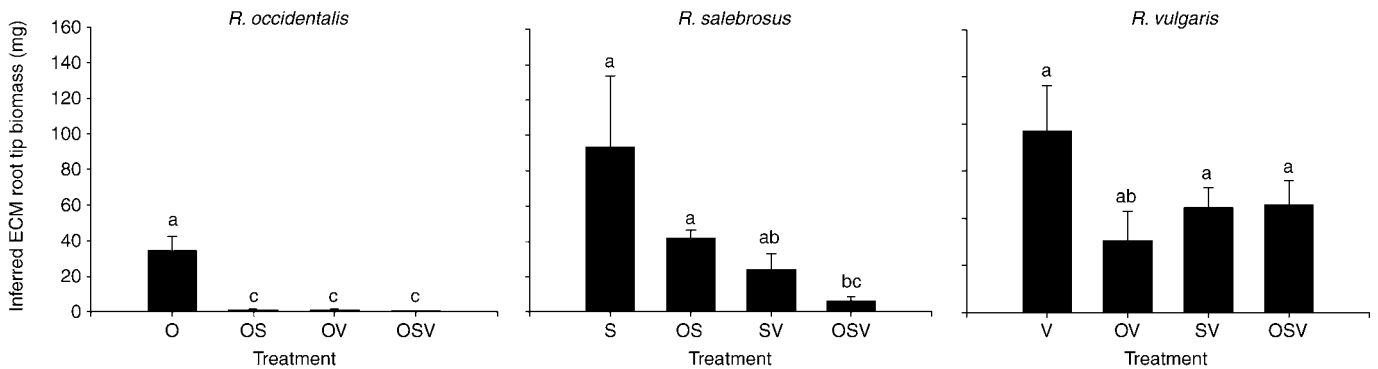


Fig. 2. Inferred ectomycorrhizal (ECM) root tip biomass of *Rhizopogon occidentalis* (O), *R. salebrosus* (S) and *R. vulgaris* (V) in the single-, two-, and three-species treatments. See methods for details about calculating inferred biomass. Bars represent means plus one standard error. Different letters indicate significant differences among data on all three graphs as determined by Tukey HSD tests ($P < 0.05$). Note that statistical analyses were conducted on $\log(x + 1)$ transformed data.

STATISTICAL ANALYSES

To assess the effects of competition, the inferred ECM biomass of each species generated from each standard were compared using a two-way fixed factor analysis of variance (ANOVA), with species and competition treatment as the predictor variables. Prior to running the ANOVA, the data were $\log(x + 1)$ transformed and the variances were determined to be homogenous by visual inspection of residual plots. Seedling shoot and root biomass, total leaf nitrogen, and percentage ECM biomass were each analysed using one-way ANOVAs. To improve variance homogeneity, shoot biomass, root biomass, and total leaf nitrogen were $\log(x + 1)$ transformed and percentage ECM root weight was arcsine transformed prior to analysis. *A posteriori* differences between treatment means were assessed using Tukey HSD tests. Analyses were performed in JMP 5.0 (Cary, NC, USA) and considered significant at $P < 0.05$.

Results

In the single-species treatment, there were no significant differences in ECM biomass among the three species, although both *R. salebrosus* and *R. vulgaris* tended to have higher ECM biomasses than *R. occidentalis* (Fig. 2). In the two-species treatments, significant

asymmetries in ECM biomass were present in two of the three species pairings (species–treatment interaction: $F_{5,116} = 30.86$, $P < 0.001$). When grown with either *R. vulgaris* or *R. salebrosus*, *R. occidentalis* had significantly lower ECM biomass than when grown without other species (Fig. 2). When *R. vulgaris* and *R. salebrosus* were grown together, both species had ECM biomasses that were not significantly different from each other or their respective single-species treatments. In the three-species treatment, *R. vulgaris* had ECM biomass similar to that of its single-species treatment, while both *R. salebrosus* and *R. occidentalis* had ECM biomasses that were significantly lower than when each were grown without other species (Fig. 2). Although three of the four multiple-species treatments had clear asymmetries in biomass, all three species were able to colonize almost all of the seedlings in each treatment, indicating complete competitive exclusion was not a common outcome (Table 1).

