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Evidence for mutualist limitation: the impacts of conspecific density on the mycorrhizal inoculum potential of woodland soils

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Abstract The ability of seedlings to establish can depend on the availability of appropriate mycorrhizal fungal inoculum. The possibility that mycorrhizal mutualists limit the distribution of seedlings may depend on the prevalence of the plant hosts that form the same type of mycorrhizal association as the target seedling species and thus provide inoculum. We tested this hypothesis by measuring ectomycorrhizal (EM) fine root distribution and conducting an EM inoculum potential bioassay along a gradient of EM host density in a pinyon–juniper woodland where pinyon is the only EM fungal host while juniper and other plant species are hosts for arbuscular mycorrhizal (AM) fungi. We found that pinyon fine roots were significantly less abundant than juniper roots both in areas dominated aboveground by juniper and in areas where pinyon and juniper were co-dominant. Pinyon seedlings establishing in pinyon–juniper zones are thus more likely to encounter AM than EM fungi. Our bioassay confirmed this result. Pinyon seedlings were six times less likely to be colonized by EM fungi when grown in soil from juniper-dominated zones than in soil from either pinyon–juniper or pinyon zones. Levels of EM colonization were also reduced in seedlings grown in juniper-zone soil. Preliminary analyses indicate that EM community composition varied among sites. These results are important because recent droughts have caused massive mortality of mature pinyons resulting in a shift towards juniper-dominated stands. Lack of EM inoculum in these stands could reduce the ability of pinyon seedlings to re-colonize sites of high pinyon mortality, leading to long-term vegetation shifts.

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Introduction

The distribution and diversity of plant communities can be affected by associations with mycorrhizal fungi (van der Heijden et al. 1998, 2003; Hartnett and Wilson 1999). Although many plant communities contain species that form different types of mycorrhizas, most studies that have examined mycorrhizal influences on community processes have focused on plant species that form the same type of mycorrhizal association. However, the relative abundance of mycorrhizal types could influence plant distributions, particularly if the quantity or diversity of the inoculum of one type becomes limited. The two dominant types of mycorrhizas in temperate ecosystems are arbuscular mycorrhizas (AM) and ectomycorrhizas (EM) (Allen 1991). Both types of mycorrhizas improve water and nutrient uptake and provide protection from pathogens resulting in significant improvements in the survivorship and growth of colonized plants (Riffle and Tinus 1982). Despite this similarity in function, only a few families of plants are capable of forming functional associations with both AM and EM fungi (Smith and Read 1997).

Because the proportion of AM to EM plant species varies dramatically among biomes (Allen 1991), some plant species may fail to thrive in an area where appropriate mycorrhizal fungi are limited. The probability that a seedling will become mycorrhizal upon germination is largely determined by the availability of the appropriate fungal inoculum in the soil (Jones et al. 2003). Inoculum sources include spores or other dormant stages of the lifecycle such as sclerotia, colonized roots and intact hyphal networks (Brundrett 1991; Smith and Read 1997). Fungal inoculum has been considered widely abundant in the soil except where disturbance is extreme, such as after fire (Stendell et al.

1999), clear cutting (Jones et al. 2003), or mining (Brundrett et al. 1996a); yet, recent studies demonstrate that the availability of inoculum can vary with season or year (Guidot et al. 2004; Swaty et al. 1998), the availability of animal vectors (Gehring et al. 2002), and proximity to plants colonized by appropriate mycorrhizal fungi (Dickie et al. 2002).

The presence of the appropriate type of mycorrhizal fungi may not be sufficient for plant establishment as recent work has shown that the species composition of mycorrhizal fungi may be as important as the level of mycorrhizal colonization to plant performance. Species of mycorrhizal fungi vary in their carbohydrate requirements (Durall et al. 1994), tolerance of environmental extremes (Bunn and Zabinski 2003), enzymatic capabilities (Finlay et al. 1992), ability to transport water and nutrients (Mitchell et al. 1984), and dispersal and colonization strategies (Jones et al. 2003). Lovelock and Miller (2002) found that the growth of red maple seedlings varied significantly when seedlings were inoculated with different AM fungal communities. Furthermore, the diversity of the EM community had a larger impact than increased root colonization on nutrient uptake in grey birch seedlings (Baxter and Dighton 2001). Therefore, the species composition and diversity of these important mutualists may also be critical for plant establishment.

