

# Below-ground interactions with arbuscular mycorrhizal shrubs decrease the performance of pinyon pine and the abundance of its ectomycorrhizas

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## Summary

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- Few studies have examined how below-ground interactions among plants affect the abundance and community composition of symbiotic mycorrhizal fungi.
- Here, we combined observations during drought with a removal experiment to examine the effects of below-ground interactions with arbuscular mycorrhizal (AM) shrubs on the growth of pinyon pines (*Pinus edulis*), and the abundance and community composition of their ectomycorrhizal (EM) fungi.
- Shrub density was negatively correlated with pinyon above- and below-ground growth and explained 75% of the variation in EM colonization. Consistent with competitive release, pinyon fine-root biomass, shoot length and needle length increased with shrub removal. EM colonization also doubled following shrub removal. EM communities did not respond to shrub removal, perhaps because of their strikingly low diversity.
- These results suggest that below-ground competition with AM shrubs negatively impacted both pinyons and EM fungi. Similar competitive effects may be observed in other ecosystems given that drought frequency and severity are predicted to increase for many land interiors.

**Key words:** below-ground competition, drought, ectomycorrhizal fungi, shrub, tree mortality.

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## Introduction

Plants frequently interact with one another, both positively and negatively (Callaway & Walker, 1997), and many of these interactions occur below-ground. For example, below-ground competition may involve more neighbors and have larger effects on plant performance than above-ground competition (Casper & Jackson, 1997). Below-ground interactions may be particularly important in dryland ecosystems where low plant densities limit above-ground interactions and below-ground dynamics can alter the relative abundance of trees, shrubs and grasses (Jurena & Archer, 2003). Anthropogenic changes in climate such as the increases in drought frequency and severity predicted by many climate models (IPCC, 2001) are likely to

alter the strength of below-ground interactions (Casper & Jackson, 1997). However, few studies have examined these interactions in the field.

Interactions among plants below-ground also may have consequences for the mycorrhizal fungi directly associated with host plant roots. Plant neighbors that form the same type of mycorrhizal association frequently overlap in mycorrhizal fungal species composition (e.g. Kennedy *et al.*, 2003; Dickie *et al.*, 2004) and form hyphal networks that may allow resource sharing (Perry *et al.*, 1989). In ecosystems with multiple types of mycorrhizal fungi that do not form hyphal networks with one another (Smith & Read, 1997), plant–plant interactions could also be important. For example, allelopathic chemicals produced by competing plants (e.g.

Bais *et al.*, 2003) could negatively impact mycorrhizal fungi directly, while competition between plants for soil resources could negatively impact mycorrhizal fungi indirectly (Urcelay *et al.*, 2003). Changes in the abundance and diversity of mycorrhizal fungi may then potentially alter plant performance (Baxter & Dighton, 2001, 2005; Jonsson *et al.*, 2001) and diversity (e.g. van der Heijden *et al.*, 1998).

In this study, we examined the relationships among plant performance, below-ground plant interactions and ectomycorrhizal (EM) fungi in the pinyon–juniper woodlands of northern Arizona during a drought (<http://www.noaa.gov/climate.html>). Pinyon pine is the only host for EM fungi in many pinyon–juniper woodlands (Haskins & Gehring, 2005). As a consequence, hyphal linkages that could facilitate resource sharing are unlikely to form between pinyon and its heterospecific neighbors, potentially increasing the likelihood of both plant competition and negative impacts on EM fungi during drought. Furthermore, from 1995 to 2004, the south-western USA experienced two extreme drought years (1996 and 2002), which resulted in widespread tree mortality including mature pinyon pine mortality levels as high as 85% in some stands (Mueller *et al.*, 2005). We used observational data and a field experiment to test two hypotheses regarding the relationship between pinyons, EM fungi, and understory shrubs that form associations with arbuscular mycorrhizal (AM) fungi: (1) below-ground interactions with AM shrubs negatively affect above- and below-ground pinyon growth; (2) below-ground interactions with AM shrubs reduce the abundance and alter the species composition of the EM fungi associated with pinyon roots.

