Plant Soil (2007) 291:155–165
DOI 10.1007/s11104-006-9183-3

ORIGINAL PAPER

Different soil moisture conditions change the outcome of the ectomycorrhizal symbiosis between *Rhizopogon* species and *Pinus muricata*

Peter G. Kennedy · Kabir G. Peay

Received: 11 October 2006 / Accepted: 12 December 2006 / Published online: 17 January 2007

Abstract The outcome of species interactions often depends on the environmental conditions under which they occur. In this study, we tested how different soil moisture conditions affected the outcome of the ectomycorrhizal symbiosis between three *Rhizopogon* species and *Pinus muricata* in a factorial growth chamber experiment. We found that when grown in 7% soil moisture conditions, ectomycorrhizal plants had similar biomass, photosynthesis, conductance, and total leaf nitrogen as non-mycorrhizal plants. However, when grown at 13% soil moisture, ectomycorrhizal plants had significantly greater shoot biomass, higher photosynthetic and conductance rates, and higher total leaf nitrogen than non-mycorrhizal plants. The differences in plant response by mycorrhizal status in the two soil moisture treatments corresponded with evidence of water limitation experienced by the fungi, which had much lower colonization at 7% compared to 13% soil moisture. Our results suggest that the outcome of the ectomycorrhizal symbiosis can be context-dependent and that fluctuating environmental conditions may strongly affect the way plants and fungi interact.

Keywords Context-dependence · Drought · Ectomycorrhiza · Fungi · Mutualism · Water

Introduction

It is widely recognized that the outcome of species interactions often varies in different environmental conditions. This was classically documented in the experimental work of Park (1954), who found that the outcome of competition among *Tribolium* beetles depended on factors such as temperature and humidity. Many subsequent studies examining a range of species interactions (e.g. competition, facilitation, predator-prey) have found that changes in abiotic and biotic environmental conditions can strongly affect the ways species interact (Hutchinson 1961; Wiens 1977; Dunham 1980; Cushman and Whitham 1989; McCreadie et al. 2005). Although environmental changes have important effects on a number of species interactions, they may be particularly important in determining the outcome of interactions among symbionts because

---

Peter G. Kennedy and Kabir G. Peay contributed equally to this work and order was determined by a coin toss.

P. G. Kennedy
Department of Plant and Microbial Biology,
University of California at Berkeley, Berkeley, USA

K. G. Peay (✉)
Department of Environmental Sciences, Policy &
Management, University of California at Berkeley,
321 Koshland Hall, Berkeley, CA 94720, USA
e-mail: kpeay@nature.berkeley.edu

there are usually direct costs associated with these types of interactions (Cushman and Whitham 1989). In some environments, benefits of interacting with symbionts outweigh costs and the net outcome is positive, while in others benefits do not exceed costs and the net outcome is negative (see Bronstein 1994 for review).

Many plant species, including many of the dominant timber species, are involved in a symbiosis with ectomycorrhizal (ECM) fungi. In this symbiosis, the plants provide carbon to fungi in exchange for nutrients. For plants, it is estimated that 10–50% of their net photosynthate is transferred to ECM fungi (Smith and Read 1997; Simard et al. 2002; Hobbie and Hobbie 2006). When soil nutrient availability is low, this cost is outweighed by benefits provided by the fungi. In these conditions, ECM plants are usually larger and/or have higher nutrient content than non-mycorrhizal plants (see Simard et al. 2002 for review). In contrast, in high soil nutrient availability conditions, there are usually no significant differences in the size or nutrient status of ECM and non-mycorrhizal plants (Scheromm et al. 1990; Quoreshi and Timmer 1998; Jonsson et al. 2001). Although ECM fungi can grow in high and low nutrient conditions, there is typically a decrease in ECM colonization of plants in high nutrient conditions (Jones et al. 1990; Dupponois and Ba 1999), presumably because for the plant the costs of the ECM association exceed the benefits.

The outcome of the ECM symbiosis may also be affected by other environmental factors aside from soil nutrient status, especially if they directly affect either symbionts' ability to provide services to its partner. For example, changes in light availability (Zhou and Sharik 1997; Weber et al. 2005) and herbivory pressure (Gehring and Whitham 2002) have both been shown to influence how plants and ECM fungi interact. Another environmental factor that has received less attention in this context is water availability. A number of studies have shown that plant water relations can be improved by ECM fungi in low water conditions (Duddridge et al. 1980; Dixon et al. 1980; Brownlee et al. 1983; Parke et al. 1983; Davies et al. 1996; Morte et al. 2001), providing evidence that ectomycorrhizal colonization can improve plant performance under certain experimental drought conditions. However, many other studies have found that ECM fungi do not provide any benefit to the plant when water becomes limiting (Sands et al. 1982; Lehto 1989; Coleman et al. 1990; Dosskey et al. 1991; Parlade et al. 2001). Lack of benefit in these studies is likely related to the fact that both symbionts are directly affected by water availability, with each having decreased performance when water is limited (Mexal and Reid 1973; Theodorou 1978; Coleman et al. 1989; Lambers et al. 1998). As a result, the fungi may be unable to provide resources to their host plants in low water conditions because their own physiological constraints (Coleman et al. 1989; Dosskey et al. 1991). Similarly, plants may not be able to satisfy carbon demand of the fungi during water limitation because photosynthetic capacity decreases due to stomatal closure (Dosskey et al. 1991). A better understanding of these mechanisms may help explain the shifts in ECM assemblages commonly observed with experimental watering or across soils with different moisture retention (Swaty et al. 1998, 2004, Gehring et al. 2006).