The possibility of mycorrhizal fungal mutualist limitation may become more important as local climates change because alterations in temperature, precipitation and carbon dioxide concentration may differentially affect host plants and their fungal mutualists (Rillig et al. 2002). Climate-induced changes in plant communities that have detrimental cascading effects on dependent mycorrhizal fungal communities could, in turn, feedback to affect subsequent host plant establishment and performance (Swaty et al. 2004). Therefore, it is increasingly important to understand the variation in mutualist abundance and diversity and how it is affected by climate change.

Here, we examine how variation in the distribution of an EM colonized tree, pinyon pine (*Pinus edulis* Engelm.), affects the abundance and species composition of EM inoculum in the soil. Pinyon-juniper woodlands are a model system for such a study for two reasons. First, they contain a dominant EM species, *Pinus edulis* or relatives, and an AM colonized co-dominant, one-seed juniper (*Juniperus monosperma* (Engelm.) Sarg.) or relatives, along with an AM colonized understory plant community. Thus, establishing pinyons can only obtain inoculum from conspecifics or long-distance dispersal, while the remainder of the plant community is likely to have multiple inoculum sources. Second, pinyons have been experiencing severe mortality as a consequence of recent droughts (Ogle et al. 2000; R. Mueller, personal communication), which could influence inoculum potential for future generations of pinyons. For example, Swaty et al. (2004) observed that EM colonization and diversity on living adult pinyons at high pinyon mortality sites was approximately half that of nearby sites with low pinyon mortality. The consequences of such losses of EM fungi to establishing seedlings are unknown. We used field observations and a growth chamber bioassay to examine EM inoculum potential in sites that varied in pinyon density prior to the pinyon mortality events of 2002 to address the following questions: (1) how are pinyon fine roots, an important source of EM fungal inoculum, distributed within the soil environment? and (2) what is the EM inoculum potential and EM community composition in zones that are juniper dominated, pinyon–juniper and predominately pinyon?

Methods

Study site

This study was conducted approximately 40 km north of Flagstaff, Arizona, USA in a pinyon–juniper woodland dominated by pinyon pine (*Pinus edulis*) and one-seed juniper (*Juniperus monosperma*). Common understory shrubs and grasses include Apache-plume (*Fallugia paradoxa* (D. Don) Endl. ex Torr.), rabbit brush (*Chrysothamnus nauseosus* (Pall.) Britt.), Mormon tea (*Ephedra trifurca* Torr. ex S. Wats.), pale lyceum (*Lycium pallidum* Miers), threeleaf sumac (*Rhus trilobata* Nutt.), blue gramma (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths), and *Aristida* sp. These species are all reputed to form arbuscular mycorrhizal associations and we have confirmed this for the most abundant species at our site (see Results). The soils are composed of basaltic ash, cinders and lava flows that resulted from the eruption of Sunset Crater in 1064 AD. They belong in the US Department of Agriculture Soil Taxonomic Sub-Group of Typic Ustorthents and are relatively low in nutrients and water storage capacity (Gehring et al. 1998). The majority of precipitation occurs in late summer during monsoonal rains or as winter snowfall. However, five of the last 8 years in northern Arizona pinyon–juniper woodlands have been moderate to extreme drought years (http://www.noaa.gov/climate.html). These droughts have resulted in widespread pinyon mortality (Swaty et al. 2004), which has converted many former pinyon–juniper woodlands into juniper-dominated woodlands (R. Mueller et al., personal communication). Loss of pinyons from these woodlands could severely reduce EM fungi and hamper re-colonization by conspecific seedlings.

Fine root distribution

To determine the distribution of pinyon fine roots that might serve as EM inoculum as well as the distribution of juniper and other unidentified roots that are likely colonized by AM fungi, we established three 50-m transects in each of three zones along a juniper–pinyon

gradient: (1) a low-elevation zone dominated by juniper (~1,735 m), (2) a mid-elevation zone (~1,751 m) where pinyon and juniper densities appeared nearly equal, and (3) a high-elevation zone (~1,781 m) where pinyon dominated. Each transect within a zone was separated by at least 200 m. We measured the basal stem areas of all junipers and pinyons within a 5,000 m^2 area encompassing each of the nine transects to compare pinyon and juniper aboveground densities.