## Materials and Methods

### Description of the study site

Below-ground interactions with shrubs were investigated near Sunset Crater National Monument, northern Arizona, between August 2002 and May 2004. The soils at this site are composed of basaltic ash and cinders and are low in nutrients and water holding capacity (Gehring *et al.*, 1998). They are classified in the US Department of Agriculture Soil Taxonomic Subgroup of Typic Ustorthents. Study pinyon pine (*Pinus edulis* Engelm.) trees were located in deep cinders, in an area with few grasses or junipers. The dominant understory species was Apache plume (*Fallugia paradoxa* D. Don Endl. ex Torr.), with skunkbush sumac (*Rhus trilobata* Torr.) and desert olive (*Forestiera neomexicana* A. Gray) occurring at lower densities (Table 1). These species of shrubs form associations with arbuscular mycorrhizal fungi (Haskins & Gehring, 2005; C. A. Gehring, unpublished work) and are slowly growing, native perennial shrubs with moderate lifespans (<http://plants.usda.gov/>). Mature pinyon pine mortality at the site was 60%, based on surveys that included more than 250 trees (Stulz, 2004).

**Table 1** Mean proportional relative abundance of the three shrub species found in the understory of pinyon pines (*Pinus edulis*) from the shrubs-intact and shrubs-removed groups

	<i>Fallugia paradoxa</i>	<i>Rhus trilobata</i>	<i>Forestiera neomexicana</i>
Shrubs-intact	0.701 ± 0.061	0.259 ± 0.046	0.040 ± 0.039
Shrubs-removed	0.740 ± 0.034	0.256 ± 0.034	0.005 ± 0.003

### AM shrub associations with pinyon performance

If shrubs competed with mature pinyons for below-ground resources, we predicted that pinyon root biomass, EM colonization and above-ground growth would be negatively associated with shrub density in the rooting zone. To determine if interactions with understory AM shrubs were associated with pinyon growth and EM colonization, we selected 18 mature pinyon pines (basal trunk diameter 16.4–27.2 cm) that were in good condition, i.e. retained a high proportion of their needles, showed little evidence of insect or mammal herbivory, and had a low proportion of branch dieback. Pinyons were growing in similar microsites as indicated by soil particle size distribution (McHugh, 2004), a measure that is highly correlated with soil nutrient content in cinder soils (Cobb *et al.*, 1997). The soil (< 2 mm particle size) fraction averaged 40.0% for all trees sampled, with little variation [standard error (SE) = 2.00].

Shrub abundance was determined by counting the number of shrub stems in the rooting zone of the study trees, an area estimated by whole root system exposure studies to be approximately half of a crown diameter beyond the dripline of a tree (C. A. Gehring, unpublished work). Root biomass was measured by collecting soil cores (15 cm length × 6 cm diameter) from the four cardinal directions at the dripline of all trees. Live roots were manually extracted from cores and classified as pinyon, shrub or other species based on color and branching structure. Roots were dried at 60°C for 24 h and weighed to the nearest 0.001 g.

Root samples for EM analysis were collected at a depth of 10–20 cm from the north aspect of each tree in May 2003. Percentage EM colonization was measured as described in Gehring & Whitham (1991). Shoot length and needle length were measured as indicators of above-ground growth at the end of the 2003 growing season. Shoot length was measured from the terminal bud scar to the branch tip for one branch at each of the four cardinal directions per tree and averaged. Fifteen needles per shoot were measured to the nearest millimeter and averaged to estimate needle length.

### AM shrub removal experiment

In order to more rigorously examine the impact of AM shrub–pinyon interactions on pinyons and their EM fungi, we

