Genetically based susceptibility to herbivory influences the ectomycorrhizal fungal communities of a foundation tree species

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Summary

• Although recent research indicates that herbivores interact with plant-associated microbes in complex ways, few studies have examined these interactions using a community approach. For example, the impact of herbivory on the community structure of ectomycorrhizal fungi (EMF) is not well known. The influence of host plant genetics on EMF community composition is also poorly understood.

• We used a study system in which susceptibility to herbivory has a genetic basis and a 20-yr insect removal experiment to examine the influence of chronic herbivory and plant genetics on the EMF community structure of Pinus edulis. We compared EMF communities of herbivore resistant trees, herbivore susceptible trees and herbivore susceptible trees from which herbivores were experimentally removed at two dates 10 yr apart.

• In both years sampled, resistant and susceptible trees differed significantly in EMF community composition. After 10 yr and 20 yr of herbivore removal, the EMF communities of removal trees were similar to those of susceptible trees, but different from resistant trees.

• The EMF community composition was more strongly influenced by innate genetic differences in plant traits associated with resistance and susceptibility to herbivory than by indirect effects of herbivory on host plant relationships with ectomycorrhizal fungi.

Introduction

A major goal in ecology is to understand how interactions among organisms influence population dynamics, community structure and ecosystem processes. While many studies focus on pairwise interactions, recent research demonstrates that interactions among multiple species frequently cannot be predicted from pairwise interactions (Stanton, 2003; Strauss & Irwin, 2005). Complex interactions among the aboveground and belowground components of terrestrial ecosystems may be particularly important to understand as they are frequently mediated by primary producers that link organisms across the soil boundary (Wardle et al., 2004). For example, mycorrhizal fungi can alter visitation rates by pollinators (Gange & Smith, 2005; Wolfe et al., 2005; Cahill et al., 2008) thereby influencing the seed set of host plants (Wolfe et al., 2005).

Mycorrhizal fungi form symbioses with plant roots in most of the world’s ecosystems, where they can promote plant growth and productivity in return for fixed carbon (Smith & Read, 2008). Because species of mycorrhizal fungi can differ widely in their physiology (Rygiewicz et al., 2000; Smith et al., 2004) and ability to promote increased nutrient uptake and productivity (Jonsson et al., 2001; Klironomos, 2003), shifts in mycorrhizal fungal community composition could have important consequences for host plants. Herbivores are also common components of terrestrial ecosystems that may compete indirectly with mycorrhizal fungi for host plant carbon, resulting in reduced mycorrhizal colonization on heavily damaged plants (Gehring & Whitham, 2002; Gange, 2007; Gehring & Bennett, 2009). However, few studies have examined the impact of herbivory on mycorrhizal fungal community structure.
Research on the effect of herbivory on mycorrhizal fungal communities has produced highly variable results. Among arbuscular mycorrhizal fungi (AMF), cattle grazing has reduced species richness and/or diversity and altered community composition (Bethlenfalvay & Dakessian, 1984; Eom et al., 2001), native ungulates have promoted AMF species richness (Frank et al., 2003) and artificial defoliation has altered the AMF communities associated with a grazing intolerant grass species, but not a grazing tolerant one (Saito et al., 2004). Similarly, changes in the abundance of ectomycorrhizal fungal (EMF) morphotypes or species following artificial defoliation were observed in Scots pine (Pinus sylvestris) (Saikkonen et al., 1999), white birch (Betula papyrifera) (Markkola et al., 2004) and lodgepole pine (Pinus contorta) (Cullings et al., 2001), but EMF community composition did not change in poplars defoliated by the gypsy moth (Kosola et al., 2004). Although herbivore resistant and susceptible individuals occur in numerous ecosystems (Fritz & Simms, 1992; Hemming & Lindroth, 1995; Lawler et al., 1998; Simchuk et al., 1999; Underwood & Rausher, 2000; O’Reilly-Wapstra et al., 2004), differences in mycorrhizal fungal communities between these two categories of plants have been described in detail only by Gehring & Whitham (2002), who observed differences in EMF community composition between pinyon pines resistant and susceptible to herbivory by the scale insect (Matsucoccus acalyptus).