Root distribution was determined by collecting soil cores in June of 2000. Soil cores (30 cm depth, 196 cm^3 V) were taken at three random locations along each transect ($n = 9$ cores per zone). Live and dead fine roots ($\leq$ 2 mm diameter) were separated from the soil cores using forceps and classified as pinyon, juniper or 'other' root. Juniper and pinyon roots could be readily distinguished from one another by diameter, color, branching pattern, and the presence of EM on pinyon fine roots. Roots were dried at 60°C for 48 h and weighed.

Differences between pinyon and juniper stem basal area within each zone were examined using *t*-tests when the assumptions of equality of variance were met as determined by a Levene's test. Root mass data within each zone were compared using a one-way ANOVA. Data were $\log(x+1)$- transformed if the data did not exhibit equal variance. All analyses were performed using SPSS for Windows v.12.0.0.

Mycorrhizal colonization of understory vegetation

To verify that understory plants in the study areas did not form EM associations, we collected roots from the dominant understory species, *F. paradoxa*, *R. trilobata*, *C. nauseosus* and *L. pallidum*, and examined them under the dissecting microscope for evidence of ectomycorrhizas. In addition, roots of these same species were assessed for the presence of AM fungi by clearing them in potassium hydroxide at 90°C, acidifying them in 1 N HCl and staining them using 0.5% trypan blue in lactoglycerol, following the methods of Phillips and Hayman (1970), but omitting phenol. Stained root samples were examined at 200X using a compound microscope to determine the presence of arbuscules or vesicles, which are indicators of AM colonization (McGonigle et al. 1990). Juniper roots were not examined for AM fungi as previous work at the study site demonstrated they were colonized exclusively by AM fungi (Gehring and Whitham 1992).

Ectomycorrhizal inoculum bioassay

We conducted a seedling bioassay to test two hypotheses. First, we hypothesized that fewer mature pinyons at a site would result in less EM inoculum in the soil. Second, we hypothesized that reduced EM inoculum would lead to poor pinyon seedling performance as measured by seedling biomass. We measured the EM inoculum potential of soils in juniper, pinyon-juniper and pinyon zones by using non-mycorrhizal pinyon seedlings as bait plants that were grown in intact field collected soil cores in a growth chamber. We used this method because intact cores include more inoculum sources than other methods, such as soil dilutions, that generally rely on spores alone (Brundrett et al. 1996b). Although field bioassays are preferable, seedlings grown in the field experience extremely high mortality due to herbivory and drought (C. Gehring, unpublished data). Pinyon seeds were cold stratified (4°C) for a minimum of 4 weeks and sterilized in 10% bleach for 30 min. Seeds were then germinated on sterile filter paper in Petri plates in a sterile growth chamber with a 16-h light: 8-h dark regime. Thirty soil cores (295 cm^3 V) per zone were collected in September 2001 from the transects described above. Cores were taken to a depth of 15 cm and then transferred intact into pots in the field. The pots were then returned to the growth chamber in the greenhouse. One non-colonized seedling was planted per pot (16×5 cm, 314 cm^3) the day after soil core collection. Seedlings were returned to the growth chamber and watered every other day for the first 2 weeks and then every fourth day for the following 5 months. Our previous studies that used pinyon seedlings as bait plants determined that 5 months was the amount of time pinyon seedlings required to establish and become mycorrhizal in growth chambers when field soil is used as inoculum.

After 5 months, seedlings were harvested. Shoots and roots were separated and oven-dried at 65°C for 48 h. Prior to drying, roots were scored for percentage EM colonization using the methods of Gehring and Whitham (1991). Ectomycorrhizal root tips were saved for molecular analyses as described below. Additionally, we took a subset of 10 seedlings from each group and examined the roots of these seedlings for AM fungi, as some pines have been shown to form AM associations (Horton et al. 1998). Pinyon roots were cleared and stained as described above to determine if AM fungi were present. Stained root samples were examined with a compound microscope (200X) for the presence of AM fungi. Differences in seedling total root length and AM colonization were examined using a one-way analysis of variance. Seedling biomass data were log transformed prior to analysis with a one-way ANOVA to achieve equality of variance. Differences in treatment means were determined using a Tukey's post-hoc test. Seedling EM colonization data did not meet the assumptions of an analysis of variance, thus differences in colonization were examined using a Kruskal-Wallis test.