initiated a shrub removal experiment in May 2003. Twenty-four mature (basal trunk diameter 15.8–26.5 cm), healthy, living pinyon pines were chosen and paired according to size, proximity, and number of shrub stems in the understory. The 12 pairs of trees were then randomly divided into two groups: shrubs-intact and shrubs-removed. Trees in the shrubs-intact group had an average of  $56 \pm 8.1$  shrub stems (mean  $\pm$  SE) in their understory, with shrubs occupying an average area of 22.3 m<sup>2</sup>. Trees in the shrubs-removed group had an average of  $60 \pm 12.7$  shrub stems in their understory, with shrubs occupying an average area of 24.3 m<sup>2</sup> before shrub removal. The shrubs-intact and shrubs-removed groups did not differ in number of shrub stems in the understory before experimental manipulation ( $t = -0.634$ ,  $P = 0.537$ ). The relative abundances of the three shrub species also were very similar in the two groups (Table 1). Shrubs within the rooting zone of the shrubs-removed group were removed by clipping their stems at the base. Additional clipping to remove re-growth was performed as needed. Shrubs within the shrubs-intact group were not altered. Although removal experiments like this one are frequently used to determine if interactions among neighboring plants are facilitative or competitive (Callaway *et al.*, 2002), shrub removal could have resulted in a nutrient release as a result of shrub root mortality and subsequent decomposition. However, shrub removal had no impact on bulk soil total nitrogen (N) after 12 months [2004 bulk soil total N =  $0.452 \pm 0.046$  mg g<sup>-1</sup> (mean  $\pm$  SE) for shrubs-intact trees and  $0.339 \pm 0.026$  for shrubs-removed trees;  $t = -0.9112$ ,  $P = 0.192$ ].

Above-ground growth of trees from the shrubs-removed group and the shrubs-intact group was measured to determine if these two groups of trees responded similarly to yearly variation in precipitation before shrub removal. Growth was assessed by measurement of annual stem length and needle length as described above (in the section 'AM shrub associations with pinyon performance') for the 5 years before shrub removal (1998–2002). To determine if pinyons showed an above-ground response to shrub removal, shoot and needle length were again measured 1 year following shrub removal (2004).

We also took soil cores to determine if shrub removal altered live shrub root biomass and to determine if pinyons responded to the reduction in shrub roots with changes in their own root biomass. Cores were collected in August 2003 and May 2004 as described previously.

### Ectomycorrhizal colonization and community structure

To experimentally determine if below-ground interactions with shrubs affected the EM colonization of pinyon pines, we compared EM colonization of pinyons from the shrubs-removed group and the shrubs-intact group. Fine roots were collected at a depth of 10–20 cm from the north side of each of the study trees in August 2003 (4 months after the removal experiment), and from the east side of each of the study trees in May 2004 (1 year after the removal experiment). Roots

were scored for percentage EM colonization as described in the 'AM shrub associations with pinyon performance' section. Measures of percentage EM colonization categorize root tips as colonized or not, but do not take into account changes in the abundance of root tips resulting from changes in root biomass. To account for any variation in abundance, we combined estimates of root biomass taken from soil cores with data on EM distribution of fine roots using the equation of Haskins & Gehring (2004):

$$\frac{\text{Number EM Tips}}{\text{Root Length (cm)}} \times \frac{\text{Root Length (cm)}}{\text{Oven Dry Root Mass (g)}} \times \frac{\text{Oven Dry Mass (g)}}{\text{Soil Area (m}^2\text{)}}$$

Live EM tips (~100 per tree per time) were classified according to morphological characteristics and then frozen (–20°C) for molecular analyses. Morphological types were determined based on branching pattern of the root tip, mantle color and texture, and the presence of hyphae when examined under a stereomicroscope at  $\times 20$  (Horton & Bruns, 1998). Morphotyping was followed by molecular analysis of EM communities for the 2004 samples. The DNA from two to three of the saved root tips of each morphotype from each tree was extracted using DNeasy Kits (Qiagen, Valencia, CA, USA), and the internal transcribed spacer (ITS) region of the fungal genome, located between the 18S and 28S rDNA genes, was amplified using PCR with the ITS1F and ITS4 primer pair (Gardes & Bruns, 1993). The amplified ITS region was characterized using restriction enzyme digestion with *Hinf*I and *Mbo*I. Restriction fragment length polymorphism (RFLP) patterns were compared with those from fungal sporocarps and RFLP patterns from previous studies for identification (Gehring *et al.*, 1998; Haskins & Gehring, 2004, 2005; Swaty *et al.*, 2004).