In this study, we conducted a growth chamber experiment to test how varying soil moisture conditions affect the outcome of the ECM symbiosis. To assess the effects of varying soil moisture conditions, we measured a number of morphological and physiological variables of the plants as well as parameters indicating the response of the fungi. We used a suite of fungal species in the genus *Rhizopogon* and the host plant *Pinus muricata*, which naturally co-occur along the coastal regions of the western United States and Mexico (Grubisha et al. 2002). In these regions, there is a prolonged period of water deficit that coincides with peak annual temperatures, during which soil water potentials can reach as low as –6 MPa (Dunne and Parker 1999). We hypothesized that ECM fungi would benefit seedling performance relative to non-mycorrhizal controls, but that their effect would be less significant in low soil moisture conditions because of the direct negative effects of water limitation on both symbionts.

Materials and methods

Experimental design

We examined the effects of soil moisture and ECM fungi on *Pinus muricata* seedling performance using a factorial experimental design. The experiment consisted of two soil moisture treatments crossed with five fungal treatments (see below). Each treatment had eight replicate seedlings (2 × 5 × 8 = 80 total seedlings). In the fall of 2004, seeds of *P. muricata* were collected from multiple trees at Point Reyes National Seashore in northern California (38°02.695′ N, 122°53.905′ W). In January 2005, seeds were surface-sterilized, germinated on moist filter paper for ~10 days, and then planted into 160 ml cone-tainers (Stuewe and Sons, Corvallis, OR) containing a two:one mix of sterile sand and autoclaved field soil taken from an area of Pt. Reyes known to have no *Rhizopogon* inoculum (T. Bruns, unpublished data). The cone-tainers were placed in racks in a growth chamber set for 16 h light at 22°C, 8 h dark at 16°C, and 60% relative humidity for the duration of the experiment. Seedlings were randomly assigned to the experimental treatments and rotated regularly throughout the experiment to insure that chamber location was not a confounding factor.

Three weeks after planting, the ECM fungal treatments were applied as follows: 16 seedlings were inoculated with spore slurries of *Rhizopogon occidentalis*, *R. salebrosus*, *R. vulgaris*, *Tomentella sublilacina* or a water control. All of these ECM species are relatively common associates of *P. muricata* at Pt. Reyes, particularly in the areas burned in the 1995 Mt. Vision fire (Taylor and Bruns 1999). The spore slurries were prepared from eight separate sporocarp collections of each species from Pt. Reyes in the winter of 2005. Each collection was made several meters apart, and all collections were typed by ITS-RFLP analysis to verify morphological species identifications. Samples from all eight collections were filtered into a single spore slurry, which was applied at a volume of ~ five million total spores per replicate. This quantity has been shown in previous studies to be sufficient inoculum to ensure maximum colonization rates of all these species (Lilleskov and Bruns 2003; Kennedy and Bruns 2005).

Two weeks after spore inoculation, the 16 seedlings in each ECM treatment were divided into two watering treatments. The two soil moisture levels, 7% and 13% volumetric water content, were chosen because they represent the upper and lower range of average soil moisture conditions experienced in the coastal grasslands during the summer months (Kennedy and Sousa 2006). In these areas, soil moisture availability declines relatively quickly after the last rain and stays at a low level for the duration of the summer (Kennedy and Sousa 2006). To simulate these conditions in the growth chamber, we chose to hold soil moisture constant at the two designated levels throughout the entire period of the experiment. Soil moisture conditions were maintained at 13% and 7% by watering with either 15 ml of dH_20 daily or 7.5 ml once a week in the two treatments, respectively. The treatments were monitored using a ‘‘Hydrosense’’ TDR moisture probe (Campbell Scientific, Logan, UT), which calculates instantaneous volumetric water content. Measurements were taken daily from one seedling in each treatment group during the first 2 months of the experiment, after which soil moisture was monitored every other week. Four ml of a 35 ppm dilution of a 10-10-10 N-P-K fertilizer (0.132 mg N, 0.06 mg P, and 0.108 mg K) was also applied once a week for the first month and every other week for the remainder of the experiment. The nutrient solution was included as part of the weekly watering quota for each plant.

After 4 months, we measured photosynthetic rate (μmol CO_2 g^{-1} s^{-1}) and stomatal conductance (mmol g^{-1} s^{-1}) of all seedlings. Measurements were made on the Li-6400 portable gas exchange system (Li-Cor, Lincoln, NE) using a modified ‘‘conifer’’ chamber. The photon flux density was held at ~1,000 μmol m^{-2} s^{-1} using an external light source, reference carbon dioxide was 400 PPM, and flow rate was 500 mmol s^{-1}. After examining gas exchange, the seedlings were clipped at the soil level and the needles removed. All leaf, shoot, and non-ECM root biomass was then placed in a drying oven at 65°C for 2 days and weighed. We also assessed the $\delta^{13}C$ of all seedling leaves because δ^{13}C provides an integrated measure of plant carbon and water relations over the life of the leaf (Farquhar et al. 1989; Dawson et al. 2002). Dried needle tissue from each seedling was placed in separate screw cap amalgamator tubes with glass beads or stainless steel ball bearings and ground in either a Wig-L-Bug Crescent Amalgamator (Dentsply International, Surrey, UK) or a Mixer Mill MM 301 (Retsch, Haan, Germany). Four milligrams were measured into a tin capsule (COSTEK, Valencia, Calif., USA) and analyzed for isotopic ratios with an ANCA/SL elemental analyzer coupled with a PDZ Europa Scientific 20/20 Mass Spectrometer at the Center for Stable Isotope Biogeochemistry, University of California, Berkeley. Carbon isotope composition is expressed in ‘‘delta’’ notation (‰), and is calculated as $\delta^{13}C = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1{,}000$. R_{sample} and R_{standard} represent the molecular ratios of ^{13}C to ^{12}C in the sample and standard (in this case V-PDB), respectively. The isotopic analyses also determined the % leaf nitrogen in each sample.