Seedling shoot biomass, root biomass and total leaf nitrogen varied significantly across the single-species ECM treatments (Fig. 3). Seedlings colonized by *R. vulgaris* had significantly greater shoot biomass, root biomass and total leaf nitrogen than those colonized by *R. occidentalis*, with *R. salebrosus* being intermediate (shoot: $F_{7,78} = 10.93$, $P < 0.001$, root: $F_{7,78} = 27.72$, $P < 0.001$, nitrogen: $F_{7,78} = 7.56$, $P < 0.001$). Multi-species

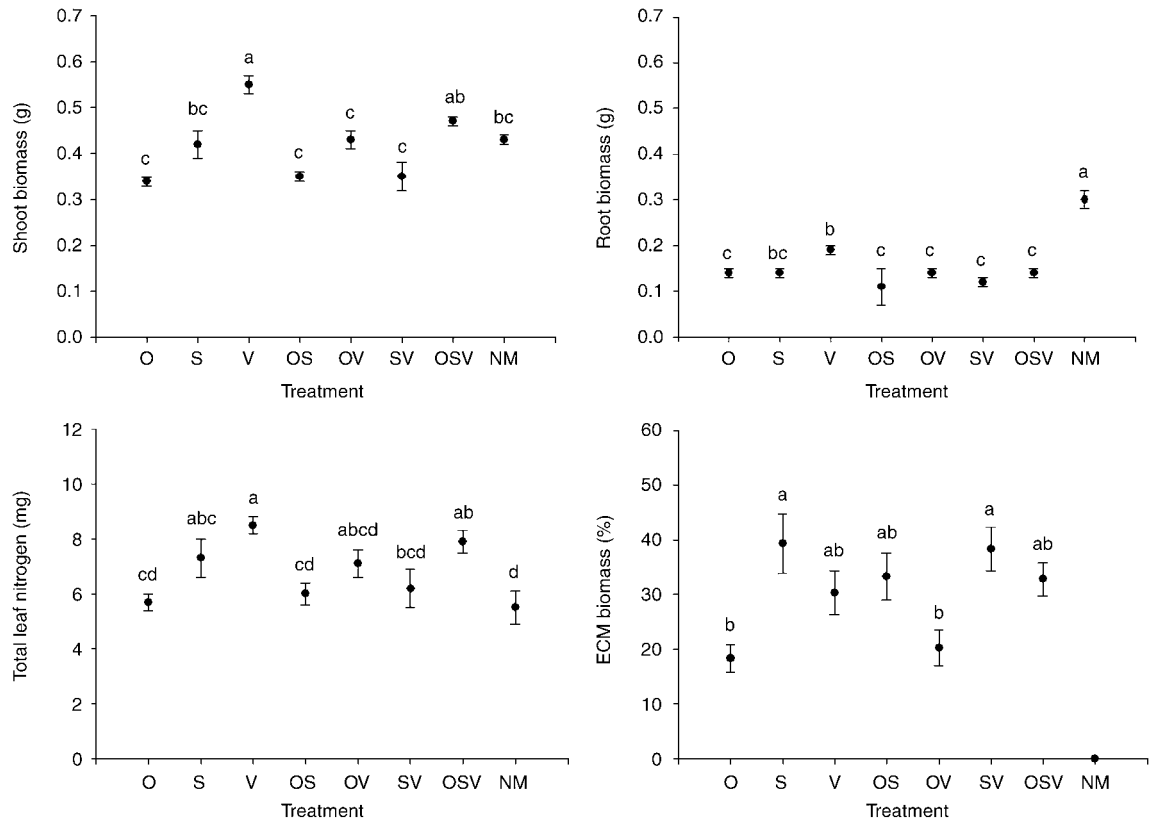


Fig. 3. Shoot biomass, root biomass, total leaf nitrogen, and percentage ECM biomass of *P. muricata* seedlings in the different treatments. O, S, and V are species abbreviations for *R. occidentalis*, *R. salebrosus* and *R. vulgaris*, respectively. Different letters within each graph indicate significant differences among means as determined by Tukey HSD tests ($P < 0.05$). Note that statistical analyses were conducted on $\log(x + 1)$ and arcsine transformed data.

treatment seedlings often had similar shoot and root biomasses and leaf nitrogen as seedlings in the single-species treatments. Patterns of percentage ECM biomass also varied significantly across treatments ($F_{6,65} = 4.95$, $P = 0.0003$). In the single-species treatment, seedlings colonized with *R. salebrosus* had significantly higher percentage ECM biomass than those with *R. occidentalis*, with *R. vulgaris* being intermediate (Fig. 3). Most of the multi-species treatments had intermediate percentage ECM biomasses, with only *R. vulgaris*/*R. occidentalis* seedlings being significantly lower than the other treatments. Non-inoculated seedlings had no ECM colonization on all 10 replicates (Fig. 3). Their root biomass was significantly higher than other treatments, while their shoot biomass was significantly lower than only the *R. vulgaris* treatment. The total leaf nitrogen of the non-inoculated seedlings was not significantly different from that of the *R. occidentalis* seedlings, but was significantly lower than both the *R. vulgaris* and *R. salebrosus* seedlings.