Molecular identification of ectomycorrhizal root tips

The DNA from one to eight of the saved root tips per seedling was extracted 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 and Bruns 1993). Restriction fragment length polymorphism (RFLP) data were obtained following the methods of Gehring et al. (1998). The amplified ITS region was characterized using restriction enzyme digestion with *Hin*fI and *Mbo*I, which have been used successfully to discriminate among fungal species associated with *P. edulis* (Gehring et al. 1998; Swaty et al. 2004). Digital images of agarose gels were recorded and analyzed using a Kodak EDAS 290 gel documentation system and accompanying software. RFLP patterns were compared to those generated from fungal sporocarps collected over the last 12 years and to samples collected previously (Gehring et al. 1998; Swaty et al. 2004; Haskins and Gehring 2004). Unique RFLP types were sequenced at the DNA Sequencing Facility at the University of Arizona on an ABI 3730xl Genetic Analyzer and analyzed using SeqMan 5.05 software (1998–2002, DNASTAR Inc.).

Results

Stem and root distribution patterns

The aboveground measures of pinyon and juniper stem basal area did not follow the same pattern as the belowground measures of fine root biomass. Mean pinyon stem basal area was significantly lower than juniper in juniper zones (Fig. 1a: $t = 10.70$, $P < 0.001$), and significantly greater than juniper in pinyon zones (Fig. 1a: $t = -2.79$, $p = 0.049$). There were no significant differences between pinyon or juniper mean basal stem areas in the pinyon–juniper zone (Fig. 1a: $t = 1.25$, $P = 0.28$). In contrast, we found significantly more juniper fine roots than pinyon fine roots in the top 30 cm of soil in both the juniper and pinyon–juniper zones (Fig. 1b: $F_{2,24} = 98.2$, $P < 0.001$; $F_{2,24} = 15.8$, $P < 0.001$, respectively). These findings are intuitive for the juniper zone, where the juniper basal stem area was nearly 15 times greater than pinyon. There were significantly fewer juniper fine roots than pinyon fine roots in the pinyon zone (Fig. 1b: $F_{2,24} = 4.8$, $P = 0.02$). In each of the three zones, the contribution made by 'other' roots was minimal (juniper zone: mean = 3.13 g/m^2 soil, SE = 0.98; pinyon-juniper zone, mean = 3.17 g/m^2 soil, SE = 1.29; pinyon zone, mean = 3.51 g/m^2 soil, SE = 1.64). The fact that there was significantly more juniper than pinyon fine root biomass in the pinyon-juniper zone and no significant differences in mean basal stem area, indicates that junipers are more prolific at fine root production than pinyons within the top 30 cm of soil. Furthermore, these findings support multiple years of observations of juniper root densities in nearby field sites (K. Haskins, personal observation)

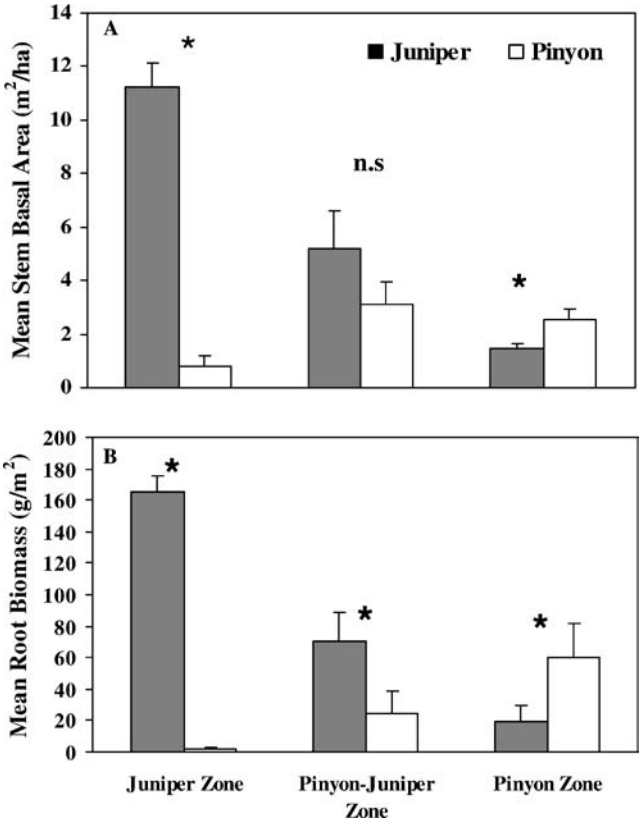

Fig. 1 Mean stem basal area (m^2/ha) (**a**) and root biomass (g/m^2) (**b**) for juniper and pinyon in the juniper, pinyon-juniper and pinyon zones. *Bars* represent means ± 1 SE. *Asterisks* indicate significant differences ($\alpha = 0.05$) between pinyon and juniper within a zone