### Data analyses

All statistical analyses were performed using SPSS for Windows, version 10.0.0 (SPSS Inc., Chicago, IL, USA) with an alpha value of 0.05. Probability values between 0.05 and 0.10 were considered marginally significant. Values are expressed as means  $\pm$  SE unless otherwise specified. Data examining the relationship between shrub density and shoot length, needle length, pinyon fine-root biomass and EM colonization were analyzed using linear regression. Shoot and needle lengths of pinyons in the shrubs-removed and shrubs-intact groups were compared using a repeated measures analysis of variance for the period 1998–2002 to test for pretreatment differences. Shoot length, needle length and EM RFLP richness data for 2004 for the shrubs-removed and shrubs-intact groups were analyzed using paired *t*-tests. Root biomass and EM colonization data for shrubs-removed and shrubs-intact groups were analyzed using a repeated measures analysis of variance. Ectomycorrhizal fungal community composition was analyzed with a multiresponse permutation procedure in PC-ORD (McCune & Medford, 1999).

## Results

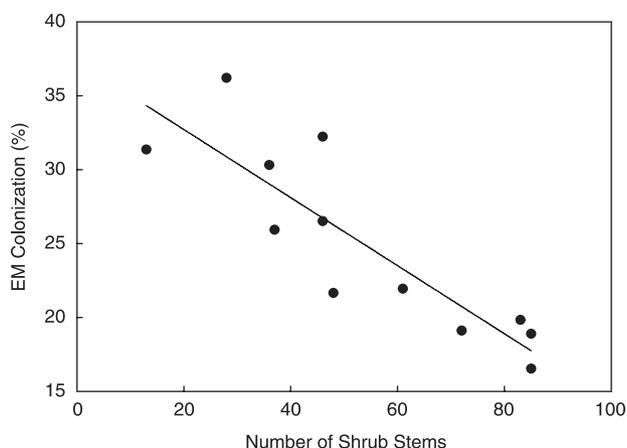
### AM shrub associations with pinyon growth and EM colonization

The number of understory shrub stems was negatively correlated with pinyon growth and EM colonization. Both stem and needle lengths were negatively correlated with shrub density (stem length  $R^2 = 0.275$ ,  $F_{1,11} = 3.80$ ,  $P = 0.07$ ; needle length  $R^2 = 0.296$ ,  $F_{1,11} = 4.20$ ,  $P = 0.06$ ), as was pinyon root biomass ( $R^2 = 0.500$ ,  $F_{1,11} = 10.00$ ,  $P = 0.010$ ). In addition, more than 75% of the variation in EM colonization was explained by shrub density ( $R^2 = 0.754$ ,  $F_{1,11} = 30.59$ ,  $P < 0.0001$ ; Fig. 1).

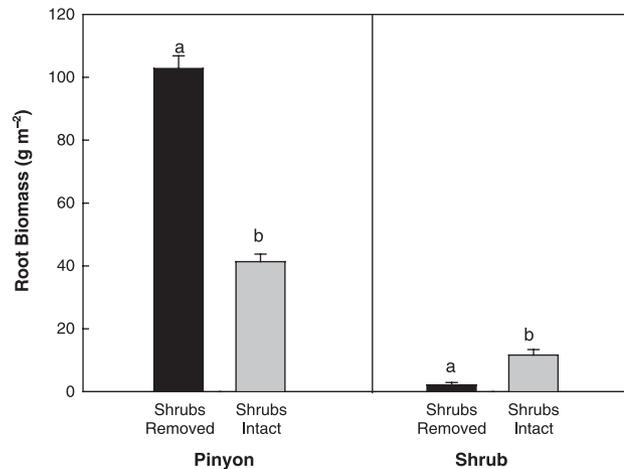
### AM shrub removal experiment

Our removal experiment successfully reduced living shrub roots in the rooting zone of focal pinyons. The mean shrub root biomass for pinyons with their shrubs removed differed significantly from the mean shrub root biomass of pinyons with their shrubs left intact both 4 months and 1 year following the onset of the experiment ( $F_{1,22} = 70.4$ ,  $P < 0.0001$ ). After 4 months, mean shrub root biomass for pinyons with shrubs removed was  $1.52 \pm 0.60 \text{ g m}^{-2}$ , compared with  $13.74 \pm 2.09 \text{ g m}^{-2}$  for pinyons with shrubs intact. Similar differences were observed 1 year after the onset of the experiment (Fig. 2). On average, nine times fewer shrub roots were found under pinyons with shrubs removed than under pinyons with shrubs intact (Fig. 2). Shrub root biomass did not vary significantly with time ( $F_{1,22} = 0.469$ ,  $P = 0.501$ ), nor was there a significant time by treatment interaction ( $F_{1,22} = 0.244$ ,  $P = 0.626$ ).