Discussion

ECM COMPETITION

We found evidence of strong competitive effects among the three ECM species in three of the four multi-species

treatments. While *R. vulgaris* and *R. salebrosus* both had strong negative effects on *R. occidentalis* in the two-species treatment, the former two species were not negatively affected by each other when grown as a pair. In the three-species treatment, however, *R. vulgaris* had significantly greater ECM biomass than both *R. salebrosus* and *R. occidentalis*. The reason for the discrepancy between these two treatments is not clear, but may be related to the timing of spore germination. In a previous microcosm study, Kennedy & Bruns (2005) found that *R. occidentalis* spores germinated faster than those of *R. salebrosus*. Although we did not sample the competitive dynamics at multiple time points in this study, an early presence of *R. occidentalis* in the three-species treatment could have shifted the competitive balance between *R. salebrosus* and *R. vulgaris* in favour of the latter. Regardless of the specific mechanism, these results suggest that the outcome of ECM competition is not necessarily predetermined by species pairings, but rather strongly influenced by the local environment under which it occurs.

Our general results are similar to the one other study that has explicitly examined competitive interactions among multiple ECM species. In that study, Wu *et al.* (1999) found non-hierarchical interactions in pair-wise comparisons of three ECM species. Although this suggests that ECM competitive interactions are not

likely to be strictly hierarchical, the competitive outcomes of this study and Wu *et al.* (1999) were not characterized by competitive networks either. In all likelihood, the patchy distribution of most ECM species combined with the spatial and temporal variability in both the root tips and soil nutrients for which they are competing make ECM competitive interactions highly stochastic in nature. As a result of this stochasticity, it is unlikely that any single model based on competition alone will accurately predict the structure of ECM assemblages. Instead, competition is likely to interact with many other factors such as dispersal limitation (Peay *et al.* 2007), nutrient availability (Lilleskov *et al.* 2002) and host plant composition (Ishida *et al.* 2007) to determine the dynamics of ECM assemblage structure at larger spatial scales.

Surprisingly, the competitive interactions between *R. occidentalis* and *R. salebrosus* in this study were reversed from those we observed in a previous growth chamber (Kennedy & Bruns 2005) and field study (Kennedy *et al.* 2007). We think this reversal is likely to be due to differences in the physical constraint of the cone-tainers in which the seedlings were grown. In both of the previous studies, the root systems of the seedlings were less constrained than they were in the cone-tainers and individual roots were more spatially separated. In addition, the root weights of seedlings in this experiment were much higher than those in the two previous studies. This resulted in a much higher effective root density that, in turn, allowed a greater number of independent root tip colonization events. In Kennedy *et al.* (2007), we observed that *R. occidentalis* often competitively excluded *R. salebrosus*, but on co-colonized seedlings *R. salebrosus* could occupy greater quantities of ECM root tips than *R. occidentalis*. We attributed this to a stronger mycelial competitive ability of *R. salebrosus* once it established on a seedling. In this experiment, the higher root densities may have allowed *R. salebrosus* to establish more frequently and then dominate competition with *R. occidentalis* by mycelial spread through the seedling root systems. If this is the case, it suggests that root density plays a key role in determining the outcome of ECM competition (Newton 1992). The observed reversal also further reiterates that the outcome of interactions among ECM fungi appears to be strongly dependent on the environmental settings in which they occur (Bronstein 1994). If competitive dominance shifts across different environmental conditions (as suggested by the compositional shifts observed in studies such as Lilleskov *et al.* 2002 and Avis *et al.* 2003), this effect, when coupled with fine-scale spatial heterogeneity, may be an important factor preventing competitive exclusion and maintaining ECM assemblage richness.