Mycorrhizal colonization of understory plants

Of the four dominant understory plant species that we examined, none were colonized by EM fungi. Furthermore, *F. paradoxa*, *R. trilobata*, *C. nauseosus* and *L. pallidum* were all colonized by arbuscular mycorrhizas, as determined by the presence of both arbuscules and vesicles within the root cortex.

Ectomycorrhizal inoculum bioassay

The percentage of pinyon seedlings colonized by EM was more than seven times greater in soils from the pinyon–juniper or pinyon zones than the juniper zone (Fig. 2a). Furthermore, the EM colonization of pinyon seedlings grown in soils from the juniper zone was significantly lower than the EM colonization of seedlings grown in soil from either the pinyon–juniper or pinyon zones (Fig. 2b: $\chi^2 = 15.10$, $P = 0.001$). Mean AM colonization of pinyon seedlings was not statistically different ($F_{2,29} = 0.12$, $P = 0.89$) in the juniper, pinyon-juniper and pinyon zones were 2.9% (SE = 1.6), 2.2% (SE = 1.2) and 1.9% (SE = 1.2), respectively, however, neither arbuscules nor coils were ever observed.

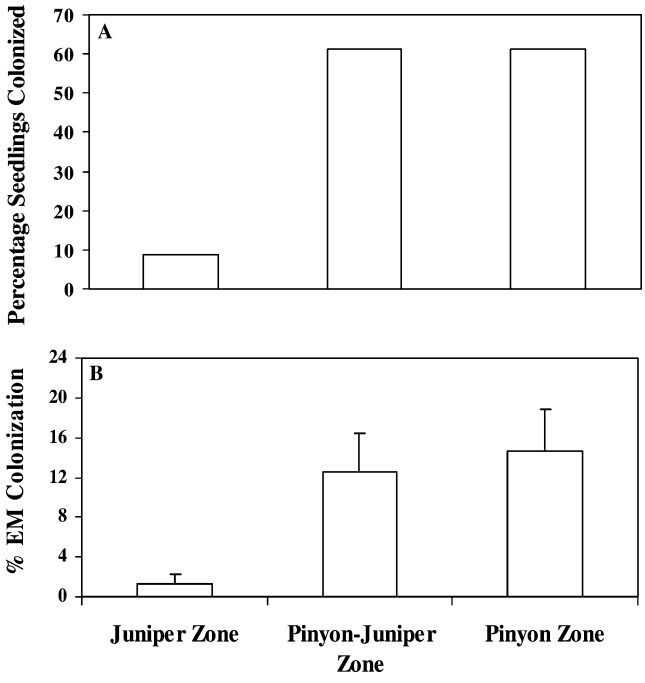

Fig. 2 The percentage of pinyon seedlings that were colonized by EM fungi (**a**) and the mean EM colonization (**b**) of the colonized seedlings in the juniper, pinyon-juniper and pinyon zones are shown. *Bars* in (**b**) represent one standard error

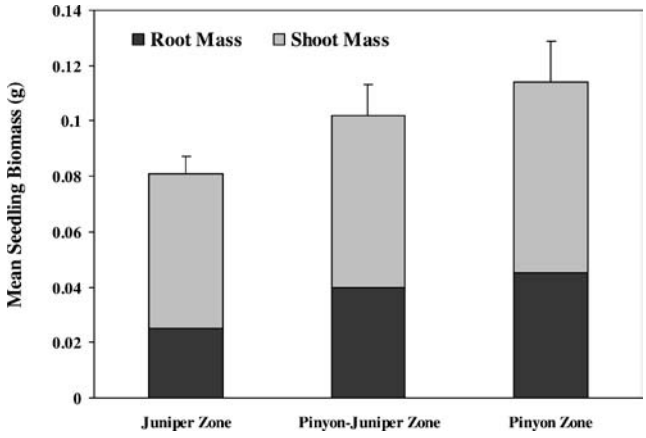

Fig. 3 Root and shoot biomass (g) and total biomass (g) of pinyon seedlings grown in soil from the juniper, pinyon–juniper and pinyon zones. Data are means ± 1SE