In support of the hypothesis that shrubs negatively affect pinyons, mean pinyon root biomass was higher in the shrubs-removed group than in the shrubs-intact group at both time periods. After 4 months, mean pinyon root biomass for pinyons with shrubs removed was  $67.84 \pm 8.64 \text{ g m}^{-2}$ ,



**Fig. 1** Percentage ectomycorrhizal colonization of mature pinyon pine (*Pinus edulis*) trees was significantly negatively associated with the number of shrub stems within the pinyon rooting zone.

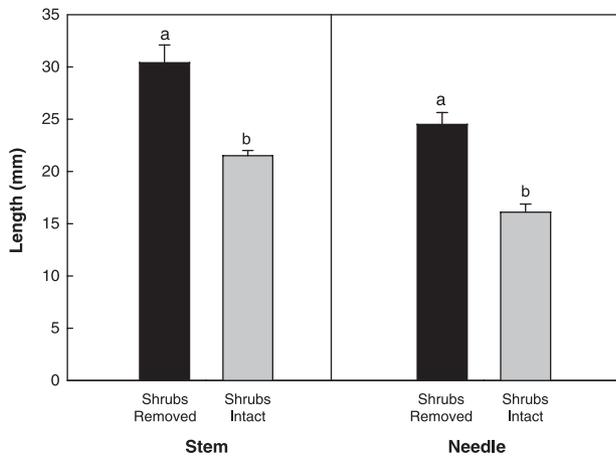


**Fig. 2** Above-ground shrub removal decreased shrub root biomass. In response, pinyon pines (*Pinus edulis*) with their shrubs removed had threefold greater pinyon root biomass than pinyons with intact shrub associations (left panel). Bars represent means + 1 standard error; the letters a and b represent significant differences. Data were collected 1 year after shrub removal.

compared with  $21.67 \pm 3.08 \text{ g m}^{-2}$  for pinyons with shrubs intact. Qualitatively similar responses were observed 1 year after the experiment (Fig. 2). On average, three times more pinyon roots were found beneath trees with shrubs removed than beneath trees with shrubs intact. There was a significant difference in pinyon root biomass with time ( $F_{1,22} = 82.9$ ,  $P < 0.0001$ ), but no significant time by treatment interaction ( $F_{1,22} = 1.66$ ,  $P = 0.210$ ). These results are consistent with a release from competition following shrub removal.

Shrubs-removed and shrubs-intact pinyons had similar growth patterns before shrub removal (1998–2002), but differed in growth 1 year after the removal experiment was initiated (2004). Stem lengths and needle lengths for the year 2004 were significantly different (Fig. 3; stems:  $t = 5.728$ ,  $P < 0.0001$ ; needles:  $t = 7.367$ ,  $P < 0.0001$ ), with trees in the shrub removal group having one and a half times longer stems and needles. Analysis of stem length data for the years 1998–2002 revealed significant year-to-year variation ( $F_{1,24} = 7.83$ ,  $P = 0.001$ ), no year by treatment interaction ( $F_{1,24} = 0.264$ ,  $P = 0.898$ ), and no difference in stem length between the shrubs-removed group and the shrubs-intact group ( $F_{1,24} = 0.509$ ,  $P = 0.483$ ). Similar patterns were observed for needle length for the years 1998–2002 with significant annual variation ( $F_{1,24} = 13.9$ ,  $P < 0.0001$ ), no year by treatment interaction ( $F_{1,24} = 0.421$ ,  $P = 0.792$ ), and no difference between the shrubs-removed and the shrubs-intact groups ( $F_{1,24} = 0.170$ ,  $P = 0.684$ ).