HOST PLANT PERFORMANCE

In the single-species treatments, we observed that seedlings colonized by *R. vulgaris* and *R. salebrosus*,

the competitive dominants, had the highest total weights and leaf nitrogen, while seedlings colonized by *R. occidentalis*, the competitive inferior, had much lower total weights and leaf nitrogen. This pattern suggests there may be a positive relationship between fungal competitive ability and host plant performance. While there are currently no studies documenting differential carbon investment among co-colonized fungal species, plants can limit the amount of carbon available to a fungus based on the amount of nutrients they are receiving from it (Nehls *et al.* 2007). If plants do limit carbon differentially, preferential support of better fungal symbionts could play a large role in determining the outcome of ECM competition (Hoeksema & Kummel 2003). We have, however, seen no evidence that the better competitor is the better symbiont in our previous studies of ECM competition (Kennedy & Bruns 2005; Kennedy *et al.* 2007). In this study there was also some relationship between percentage ECM biomass and species composition in the single-species treatments, so variation in seedling performance may have been caused as much by differences in percentage ECM biomass as species composition. Although our combined work does not indicate that there is a clear relationship between fungal competitive ability and host plant benefit, the *Rhizopogon* species we are studying are closely related and may have similar functional relationships with *P. muricata* seedlings. Future studies examining a wider range of functionally diverse species (Agerer 2001) in conjunction with isotopic methods that allow researchers to track carbon allocation will greatly improve our understanding of how plants may influence ECM competitive dynamics.

In the multi-species treatments, seedlings had shoot biomasses and leaf nitrogen that were often somewhat lower than their respective single-species treatments. These findings are consistent with other studies showing that co-colonization does not typically increase seedling growth or nitrogen uptake (Chu-Chou & Grace 1985; Parlade & Alvarez 1993; Baxter & Dighton 2001; but see Reddy & Natajara 1997) and suggest that competition may negatively affect the ability of ECM fungi to provide resources to their host plants. If, for example, fungi invest in nitrogen-based defensive compounds used for direct antagonistic interactions, they may provide less nitrogen to their host. Little is known about the defense compounds produced by ECM fungi, but nitrogen-rich phenoloxidase and peroxidase enzymes are produced in response to mycelial interactions (A.D.N. Rayner, pers. comm.), suggesting nitrogen may be diverted away from the host when ECM fungi are directly interacting. Alternatively, co-colonized plants may benefit only up to the level of colonization by the best symbiont regardless of the total number of ECM species that are present. This type of response is supported by the results of a recent meta-analysis by Cardinale *et al.* (2006), who found that resource depletion in species rich polycultures tended to be no different from that of the single

most productive species used in the experiments they surveyed.

REAL-TIME PCR

Real-time PCR allowed us to successfully estimate the relative abundance of each species in the different competition treatments. In general, our estimates of inferred ECM biomass corresponded well with our measures of actual ECM biomass as well as percentage ECM biomass. However, we did encounter a significant limitation with this method. Our experiment originally included a fourth *Rhizopogon* species, *R. evadens*, which is a less abundant species encountered in mature *P. muricata* forests in our study area. This species did not colonize seedlings well in the single-species treatment and was never detected in the multi-species treatments. Although we wanted to include data on this species, the predicted ECM biomass of samples with similar actual ECM root tip biomasses were highly variable, so we could not obtain a reliable relationship from which to calculate inferred ECM biomass. Therefore we excluded *R. evadens* from all analyses. We believe that the higher variability of predicted biomass was due to greater variation in root tip age. We made a concerted effort to sample only live ECM root tips, but *R. evadens* had more variation in tip colour and texture than the other three species and we may have included older tips that contained fewer viable nuclei. Parlade *et al.* (2007) observed a similar phenomenon, where the age of *Lactarius deliciosus* mycelium had a significant effect on C_i value. In their study, older mycelial aliquots had higher C_i values than younger aliquots of identical weight. Although this issue is not easily resolved for samples of bulk root tips, we recommend that future studies either use larger sample sizes in the single-species treatments to help overcome this type of variation or other methods such as typing samples of individual root tips to estimate species abundance (Kennedy & Bruns 2005).

Conclusions

This study adds to a rapidly expanding body of research documenting that competition can play an important role in ECM species interactions (Wu *et al.* 1999; Landeweert *et al.* 2003; Lilleskov & Bruns 2003; Mahmood 2003; Kennedy & Bruns 2005; Koide *et al.* 2005; Kennedy *et al.* 2007). Although we found that ECM interactions were characterized by neither strict competitive networks nor hierarchies, we examined only a very small suite of closely related species and future research examining a broader range of species is needed to better understand the influence of competition on ECM assemblage dynamics. In comparison with our previous studies, our current results suggest that environmental conditions may play an important role in determining the outcome of ECM competition. As such, future research manipulating both species

composition and factors such as root density and nutrient conditions will help broaden our knowledge of how ECM competitive interactions are structured.

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