Finally, to assess how the ECM fungi were affected by soil moisture, we measured the % ECM root weight of the colonized seedlings. This measure is similar to % ECM root tip colonization, but avoids the difficulty of defining an individual root tip (which for *Rhizopogon* species is difficult due to their typically coralloid ECM morphology). To measure % ECM root weight, ECM seedling root systems were carefully washed to remove all soil and then placed under a 10× dissecting microscope. All of the ECM root tips and non-ECM root biomass were separately removed from each seedling and dried for two days at 65°C. % ECM root weight was then calculated as ([ECM biomass/ECM biomass + non-ECM root biomass] × 100).

Statistics

At the time of ECM sampling, we found that there was no ECM colonization in the *T. sublilacina* treatment. We confirmed this using both morphological and molecular analyses (i.e. root tip observation and PCR and DNA sequencing of fungal ITS rDNA). This treatment was therefore grouped with the non-mycorrhizal treatment for all statistical analyses. Preliminary analyses also revealed there were no differences in the response of the three ECM species treatments, so we grouped all ECM inoculated seedlings together into a single ECM treatment for all statistical analyses. To analyze *P. muricata* performance, we used a series of two-way fixed-factor analyses of variance (ANOVA), with moisture level (13% and 7%), and ECM status (ECM or non-mycorrhizal) as the predictor variables. The response variables were shoot biomass, root biomass, total plant biomass, root:shoot ratio, photosynthetic rate, stomatal conductance, δ^{13}C, water use efficiency, % leaf nitrogen, and total leaf nitrogen. Prior to analysis, the raw photosynthetic and conductance rate values were divided by leaf weight to account for differences in plant size between treatments. Water use efficiency was calculated by dividing photosynthesis by conductance. Total leaf nitrogen was calculated by multiplying % leaf nitrogen by leaf weight. We used a one-way ANOVA to analyze % ECM root weight, with moisture level as the predictor variable. For all the ANOVAs, variances were visually checked and log transformed when necessary to meet assumptions of homogeneity. Tukey HSD tests were used for a posteriori comparisons among means. The relationships between % leaf nitrogen and % ECM root weight at each moisture level was analyzed using simple linear regression. All analyses were conducted in JMP 5.0 (SAS Institute, Inc., Cary, NC) and considered significant at $P \le 0.05$.

Results

At 7% soil moisture, there was no significant difference in the seedling biomass of ECM and non-mycorrhizal seedlings (Table 1). In contrast, at 13% soil moisture, ECM seedlings had significantly greater shoot biomass than non-mycorrhizal seedlings (log shoot biomass ECM status x soil moisture interaction: $F_{1,72} = 6.7464$, $P = 0.011$). Total biomass was also higher for ECM seedlings at 13% soil moisture, but not significantly different from non-mycorrhizal seedlings (log total biomass ECM status × soil moisture interaction: $F_{1,72} = 2.437$, $P = 0.123$). This difference in the

Table 1 Effects of soil moisture and ectomycorrhizal (ECM) status on the biomass of *Pinus muricata* seedlings

Soil moisture	7%		13%	
ECM status	Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal
Total biomass (g)	0.53 (0.02) b	0.54 (0.03) b	0.94 (0.1) a	1.19 (0.08) a
Shoot biomass (g)	0.31 (0.01) bc	0.31 (0.02) c	0.51 (0.06) b	0.81 (0.07) a
Root biomass (g)	0.22 (0.01) b	0.23 (0.01) b	0.43 (0.05) a	0.38 (0.03) a
Root:shoot ratio	0.70 (0.04) ab	0.78 (0.05) a	0.86 (0.03) a	0.55 (0.07) b

Mycorrhizal seedlings were colonized by three different *Rhizopogon* species (*R. occidentalis*, *R. salebrosus*, and *R. vulgaris*), which were grouped for all analyses (see Methods). Statistical tests of significance were done on log-transformed values of all variables except root:shoot ratio. Treatment means are shown ±1 SE in parentheses with different letters representing significantly different means ($P < 0.05$) using Tukey HSD tests

response of ECM seedling shoot versus total biomass was caused by a difference in root:shoot allocation at 13% soil moisture, with ECM seedlings having significantly lower ratios than non-mycorrhizal seedlings (ECM status × soil moisture interaction: $F_{1,72} = 10.694$, $P = 0.002$). Similar to biomass, seedling physiological performance also varied by both soil moisture and ECM status (Table 2). At 7% soil moisture, there were no significant differences in photosynthetic or conductance rates between ECM and non-mycorrhizal seedlings, however, at 13% soil moisture, ECM seedlings had significantly higher rates of both photosynthesis and conductance than non-mycorrhizal seedlings (log photosynthetic rate ECM status × soil moisture interaction: $F_{1,72} = 4.993$, $P = 0.029$; log conductance rate ECM status × soil moisture interaction: $F_{1,72} = 37.600$, $P < 0.001$).