As predicted by our second hypothesis, percentage EM colonization of pinyons with shrubs removed was significantly higher than EM colonization of pinyons with shrubs intact at both sample periods. Four months after the removal, EM colonization of pinyons with shrubs removed was  $21.1 \pm$

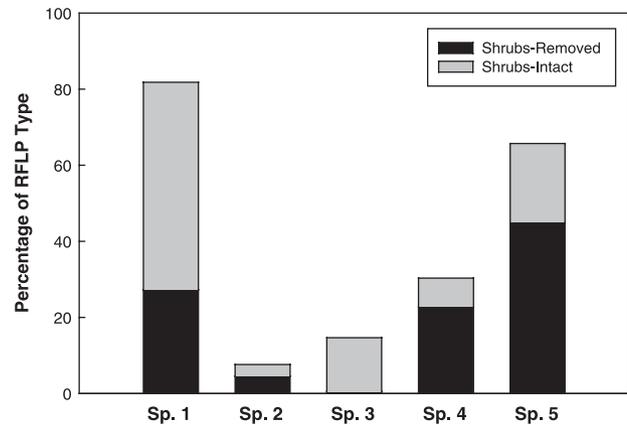


**Fig. 3** Stem lengths (left panel) and needle lengths (right panel) were one and a half times longer for pinyon pines (*Pinus edulis*) with their shrubs removed than for pinyons with intact shrub associations. Bars represent means + 1 standard error; the letters a and b represent significant differences.

0.70%, compared with  $9.51 \pm 0.60\%$  for pinyons with shrubs intact. Similarly, after 1 year, EM colonization of pinyons with shrubs removed was  $54.5 \pm 2.52\%$  compared with  $25.3 \pm 1.98\%$  for pinyons with shrubs intact ( $F_{1,22} = 151.6$ ,  $P < 0.0001$ ). There was a significant difference in EM colonization with time ( $F_{1,22} = 195.9$ ,  $P < 0.0001$ ), and also a significant time by treatment interaction ( $F_{1,22} = 28.1$ ,  $P < 0.0001$ ). The dramatic increase in EM colonization during the second sampling period was likely a result of much wetter conditions at that time (Swaty *et al.*, 1998). When the near tripling of pinyon fine-root biomass associated with shrub removal was considered, shrubs-removed pinyons had four and a half times more EM tips per square meter of soil than did shrubs-intact pinyons, 1 year after the removal (EM tips  $m^{-2}$  soil for shrubs-intact group =  $1210.8 \pm 151.25$ , compared with  $6729.1 \pm 621.97$  for the shrubs-removed group;  $t = -8.24$ ,  $P < 0.0001$ ).

One dominant morphotype was observed in both the shrubs-removed group and the shrubs-intact group at both sampling periods, comprising 97 and 96% of all EM tips, respectively. This morphotype was characterized by a smooth reddish-brown mantle with short, stubby bifurcated root tips, and represented five distinct RFLP types which matched those observed previously at nearby sites (Gehring *et al.*, 1998; Haskins & Gehring, 2004). These RFLP types were sequenced by Haskins & Gehring (2004) and tentatively placed by them in the order Pezizales, family Pyrenomataceae based on correspondence with sequences in GenBank (Bidartondo *et al.*, 2001).

The EM community compositions of shrubs-intact pinyons and shrubs-removed pinyons were very similar ( $a = 0.41$ ,  $P = 0.13$ ) (Fig. 4). No significant differences were observed in the overall species richness of either individual trees ( $1.81 \pm 0.208$  species for pinyons with shrubs intact and  $1.55 \pm 0.153$  for



**Fig. 4** The percentage of ectomycorrhizal (EM) fungal species as determined by restriction fragment length polymorphism (RFLP) analysis did not differ between pinyon pines (*Pinus edulis*) with their shrubs removed and pinyons with their shrubs intact. Data were collected in 2004 only. All five species were tentatively identified as members of the order Pezizales.

pinyons with shrubs removed;  $t = 0.867$ ,  $P = 0.44$ ), or of the groups as a whole, as the same five species were observed in both groups.

## Discussion

### Observational relationships between AM shrub abundance, pinyon performance, and EM fungi

Our observational studies during a time of extreme drought demonstrated that pinyon performance was negatively associated with the abundance of AM shrubs. As the number of shrub stems in the rooting zone of pinyons increased, pinyon root biomass, shoot growth and needle length all declined. These data suggest that shrubs compete for below-ground resources with mature pinyons, and are consistent with models of nutrient foraging, whereby the presence of the roots of a competitor is expected to reduce nutrient availability and foraging efficiency, thus retarding root production of the inferior competitor (Schenk & Jackson, 2002). Ectomycorrhizal colonization also showed a sharp, linear decline as the number of shrub stems increased. The strength of the negative relationship between shrub density and EM colonization suggests that competition may be mediated partly through EM symbionts. Our findings also support the results of McHugh (2004), who observed that pinyon pines that died during the drought had more shrub stems and roots in their rooting zones than pinyon pines that survived.