Differences in the mycorrhizal fungal communities of resistant and susceptible trees could be caused by herbivory itself or be the result of other genetic influences that are associated with herbivore resistance. Plant genetics can affect the ability of plants to form symbioses with mycorrhizal fungi (Smith & Read, 2008), the relative benefits they receive from mycorrhizal colonization (Barker et al., 2002), and the level of colonization of roots by mycorrhizal fungi (Tagu et al., 2001, 2005; Van der Heijden & Kuyper, 2001; Khaza et al., 2002; Gehring et al., 2006). However, the influence of plant genetics on mycorrhizal fungal community composition is not well understood. In this study, we compared the EMF communities of trees that were either resistant or susceptible to insect herbivory and used a long-term herbivore removal experiment to determine if herbivory was the direct cause of differences in EMF communities.

Mature Pinus edulis in northern Arizona provides a model system to test the influence of plant genetics on complex herbivore–mycorrhizal-plant interactions for five reasons. First, some mature P. edulis suffer consistently high rates of herbivory by a stem-boring moth (Dioryctria albivittella) while others do not, creating readily identifiable resistant and susceptible trees across the landscape (Whitham & Mopper, 1985). Second, resistance and susceptibility to moth herbivory has a genetic basis (Mopper et al., 1991). Third, chronic moth herbivory alters EMF abundance (Gehring & Whitham, 1991), and preliminary data at a coarse scale of taxonomic resolution suggest that resistant and susceptible trees differ in EMF community composition (Brown et al., 2001). Fourth, our study includes susceptible trees from which moths have been experimentally removed for approx. 20 yr, allowing comparison of trees with similar genetics but dramatically different levels of herbivory. The moth removal trees allowed us to determine if differences in the EMF communities of resistant and susceptible trees were caused by herbivore effects on host plant quality that affected EMF, or resulted from innate differences between resistant and susceptible P. edulis that more directly affected the EMF community. Fifth, we collected two sets of samples over a 10-yr period to examine the consistency of interactions between resistance to herbivory and EMF over time.

Materials and Methods

Study system

We conducted this research near Sunset Crater National Monument, 33 km north-east of Flagstaff, AZ, USA. Soils in this area are dominated by 1200-yr-old cinder deposits (Hooten et al., 2001) that include basaltic ash, cinders and lava flows, and belong in the US Department of Agriculture Soil Taxonomic Sub-Group of Typic Ustorthents. This site is dominated by pinyon pine (P. edulis Engelm) but also includes one-seed juniper (Juniperus monosperma Engelm) and Apache plume (Fallugia paradoxa D. Don). Vegetation cover is relatively low with large areas of bare ground. This young nutrient-poor soil also has low water-holding capacity and represents a stressful site for P. edulis growth where associations with mycorrhizal fungi may be especially important (Gehring & Whitham, 1994). Furthermore, P. edulis is the only host for EMF in this site as other common species (juniper and Apache plume) support arbuscular mycorrhizal fungi (Haskins & Gehring, 2005). Thus, any effects that herbivory, genetics or drought have on P. edulis EMF may also affect fungal biodiversity in this region of northern Arizona that is considered semi-arid (averaging c. 400 mm of precipitation per year, approximately evenly divided between summer rainfall and winter rain and snow). The first collection of EMF data (1994) was gathered after a decade of climate conditions that were wetter than average. The second collection of EMF data (2004) was gathered when the study site, along with much of the southwestern US, had suffered from a decade-long drought that resulted in widespread P. edulis mortality (Mueller et al., 2005a). Average early year (January–May) precipitation totalled 188.4 mm for the 5 yr before the 1994 collection and 86.6 mm for the 5 yr before the 2004 collection (http://www.wrcc.dri.edu/cgi-bin/cliMONtpre.pl?azsuns).