Seedling water relations, as measured by water use efficiency and δ^{13}C, did not differ by ECM status, but did differ by soil moisture treatment (Table 2). Seedlings had significantly higher water use efficiencies at 7% soil moisture compared to 13% soil moisture (soil moisture: $F_{1,72} = 658.180$, $P < 0.001$) and δ^{13}C values of seedlings at 7% soil moisture were significantly less enriched than those at 13% soil moisture (soil moisture: $F_{1,71} = 1070.887$, $P < 0.001$). While water relations differed only by moisture treatment, seedling nitrogen levels were strongly affected by ECM status. Seedling % leaf nitrogen was significantly higher for ECM seedlings than non-mycorrhizal seedlings regardless of soil moisture treatment (Table 2). For the non-mycorrhizal seedlings, % leaf nitrogen was significantly higher at 7% soil moisture than 13% soil moisture (ECM status × soil moisture interaction: $F_{1,71} = 15.672$, $P < 0.001$). This latter difference was caused not by a change in total seedling nitrogen between the two treatments, but rather differences in seedling size (seedlings at 13% soil

Table 2 Effects of soil moisture and ectomycorrhizal (ECM) status on the physiological performance and nutrient status of *Pinus muricata* seedlings

Soil moisture	7%		13%	
ECM status	Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal
Photosynthetic rate (μmol g^{-1} s^{-1})	41.4 (2.1) ab	40.1 (2.3) ab	37.1 (5.4) b	49.6 (4.0) a
Conductance rate (mmol g^{-1} s^{-1})	0.52 (0.03)	0.47 (0.03) c	1.54 (0.13) b	3.72 (0.24) a
δ^{13}C	–26.31 (0.16) b	–26.50 (0.12) b	–31.23 (0.24) a	–31.45 (0.1) a
WUE	82.1 (4.6) b	86.7 (2.6) b	23.27 (1.4) a	14.08 (1.2) a
Leaf N (%)	1.20 (0.04) b	1.44 (0.06) a	0.73 (0.07) c	1.40 (0.04) a
Total leaf N (g)	0.32 (0.02) b	0.37 (0.02) b	0.36 (0.02) b	1.0 (0.11) a

Water use efficiency is abbreviated as WUE and is calculated by dividing photosynthetic rate by conductance rate. δ^{13}C values are presented in units of parts per thousand (see text). Statistical comparisons of conductance were conducted on log-transformed values. Treatment means are shown ±1 SE in parentheses, with different letters representing significantly different means ($P < 0.05$) using Tukey HSD tests moisture were much larger and therefore had lower % leaf nitrogen). In contrast to % leaf nitrogen, ECM seedling total leaf nitrogen was significantly higher than that of non-mycorrhizal seedlings at 13% soil moisture, but not at 7% soil moisture (ECM status × soil moisture interaction: $F_{1,71} = 33.053$, $P < 0.001$).

Soil moisture also affected % ECM root weight. % ECM root weight was significantly lower in the 7% soil moisture treatment (19.3 ± 2.8 (mean ± s.e %)) compared to the 13% soil moisture treatment (27.3 ± 2.6 %) ($t = 2.074$, df = 42, $P = 0.044$). In addition, the relationship between % ECM root weight and % leaf nitrogen also differed between the soil moisture treatments (Fig. 1). At 13% soil moisture, there was a significant positive correlation between % ECM root weight and % leaf nitrogen ($F_{1,23} = 6.351$, $P = 0.020$). At 7% soil moisture, however, there was no significant relationship between % ECM root weight and % leaf nitrogen ($F_{1,19} = 0.354$, $P = 0.559$).

Discussion

Seedling performance

In our study, ECM fungi did not improve overall plant performance when water quantity was most limited. Despite increased % leaf nitrogen, ECM seedlings at 7% soil moisture performed no better than non-mycorrhizal seedlings, as evidenced by the lack of significant differences in biomass, photosynthetic rate, conductance, and water use efficiency (both instantaneous and ^{13}C). However, when soil moisture levels were increased, the presence of ECM fungi had large, positive impacts on a range of seedling properties. Compared with non-mycorrhizal seedlings, ECM seedlings at 13% soil moisture were larger and able to invest significantly more resources into shoot growth than non-mycorrhizal seedlings. They also had higher rates of photosynthesis, conductance, % and total leaf nitrogen. The overall improvements in ECM seedling performance at 13% soil moisture are consistent with many lab and field studies showing that ECM seedlings can have a significant advantage over non-mycorrhizal seedlings in a number of morphological and physiological parameters (Smith and Read 1997; Nara and Hogetsu 2004; Nara 2006).

Despite the fact that ECM fungi are often assumed to improve plant performance under low water conditions, other studies have reported similar results to those that we observed. For example, Parlade et al. (2001) observed no difference in the growth of ECM and non-mycorrhizal *Pinus pinaster* seedlings under 5% soil moisture, but significantly better growth of ECM seedlings in higher soil moisture conditions. Similarly, Zerova (1955), Theodorou (1978), and Dosskey et al. (1991) found that seedlings responded more positively to mycorrhizal colonization at intermediate rather than lower levels of soil moisture. These results suggest that broad generalizations about the role of ECM fungi in plant water relations may not be warranted, and support the context dependence reported for the symbiosis with other environmental variables, such as nutrients or light (Scheromm et al. 1990; Smith and Read 1997; Quoreshi and Timmer 1998; Jonsson et al. 2001; Treseder 2004).