Although there were no significant differences in seedling total root length, total biomass or shoot biomass across the three zones ($F_{2,56} = 0.44$, $P = 0.64$; $F_{2,56} = 1.38$, $P = 0.32$, $F_{2,56} = 0.43$, $P = 0.65$, respectively), seedlings grown in soil from the juniper zone had significantly lower root mass than seedlings grown in soil from the pinyon zones (Fig. 3: $F_{2,56} = 3.39$, $P = 0.04$).

Resolution of the EM fungal composition of seedlings was hampered by poor DNA extraction success, likely due to the very young age of the seedlings. For this reason, our results must be considered preliminary and we have not made statistical comparisons. In total, we examined 46 EM tips using molecular tools; 17, 26 and three EM tips were obtained from five, eight and three seedlings grown in soil from the pinyon, pinyon-juniper and juniper zones, respectively (only three seedlings formed EM in the juniper zone). We observed 18 different RFLP types, only six of which could be assigned to taxa even with DNA sequence data (Fig. 4). Seedlings grown in soil from the pinyon zone had the highest richness (11 RFLP) types, followed by seedlings grown in soil from the pinyon–juniper zone with 8 RFLP types. These two groups of seedlings had three RFLP types in common. The three EM root tips from the seedlings grown in soil from the juniper zone were of different RFLPs and only one of these was shared with the pinyon and pinyon–juniper zones. Identified taxa include *Rhizopogon rubescens, Rhizopogon sp.*, members of the order Pezizales, and an unidentified member of the Basidiomycota. These taxa have been found in association with juvenile and mature pinyons in studies conducted near this field site (Haskins and Gehring 2004; Swaty et al. 2004).

Discussion

Our examination of pinyon and juniper fine root distribution across a pinyon-juniper gradient revealed juniper root dominance in the top 30 cm of soil in the pinyon–juniper and juniper zones. Previous work has shown that junipers are better able to access intercanopy water than pinyons (Breshears et al. 1997) and thus, are better competitors for this limiting resource. Our root biomass data provide a potential mechanism for this finding. In contrast, pinyon root biomass was three-fold greater than juniper root biomass in the pinyon zone despite only twofold greater stem basal area. This result suggests that pinyon roots proliferate in the upper 30 cm of soil when juniper density is low, a finding that supports the work of Haskins and Gehring (2004) showing a sharp increase in pinyon root biomass following trenching to exclude juniper roots. One caveat of these results is that belowground sampling intensity was by necessity much lower than aboveground sampling intensity, which could have contributed to the discrepancy observed between aboveground and belowground views of pinyon vs. juniper dominance. However, if our root biomass data are representative, we predict that pinyon seedlings are more likely to come into contact with juniper roots than pinyon roots in the pinyon–juniper and juniper zones, and thus, are less likely to develop mycorrhizas from EM colonized roots and hyphae in these two areas.

The inoculum potential bioassay confirmed that pinyon seedlings are less likely to become colonized in soils that are dominated by juniper, but are equally likely to become colonized in the pinyon-juniper and pinyon zones. Pinyon seedlings grown in soils from