Mature P. edulis growing at the study site exhibited variation in resistance to the stem-boring moth, Dioryctria albivittella, which resulted in two distinct tree architectural
phenotypes (see Brown et al., 2001). Because the moth attacks the terminal shoots of the tree, susceptible trees have a short, shrubby architecture compared with the tall, upright architecture of resistant trees (Whitham & Mopper, 1985). Chronic herbivory by the moth resulted in increased branch mortality, reduced stem growth, reduced cone production, and reduced ectomycorrhizal colonization in susceptible trees (Table 1).

Resistant and susceptible tree EMF communities

We determined whether species richness and community composition differed between moth-resistant and moth-susceptible trees by collecting roots from 10 mature trees of each type in late May of 1994 and 2004. The study trees were c. 60–65 yr old in the first sampling year and extended over an area of 0.8 km². Previous observations show that the EMF communities of P. edulis in this age range were similar to those found on trees c. 160 yr old (C. Gehring, unpublished), therefore, we are confident that the 10 yr between sample dates had minimal ontogenetic effects. Moth resistant vs susceptible tree status was determined using annual censuses of moth herbivory conducted since 1983 (Whitham & Mopper, 1985; Brown et al., 2001; Mueller et al., 2005b) (Table 1). Using a hand-trowel, we collected fine roots (< 2 mm diameter) from the dripline of the north side of each tree at a depth of 0–30 cm, as in Gehring & Whitham (1991). The roots were transported back to the laboratory and about 75 living EMF tips per tree were classified based on the morphology of the EMF mantle and emanating hyphae as described by Agerer (1991).

Because samples were taken 10 yr apart and some different methods were used, care was taken to ensure that the two sets of samples could be reliably compared. One author (C.A.G.) led the collection effort during both time periods. She morphotyped the 1994 samples and C.M.S. morphotyped the 2004 samples. To ensure that morphotyping was consistent among years, C.A.G. and C.M.S. worked together on the 2004 samples until their morphotyping was completely consistent. Following morphotyping, EMF root tips were stored by tree and morphotype in 1.5 ml microcentrifuge tubes at −20°C. We extracted the DNA from a minimum of two to three root tips of each morphotype per tree using the mini-prep method of Gardes & Bruns (1993) as modified by Gehring et al. (1998) for the 1994 samples, but used DNeasy Kits (Qiagen) for the 2004 samples. We amplified the internal transcribed spacer (ITS) region of the fungal genome, located between the 18S and 28S rRNA, using PCR with the ITS1F and ITS4 primer pair (Gardes & Bruns, 1993). An identical PCR program was used for samples collected in 1994 and 2004. DNA extraction and amplification success was similar for samples collected during both years (91% for 1994 samples and 93% for 2004 samples). A subset of the samples collected in 1994 was not analysed immediately and instead was frozen at −80°C until analysis in 2001. The DNA extraction and amplification success of these samples did not differ from that of samples analysed within 6 months of collection.

We obtained restriction-fragment length polymorphism (RFLP) data following the methods of Gehring et al. (1998) for samples collected during both years. The amplified ITS region was characterized using restriction enzyme digestion with HindIII and Mbol, which have been used to discriminate among fungal species in P. edulis (Gehring et al., 1998). For samples collected during both years, we photographed gels under UV illumination, but used a Polaroid camera for 1994 samples and a Kodak EDAS 290 gel documentation system and accompanying software (Eastman Kodak Company, Rochester, NY, USA) for the 2004 samples. We compared RFLP patterns with those generated from fungal sporocarps and with a RFLP/sequence database generated using previous studies on P. edulis. The ITS regions of two or more DNA samples from each unidentified RFLP type (at least one from 1994 and one from 2004, where possible) were subject to forward and reverse sequencing using an ABI 3730 Genetic Analyser (Applied Biosystems, Foster City, CA, USA) at the University of Arizona sequencing facility. The sequences were aligned and edited using BioEdit version 7.0.9 (Hall, 2007). We compared these sequences with reference sequences in the GenBank (http://www.ncbi.nlm.nih.gov/) and UNITE (http://unite.ut.ee/analysis.php) databases. In addition, because variation in the prevalence of ascomycete and basi-