Fungal performance

Our data on % ECM root weight suggests that growth conditions in the 7% water treatment were also unfavorable for the *Rhizopogon* species used in our study. Because it is still unclear to what extent root colonization is controlled by plants versus ECM fungi, it is impossible to completely separate ECM responses to decreasing water availability from changes in host plant carbon allocation. Water-stressed plants may be unable to support high levels of ECM colonization due to stomatal closure, which reduces the total amount of carbon available to the plant (Dosskey et al. 1991). However, a number of different types of studies support the idea that ECM abundance is also affected directly by water availability. A pure culture study by Coleman et al. (1989) found that all ECM species exhibited growth decreases at low water potentials, and that for 87% of the species used in the study, optimum growth occurred at the lowest level of water stress tested (–0.2 MPa). Similar results were obtained

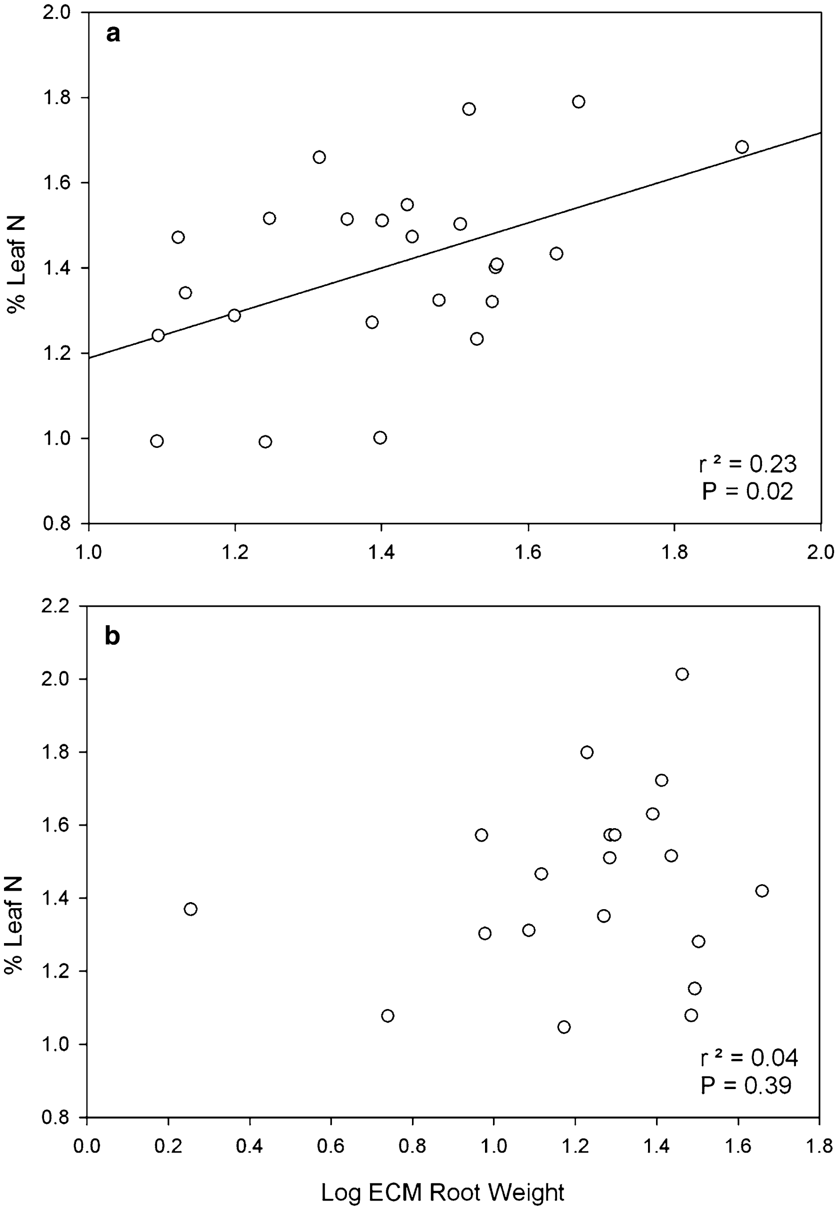

Fig. 1 Relationships between log ($x + 1$) transformed % ECM root weight and % leaf nitrogen of *P. muricata* at 13% soil moisture (**a**) and 7% soil moisture (**b**)

by Mexal and Reid (1973), who concluded that in culture ECM fungi may be even more susceptible to drought than most other fungi. A large number of greenhouse studies provide corroborating evidence that fungal limitation leads to decreased abundance during times of water stress (Worley and Hacskaylo 1959; Mexal and Reid 1973; Theodorou 1978; Lehto 1992; Lansac et al. 1995; Nilsen et al. 1998). For example, Theodorou (1978) found that abundance of ECM fungi was reduced at both very low and very high levels of soil moisture (7% and 27%, respectively) (the high levels were associated with very limited soil oxygen availability). Analogous observations have also been reported from environmental studies (Swaty et al. 1998, 2004; Bell and Adams 2004). In an experimental test of water availability on ECM fungal performance, Swaty et al. (1998) found that % ECM colonization on a cinder soil was highly correlated with % soil moisture, and that watering significantly increased ECM colonization. While host response certainly plays some role in controlling ECM abundance in our study, it also seems very likely that decreases in colonization were caused at least in part by fungal water limitation.