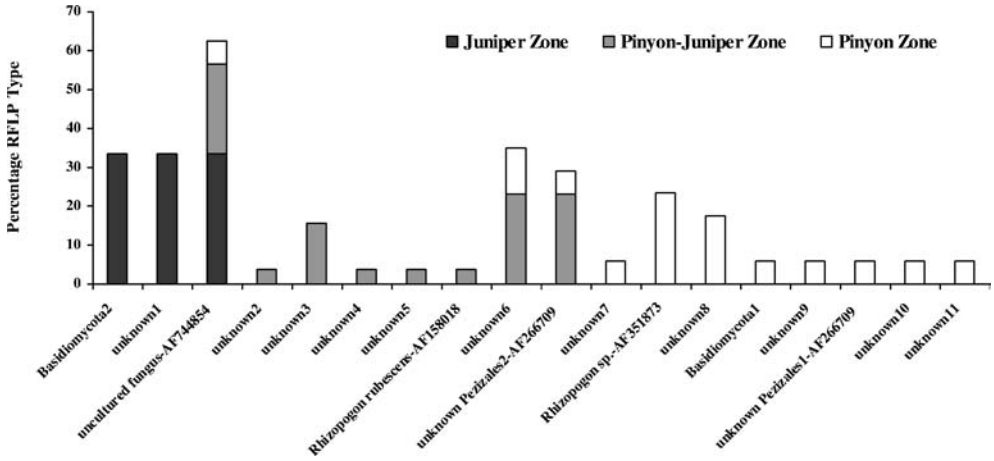

Fig. 4 The percentage of each RFLP type from seedlings grown in soil from juniper, pinyon-juniper and pinyon zones. RFLP types that were successfully sequenced and matched to a taxon are listed with their NCBI (http://www.ncbi.nlm.nih.gov) accession numbers. There were two RFLP types that were matched to known basidiomycetes based on previous work. All other unique RFLP types are unidentified

juniper zones were 86% less frequently colonized by EM fungi than seedlings grown in soils from either the pinyon-juniper or the pinyon zones. Additionally, the seedlings in the juniper soils that were colonized by EM fungi exhibited 90% lower levels of colonization than seedlings grown in soils from either the pinyon–juniper or the pinyon zones. These findings are similar to studies that have examined EM inoculum potential in sites devoid of EM hosts (Terwilliger and Pastor 1999) and to studies demonstrating the importance of living EM hosts as sources of inoculum for establishing seedlings. Dickie et al. (2002) showed that mature *Quercus montana* hosts (an EM species) indirectly facilitated seedlings of *Q. rubra* (another EM species) by increasing mycorrhizal infection and by altering the EM community of the seedlings.

It is not uncommon to find AM fungi colonizing seedlings of *Pinus* species (Cazares and Smith 1996; Smith et al. 1998), though the function of this association is still unclear (Horton et al. 1998). It has been suggested that AM fungal colonization of typically EM host plants may be important in nutrient poor sites (Lapeyerie and Chilvers 1985), such as ours. However, Egerton-Warburton and Allen (2001) demonstrated that dual colonization of roots by both types of fungi in oak seedlings (<1 year) did not increase nutrient uptake or plant biomass in comparison to non-inoculated controls, whereas oak seedlings colonized by either AM or EM performed better than non-inoculated controls. Because we observed little AM colonization in our pinyon seedlings and never observed arbuscules or coils, the diagnostic structures of AM fungi that function in materials exchange between fungus and host, we feel that AM fungi likely did not have a significant effect on pinyon seedling growth.

Our seedling bioassay was effective at assessing EM inoculum from dying roots, recently disrupted hyphal networks, spores and sclerotia. Because removing a core from the soil disrupts living hyphal networks, our methods may have reduced the inoculum potential compared to levels we might observe had we planted seedlings in the field. Additionally, because we collected soil cores in September, fungal species whose spores required cold stratification for germination may not have been represented in our study. However, we feel that our findings are robust for three reasons. First, we planted actively growing seedlings immediately in soil cores, allowing opportunities for colonization from hyphae and roots that had just been removed from the field and had not senesced to any great extent. Second, 78% (seven of nine soil cores) of the soil cores taken from the juniper zone had no pinyon fine roots, suggesting that pinyon roots are rare in juniper-dominated zones, potentially reducing the importance of hyphal networks attached to living pinyon roots as sources of inoculum. Third, problems associated with the use of soil cores and spore dormancy are likely to be equally important at all of the study sites and thus are unlikely to explain the dramatic differences we observed.

The prevalence of EM spores in juniper-dominated zones is unknown, but we suspect that it is limited compared to more mesic environments. A 6-year survey conducted in nearby field sites by Gehring et al. (1998) found a total of 22 sporocarp species, only four of which were hypogeous, and only seven of which matched the 51 RFLP types observed on pinyon EM root tips. These sporocarp data were collected primarily before the onset of the current drought in 1996. Since 2002, we have not observed any epigeous EM fungal sporocarps at our field sites that could provide windborne inoculum. We believe that hypogeous sporocarp production could still be occurring despite the dry conditions, but the spores of these species may have a more limited distribution due to their reliance on animal vectors. Further studies examining hypogeous sporocarp production in these areas are required.