### Pinyon and EM responses to AM shrub removal

The results of our shrub removal experiment are consistent with our observational studies on above- and below-ground

pinyon growth and EM colonization. Removing the above-ground portion of shrubs significantly reduced the amount of shrub roots found within the rooting zone of pinyons. Just 4 months after the removal experiment was performed, pinyons tripled their fine-root biomass, and this same pattern was again observed 1 year after shrub removal. Similarly, Haskins & Gehring (2004) found a doubling in pinyon fine-root production after trenching to exclude juniper roots from the pinyon rooting zone. These experimental data strongly argue that shrubs and junipers limit the amount of resources that pinyon roots can acquire from the soil, and support the findings of Fredericksen & Zedaker (1995), who observed reduced root biomass of the inferior competitor in young pine–hardwood stands. Studies looking at the effects of understory competition in *Pinus densiflora*, an arid land plant, also found that co-occurring plants negatively affected pine growth through below-ground interactions (Kume *et al.*, 2003).

As with root trenching in nutrient-poor soils, removal of a competitor is expected to have a positive effect on above-ground growth (reviewed by Coomes & Grubb, 2000). Such was the case in our study, where we found a 1.5-fold increase in both the stem lengths and needle lengths of pinyons with understory shrubs removed after 1 year. These findings are consistent with observational data demonstrating a negative association between shrub density and pinyon stem and needle growth, and indicate that shrubs limited above-ground as well as below-ground growth.

Root colonization by EM fungi doubled in the shrubs-removed group, suggesting that these mutualists were suppressed by the below-ground competitors of their host plant. When combined with root biomass data, these reductions in colonization translated into a 4.5-fold increase in EM abundance with competitor removal. Haskins & Gehring (2004) also found that trenching to reduce below-ground competition with juniper resulted in a twofold increase in EM abundance. However, Haskins & Gehring (2004) found no change in EM colonization, suggesting that the changes in EM abundance they observed were primarily a result of changes in root abundance. These differences in response between the two studies may be attributable to sampling location. While the two studies took place on cinder soils near one another, the present study took place within a stressful site at the lower elevational range of pinyon pine. Swaty *et al.* (2004) also observed that EM colonization declined at stressful sites.

It is also possible that shrubs have greater negative effects on EM fungi than junipers, despite lower root biomass in the soil. Previous studies have suggested that allelopathic chemicals produced by shrubs can reduce mycorrhizal colonization of heterospecifics (e.g. Nilsson *et al.*, 1993), although this mechanism has been difficult to distinguish from competition for resources (Michelsen *et al.*, 1995). The dominant shrub species in our study, *Fallugia paradoxa*, produces a variety of phytochemicals (Lucero *et al.*, 2002), but their potential function in plant–plant interactions remains poorly understood.

The hyphae of the AM fungi associated with shrubs and the EM fungi associated with pinyon pines also may interact with one another negatively in the soil. Several studies have documented hyphal interactions among EM fungi and other soil fungi (e.g. Shaw *et al.*, 1995; Baar & Stanton, 2000) and AM and EM fungi were hypothesized to compete with one another for root colonization sites in plants colonized by both types of fungi (Lodge & Wentworth, 1990). The role of these potential interactions among AM and EM fungi deserves further study.

The negative effects of shrubs on EM fungi in our study contrast with the results of Urcelay *et al.* (2003), who found that EM colonization of *Betula nana* declined 4 years after neighbor removal in a nutrient-poor tundra ecosystem, a result consistent with those of fertilization studies at the same site which also found decreased EM colonization. We believe that the difference between our results and those of Urcelay *et al.* (2003) may be a result of the importance of water limitation at our sites. The increased EM colonization we observed in response to competitor removal is consistent with studies at nearby sites which showed that supplemental water resulted in rapid increases in EM colonization (Gehring, 1991; Swaty *et al.*, 1998), while addition of supplemental nutrients alone had no effect (Gehring, 1991). Thus, although our results differ from those of Urcelay *et al.* (2003), they are consistent with the biology of the study system in which water is the key limiting resource.