In addition to the differences in % ECM root weight, the idea that fungi performed poorly in the 7% treatment was supported in our study by the interaction between % ECM root weight and % leaf nitrogen. At 7% soil moisture, the level of colonization of ECM fungi was not correlated with seedling nitrogen status; however, at 13% soil moisture, increases in % ECM colonization were significantly correlated with increased % leaf nitrogen. Additionally, despite the fact that ECM seedlings at 7% soil moisture had significantly higher %leaf nitrogen than non-mycorrhizal seedlings, total leaf nitrogen did not differ between the two ECM treatments (Table 2) This is in contrast to the 13% soil moisture treatment, where ECM seedlings had significantly greater total leaf nitrogen than all other treatments (Table 2). The lack of a biologically significant increase in leaf nitrogen may explain why seedlings colonized by ECM fungi at 7% soil moisture had higher % leaf nitrogen but similar photosynthetic rates and biomass. Furthermore, the overall lack of a strong relationship between ectomycorhizal colonization and seedling nitrogen only in the 7% soil moisture treatment suggests that significant portions of the ECM mycelium were physiologically inactive. While this outcome has not been widely documented, there is some evidence that fungal water limitation may reduce ECM benefits in certain soil moisture conditions. For example, Dosskey et al. (1991) concluded that decreasing fungal activity led to the convergence of photosynthetic rates between *Rhizopogon* colonized and non-mycorrhizal seedlings as soil water potential decreased. Similarly, Shi et al. (2002) found that fungal-specific sugars that help plants maintain positive osmotic potentials during water limitation were increased during moderate drought but absent during strong drought. Their study suggests that under very low soil moisture conditions (i.e. strong drought), the fungi were not active and therefore provided no benefit to the plant.

Water versus nutrient effects

There is considerable debate in the ECM literature about the ability of ECM fungi to directly improve plant water uptake. Support for this idea comes from Duddridge et al. (1980) and Brownlee et al. (1983), who showed that ECM fungi can transport physiologically significant quantities of water to a host when the fungus has access to an abundant water source unavailable to the host. However, in most studies where water is limited for both plants and the fungi, it is unclear how much improved plant performance is due to increased uptake of water versus nutrients (Dixon et al. 1980; Parke et al. 1983; Davies et al. 1996; Morte et al. 2001; but see Hasselquist et al. 2005). In fact, one study has shown that the assumed roles of plant and fungus may reverse in some soil moisture conditions, and that plants can use hydraulic lift to transport water to drought stressed ECM fungi (Querejeta et al. 2003). While our study did not explicitly attempt to separate the effects of ECM-related water and nutrient uptake, our results support the idea that improved plant performance is related to improved nutrient rather than water uptake. While stomatal conductance rates were higher in ECM plants in the 13% soil moisture treatment, plant water use efficiency (both instantaneous or $\delta^{13}C$) did not differ by ECM status in either soil moisture treatment, suggesting that ECM fungi did not improve water uptake for their hosts. In contrast, % leaf N content was higher for ECM seedlings relative to non-mycorrhizal seedlings in both soil moisture treatments and total leaf N was higher at 13% soil moisture. Since nutrients are taken up in solution in natural settings, however, we believe that separating nutrient versus water benefits is less important than determining the soil moisture conditions under which ECM fungi improve plant performance.

Implications

Our results clearly demonstrate that the outcome of ECM symbioses can be strongly influenced by different soil moisture conditions, but we recognize that they should be assessed with some caution due to the artificial growth environment of the study. While growth chamber environments provide a number of advantages for experimental manipulation, the growing conditions for both the plant and the fungi rarely mimic those of the field. In particular, the physical constraints of the seedling cone-tainers may not have allowed the fungi to explore the same volume of soil as they would in natural environments, and could have decreased their ability to benefit the plants in the 7% soil moisture treatment. In addition, our experiment examined only one aspect of the plant's life stage, i.e. the seedling life stage, and larger plants may be less sensitive to fluctuating soil moisture conditions due to their larger and deeply located root systems (Quejereta et al. 2003). Finally, our study only examined the effects of chronic water stress on the ECM symbiosis; however, other studies have indicated that ECM fungi may increase the speed of photosynthetic recovery in systems where frequent wetting and drying is common (e.g. Parke et al. 1983).

While our results do show that there are times in which ECM fungi provide no benefit, they also show that having ECM fungi is never detrimental to the plant. This has important consequences for the long-term stability of the ECM symbiosis. Our data indicate that during dry years, ECM fungi may not be able to help plants grow, but during wet years, they provide a significant growth advantage. The same idea can be applied spatially, with ECM plants doing better than non-ECM plants in areas where there is sufficient water for both symbionts. Given the significant temporal and spatial variation observed in most systems, plants are likely to do better if they associate with ECM fungi than if they do not. These results are, however, based on a single host plant and suite of closely related fungal species. In our system, *Rhizopogon* species are among the dominant species associating with young *P. muricata* (Horton et al. 1998; Taylor and Bruns 1999), so associating with ECM fungi appears to be consistently advantageous for *P. muricata* seedlings. But ECM fungal species differ considerably in hyphal and root tip morphology (Agerer 2001) as well as water stress tolerance (Mexal and Reid 1973; Theodorou 1978; Coleman et al. 1989; Dosskey et al. 1991), so tests of additional fungal–host pairings are needed to provide a more complete understanding of how ECM fungi influence host plant performance in different soil moisture environments.