The persistence of mycorrhizal fungal propagules is unknown for many species, but is likely to span a large range. For example, without a host plant, the EM fungal mycelia of *Laccaria bicolor* lived up to 23 weeks in autoclaved field soil (Brulé et al. 2001). Sclerotia of *Paxillus involutus* that were buried in unsterile, brown earth were found to be nearly completely degenerated and unfit for infectivity after only 40 weeks (Fox 1986). Alternatively, the lifespan of *Rhizopogon* spores could be extensive, as suggested by the homogenized spore banks found in coastal California (Kjøller and Bruns 2003). *Rhizopogon* species made up nearly 25% of the EM fungal community of pinyon bioassay seedlings found in the pinyon zone. However, further work is needed to determine what kind of spore bank currently exists, and whether or not pinyon seedlings can rely on resistant EM fungal propagules when they attempt to re-establish.

Preliminary comparison of the EM fungal communities, as defined by RFLP types, between the pinyon and pinyon–juniper zones revealed minimal overlap (19%) in RFLP types. These substantial differences were surprising given that all collection sites were located within a 50 m span of altitude and were spread across a distance of approximately 400 m. Four of the six taxa identified (22% of all RFLP types) in our bioassay were either hypogeous basidiomycetes (*Rhizopogon*) or ascomycetes of the order Pezizales. These fungi are commonly associated with semi-arid or disturbed environments (Danielson and Pruden 1989; Horton et al. 1998). At the same study site, Haskins and Gehring (2004) found that 73 and 100% of EM fungal communities of trenched and control mature pinyons respectively, consisted of hypogeous basidiomycetes and ascomycetes. Some of the differences between previous studies on older trees and the results we present here may be due to the age of the tree sampled. Differences between the community composition of seedlings and mature trees is well-documented (Fleming 1983; Dickie et al. 2002) and likely driven by differences in physiological requirements (Smith and Read 1997). Although 61% (11 of 18) of the RFLP types observed here have also been observed in other studies on older pinyons (Haskins and Gehring 2004; Swaty et al. 2004), seven RFLP types are new and could be important to establishing pinyon seedlings.

Implications

Drought in the Southwest has been one of the most important determinants of pinyon–juniper woodland community structure for the past 8 years (R. Mueller, personal communication). The loss of mature pinyon pines may be directly linked to drought via xylem cavitation, as was seen in juvenile and mature eucalypts in Australia (Rice et al. 2004), or indirectly linked via outbreaks of insect pests, such as bark beetles (Hanson and Weltzin 2000) or competition with junipers and shrubs (McHugh 2004). We believe the large-scale mortality events that have occurred in pinyon–juniper woodlands will be detrimental to pinyon seedling recruitment and long-lasting for two reasons. First, pinyons do not reach reproductive maturity until they are nearly 40-years-old, so seed production will be drastically reduced for many years. Second, because pinyon pine is often the only EM host species in these environments, the loss or reduction of mature pinyons also means the loss of an important inoculum source in the form of active EM vegetative mycelium and hyphal networks (Onguene and Kuyper 2002; Read and Birch 1988), and potentially the eventual loss of spores and sclerotia as well. The hypothesis that reduced pinyon root abundance would lead to reduced EM inoculum was supported by the bioassay study. The high pinyon mortality sites support fewer living ectomycorrhizas (Swaty et al. 2004), and thus more closely resemble the juniper-dominated site described in this paper. We believe this pattern could have important long-lasting effects on the expansion of juniper woodlands and for the thousands of species dependent on pinyon pine.

Inoculum limitation is a relatively unexplored phenomenon that could be important in determining host plant community structure. For example, if inoculum levels drop below a threshold, then certain mycorrhiza-dependent species could be prevented from establishing. Our data show evidence of a threshold as pinyon seedlings are equally likely to become colonized by EM fungi in areas where pinyon dominates or co-dominates, but are highly unlikely to become colonized in juniper dominated areas. A similar scenario could be true in other systems where a plant community member is colonized by a different type of mycorrhizal association from other plant community members, such as in ponderosa pine forests (Kovacic et al. 1984) or oak savannahs (Allen 1991). Such plant communities could be susceptible to inoculum limitation and plant community shifts that favor low dependency on the surbordinate mycorrhizal association or that form the dominant mycorrhizal association (Urcelay and Díaz 2003).

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