The large increases in EM colonization with AM shrub removal that we observed were not accompanied by changes in EM fungal diversity and community composition. This is not surprising given that only five putative species were observed, with each tree averaging less than two species. All EM species were members of the order Pezizales that matched the same GenBank sequence (AF266709) with high affinity. This low species richness contrasts with a previous study at a nearby site where both average richness per tree and total species richness across all trees were more than twofold higher (Gehring & Whitham, 2002). Extreme drought stress may contribute to this pattern (Swaty *et al.*, 2004), and we plan to sample again during years of higher moisture if they become available. In addition, although ascomycete fungi have been shown to dominate pinyon roots at stressful sites (e.g. Gehring *et al.*, 1998) and under stressful conditions (Brown *et al.*, 2001; Haskins & Gehring, 2004), basidiomycete fungi have been observed in these previous studies of this species. Ascomycete fungi have also been associated with stressful and/or disturbed sites in other studies, including prescribed fire (Fujimura *et al.*, 2005), glacial forefronts (Trowbridge & Jumpponen, 2004), and forest edges (Dickie & Reich, 2005). There are over 5000 species of EM fungi, mostly basidiomycetes (Molina *et al.*, 1992), which vary in attributes such as the ability to utilize organic nutrient sources, or to transport water (Bougher & Malajczuk, 1990; Anderson *et al.*, 1999). Furthermore, EM fungal species richness can contribute

positively to tree seedling performance under some conditions (Jonsson *et al.*, 2001; Baxter & Dighton, 2001, 2005). Therefore, the depauperate community of closely related ascomycete fungi colonizing pinyons in this study may not be as beneficial as the more species-rich communities observed in previous studies and in other systems.

### Potential problems with removal experiments

Although removal experiments are widely used to demonstrate the nature of the relationships between plants (e.g. Coomes & Grubb, 2000; Callaway *et al.*, 2002), removal of the above-ground portion of the plant alone leaves roots behind that may decompose and alter nutrient dynamics for focal plants and their associated EM fungi. We believe this potential alternative explanation of the patterns we observed is unlikely in our study for three reasons. First, the direction and magnitude of the changes we observed with shrub removal were similar to those observed in response to natural variation in shrub density. For example, in our observational study, pinyons with high numbers of shrubs had approximately half the EM colonization of pinyons with low densities of shrubs, a difference similar to that observed between the shrub-removal and shrub-intact groups within the experiment. The similarity of our observational and experimental data sets also argues that pinyon responses to shrub removal were not the result of disturbance during the establishment of the experiment (Díaz *et al.*, 2003). Secondly, the arid conditions in the woodlands we studied are unlikely to lead to significant changes in soil nutrients during the time-course of our study, as decomposition occurs slowly (82% of initial root mass remained after one relatively moist year; A. Classen, unpublished results), and we found no differences in soil nitrogen following shrub removal. Thirdly, in their review, Coomes & Grubb (2000) stated that 'limited evidence suggests that the supply by root decay is relatively small during the time period in which many experiments are conducted.' Thus, although we cannot rule out the possibility that pinyons or their EM fungi received some benefit from nutrient release associated with root decay, we conclude that the greatest benefit they received from shrub removal was release from competition.

### Below-ground competition as a driver of change

The results of our study indicate that AM shrubs can represent important competitors for mature EM trees during times of drought. The impact of AM shrubs on trees and their EM fungi may lead to long-term impacts on the EM community that could feed back to affect host plants. Reduced EM abundance with competition, combined with losses of EM diversity as a result of drought (Swaty *et al.*, 2004), could lead to local extinctions of fungal species. Drought conditions also contribute to poor sporocarp production (e.g. Gehring *et al.*, 1998) that could limit colonization by fungi from

neighboring sites. The long-term impacts of drought and plant–plant competition on mycorrhizal fungi have not been explored, so the resilience of these systems to such perturbations is unknown. Reduced EM abundance could result in decreased plant performance and reduced competitive ability. For example, EM fungi can improve drought tolerance (e.g. Parke *et al.*, 1983), a benefit that could be reduced with declines in EM abundance. Lower EM abundance on trees also could reduce the amount of EM inoculum available in the soil, potentially limiting tree recruitment in areas where EM host plant density is low (Haskins & Gehring, 2005).

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