Conclusion

This study lends supports to the view that the outcome of the ECM symbiosis is dynamic in space and time (Johnson et al. 1997). In particular, we found that many of the positive effects of ECM fungi may be negated when environmental conditions are not favorable to both symbionts. While the exact mechanism of this effect is unclear, it is likely that physiologically stressed ECM fungi provide limited benefit to their host in unfavorable conditions. It is important to note that had we looked at only one level of soil moisture, we would have drawn very different conclusions about the influence of ECM fungi on seedling performance, suggesting that future studies of fungal–plant interactions should be conducted over a wide range of environmental conditions to encompass variation in the outcome of the symbiosis.

Acknowledgments We thank the Pt. Reyes National Seashore for use of their land; Tom Bruns, Todd Dawson, and Matteo Garbelotto for their advice and logistical support; C. Benneman for assistance with the growth chamber experiment; S. Mambelli for assistance with the isotopic analyses. M. Bidartondo and members of both the Bruns and Garbelotto labs at UC Berkeley for constructive criticism on earlier drafts of this manuscript. Financial support for this project was provided by a National Science Foundation doctoral dissertation improvement grant to P.G. Kennedy, T.D. Bruns, and W.P. Sousa (DEB 0309152), a National Parks Ecological Research Fellowship to P.G. Kennedy, and a NASA Earth Systems Science Fellowship to K.G. Peay.

References

Agerer R (2001) Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114

Bell TL, Adams MA (2004) Ecophysiology of ectomycorrhizal fungi associated with *Pinus* spp. In low rainfall areas of Western Australia. Plant Ecol 171:35–52

Bronstein JL (1994) Conditional outcomes of mutualistic interactions. Trends Eco Evol 9:214-217

Brownlee C, Duddridge JA, Malibari A, Read DJ (1983) The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. Plant Soil 71:433–443

Coleman MD, Bledsoe CS, Lopushinsky W (1989) Pure culture response of ectomycorrhizal fungi to imposed water stress. Can J Bot 67:29–39

Coleman MD, Bledsoe CS, Smit BA (1990) Root hydraulic conductivity and xylem sap levels of zeatin riboside and abscisic acid in ectomycorrhizal Douglas fir seedlings. New Phytol 115:275–284

Cushman JH, Whitham TG (1989) Conditional mutualism in a membracid-ant association: temporal, age-specific, and density-dependent effects. Ecology 70:1040–1047

Davies FT, Svenson SE, Cole JC, Phavaphutanon L, Duray SA, OlaldePortugal V, Meier CE, Bo SH (1996) Non-nutritional stress acclimation of mycorrhizal woody plants exposed to drought. Tree Physiol 16:985–993

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. Ann Rev Ecol Evol S33:507–559

Dixon RK, Wright GM, Behrns GT, Teskey KO, Hinckley TM (1980) Water deficits and root growth of ectomycorrhizal white oak seedlings. Can J For Res 10:545–548

Dosskey MG, Boersma L, Linderman RG (1991) Role for the photosynthate demand of ectomycorrhizas in the response of Douglas fir seedlings to drying soil. New Phytol 117:327–334

Duddridge JA, Malibari A, Read DJ (1980) Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. Nature 287:834–836

Dunham AE (1980) An experimental study of interspecific competition between the iguanid lizards Sceloporus merriami ad Urosaurus ornatus. Eco Mono 50:309–330

Dunne JA, Parker VT (1999) Species-mediated soil moisture availability and patchy establishment of *Pseudotsuga menziesii* in chaparral. Oecologia 119:36–45

Dupponois R, Ba AM (1999) Growth stimulation of *Acacia mangium* Willd. By *Pisolithus* sp. in some Senegalese soils. For Eco Manage 119:209–215

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Ann Rev Plant Biol 40:503–537

Gehring CA, Whitham TG (2002) Mycorrhizae–herbivore interactions: population and community consequences. In: van der Heijden MGA, Sanders IR (eds) Mycorrhizal ecology. Springer, New York, pp 295–320

Gehring CA, Mueller RC, Whitham TG (2006) Environmental and genetic effects on the formation of ectomycorrhizal and arbuscular mycorrhizal associations in cottonwoods. Oecologia 149:158–164

Grubisha LC, Trappe JM, Molina R, Spatafora JW (2002) Biology of the ectomycorrhizal genus *Rhizopogon* VI Re-examination of infrageneric relationships inferred from phylogenetic analyses from phylogenetic analyses of ITS sequences. Mycologia 94:607–619

Hasselquist N, Germino MJ, McGonigle T, Smith WK (2005) Variability of Cenococcum colonization and its ecophysiological significant for young conifers at alpine-treeline. New Phytol 165:867–873

Hobbie JE, Hobbie EA (2006) ^{15}N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in Artic tundra. Ecology 87:816–822

Horton TR, Cazaves E, Bruns TD (1998) Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first five months of growth after wildfire. Mycorrhiza 8:11–18

Hutchinson GE (1961) The paradox of the plankton. Am Nat 93l:145–159

Johnson NC (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phyto 135:575–586

Jones MD, Durall DM, Tinker PB (1990) Phosphorus relationships and production of extramatrical hyphae by two types of willow ecto-mycorrhizas at different soil phosphorus levels. New Phytol 115:259–267

Jonsson LM, Marie-Charlotte N, Wardle DA, Zackrisson O (2001) Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. Oikos 93:353–364

Kennedy PG, Bruns TD (2005) Priority effects determine the outcome of ectomycorrhizal competition between two *Rhizopogon* species colonizing *Pinus muricata* seedlings. New Phytol 166:631–638

Kennedy PG, Sousa WP (2006) Forest encroachment into a Californian grassland: examining the simultaneous effects of facilitation and competition on tree seedling recruitment. Oecologia 148:464–474

Lambers H, Chapin FS, Pons TL (1998) Plant physiological ecology. Springer-Verlag, New York

Lansac AR, Martin A, Roldan A (1995) Mycorrhizal colonization and drought interactions of Mediterranean shrubs under greenhouse conditions. Arid Soil Res 9:167–175

Lehto TH (1989) Effects of mycorrhiza and drought on photosynthesis and water relations of Sitka Spruce. Agr Ecosyst Environ 28:299–303

Lehto T (1992) Effect of drought on *Picea sitchensis* seedlings inoculated with mycorrhizal fungi. Scand J For Res 7:177–182

Lilleskov EA, Bruns TD (2003) Root colonization dynamics of two ectomycorrhizal fungi of contrasting life history strategies are mediated by addition of organic nutrient patches. New Phytol 159:141–151

McCreadie JW, Beard CE, Adler PH (2005) Context-dependent symbiosis between black flies (Diptera: Simuliidae) and trichomycete fungi (Harpellales: Legeriomycetaceae). Oikos 108:362–370

Mexal J, Reid CPP (1973) Growth of selected mycorrhizal fungi in response to induced water stress. Can J Bot 51:1579–1588

Morte A, Diaz G, Rodriguez P, Alarcon JJ, Sanchez-Blanco MJ (2001) Growth and water relations in mycorrhizal and non-mycorrhizal *Pinus halpensis* plants in response to drought. Biol Plantarum 44:263–267

Nara K, Hogetsu T (2004) Ectomycorrhizal fungi on established shrubs facilitate subsequent seedling establishment of successional plant species. Ecology 85:1700–1707

Nara K (2006) Ectomycorrhizal networks and seedling establishment during early primary succession. New Phytol 169:69–78

Nilsen P, Borja I, Knutsen H, Brean R (1998) Nitrogen and drought effects on ectomycorrhizae of Norway spruce [Picea abies L. (Karst.)]. Plant Soil 198:179–184

Park T (1954) Experimental studies of interspecies competition. II. Temperature, humidity, and competition in two species of Tribolium. Physiol Zool 27:177–238

Parke J, Linderman R, Black C (1983) The role of ectomycorrhizas in drought tolerance of Douglas-fir seedlings. New Phytol 95:83–95

Parlade J, Cohen M, Doltra J, Luque J, Pera J (2001) Continuous measurement of stem-diameter growth response of *Pinus pinea* seedlings mycorrhizal with *Rhizopogon roseolus* and submitted to two water regimes. Mycorrhiza 11:129–136

Querejeta JI, Egerton-Warburton LM, Allen MF (2003) Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe soil drying. Oecologia 134:55–64

Quoreshi AM, Timmer VR (1998) Exponential fertilization increases nutrient uptake and ectomycorrhizal development of black spruce seedlings. Can J For Res 28:674–682

Sands R, Fiscus EL, Reid CPP (1982) Hydraulic properties of pine and bean roots with varying degrees of suberization, vascular differentiation, and mycorrhizal infection. Aust J Plant Physiol 9:559–569

Scheromm P, Plassard C, Salsac L (1990) Nitrate nutrition of maritime pine (Pinus pinaster Soland in Ait.) ectomycorrhizal with Hebeloma cylindrosporum Romagn. New Phytol 114:93–98

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

Simard SW, Jones MD, Durall DM (2002) Carbon and nutrient fluxes within and between mycorrhizal plants. In van der Heijden MGA, Sanders IR (eds) Mycorrhizal ecology. Springer, New York, pp 34–74

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic Press, New York

Swaty RL, Gehring CA, Van Ert M, Theimer TC, Keim P, Whitham TG (1998) Temporal variation in temperature and rainfall differentially affects ectomycorrhizal colonization at two contrasting sites. New Phytol 139:733–739

Swaty RL, Deckert RJ, Whitham TG, Gehring CA (2004) Ectomycorrhizal abundance and community composition shifts with drought: predictions from tree rings. Ecology 85:1072–1084

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 Eco 8:1837–1850

Theodorou C (1978) Soil-moisture and mycorrhizal association of *Pinus radiata* D. Don. Soil Biol Biochem 10:33–37

Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytol 164:347–355

Weber A, Karst J, Gilbert B, Kimmins JP (2005) Thuja plicata exclusion in ectomycorrhizal-dominated forests: testing the role of inoculum potential of arbuscular mycorrhizal fungi. Oecologia 143:148–156

Wiens JA (1977) On competition in variable environments. Am Sci 65:590–597

Worley JF, Hacskaylo E (1959) The effect of available soil moisture on the mycorrhizal association of Virginian pine. Forest Sci 5:267–268

Zerova MY (1955) Mykorrhiza formation in forest trees of the Ukranian SSR. In: Imshenetskii A (ed) Mycotrophy in plants. USDA and NSF, Washington DC, pp 39–59

Zhou M, Sharik TL (1997) Ectomycorrhizal associations of northern red oak (*Quercus rubra*) seedlings along a environmental gradient. Can J For Res 27:1705–1713