Table 1 Effects of moth herbivory and experimental moth removal on pinyon performance and ectomycorrhizal fungal (EMF) colonization

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Moth resistant</th>
<th>Moth susceptible</th>
<th>Moth removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot mortality¹ (no. dead shoots)</td>
<td>8.8ᵃ</td>
<td>115.5ᵇ</td>
<td>4.0ᵃ</td>
</tr>
<tr>
<td>Cone production² (no. cones/tree)</td>
<td>60ᵇ</td>
<td>20ᵃ</td>
<td>120ᵇ</td>
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<td>Shoot growth (mm)³</td>
<td>42ᵃ</td>
<td>35ᵇ</td>
<td>47ᵇ</td>
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<tr>
<td>EMF colonization (%)⁴</td>
<td>51⁴</td>
<td>34ᵇ</td>
<td>56ᵇ</td>
</tr>
</tbody>
</table>

¹Data from Mueller et al. (2005b) on the number of shoots killed by the stem-boring moth in 1992. Different letters represent significant differences among groups. Similar differences among groups have been observed over 20 yr of measurement.

²Data from Mueller et al. (2005b) on the number of cones produced per tree in 1992. Different letters represent significant differences among groups.

³Unpublished data on average shoot growth per year from 1986 to 2003 resulting from a long-term monitoring project at the study site. Different letters represent significant differences among groups.

⁴Data from Gehring & Whitham (1991) representing an average of two time periods. Different letters represent significant differences among groups.
diomycete fungi has been observed in pinyon pine in association with interspecific competition (Haskins & Gehring, 2004), herbivory (Brown et al., 2001) and mistletoe infection (Mueller & Gehring, 2006), we compared the percentage of EMF belonging to the division Ascomycota in resistant and susceptible trees.

**EMF communities on herbivore removal trees**

We tested if removal of the herbivore would result in altered EMF richness, a change in the percentage of Ascomycota and a community composition similar to moth-resistant trees by collecting fine roots from 10 trees that had moths experimentally removed for 10 yr (1994 sample) or 20 yr (2004 sample). Including these trees allowed us to compare the EMF communities of trees with similar genetics but dramatically different levels of herbivory. We characterized the EMF communities of moth removal trees at the same time as those of resistant and susceptible trees using the methods described earlier.

We removed moths once annually in September beginning in 1984 using the systemic insecticide, Cygon (C₆H₁₂NO₃PS₂; BASF Corp., Agricultural Product Group, Research Triangle, NC, USA). We sprayed Cygon at this time of the year to kill the moth larvae before they attacked terminal shoots. Previous studies indicated that Cygon did not have a direct fertilizer effect on pinyon growth (Whitham & Mopper, 1985), and it did not alter the abundance of belowground microarthropods that could influence ectomycorrhizas (Gehring & Whitham, 1991). Annual application of the insecticide over time has altered susceptible moth removal tree architecture so that it resembles that of naturally resistant trees (Brown et al., 2001). This change in architecture was associated with reduced mortality of branches, a dramatic increase in cone production and increased ectomycorrhizal colonization on moth removal trees (Table 1), indicating that the original differences observed between resistant and susceptible trees in these variables was caused by the presence of the herbivore. If moth herbivory also drives EMF community structure, then the moth removal trees should have similar EMF species richness and community composition as moth-resistant trees. Alternatively, EMF communities could be influenced directly by differences in plant genetics associated with resistance and susceptibility to moth herbivory. This hypothesis would be supported if the EMF communities of moth removal trees remained similar to moth-susceptible trees despite removal of the moth.

**Data analysis**

We analysed community composition data using PRIMER 5 (2002 PRIMER-E Ltd, Lutton Ivybridge, UK), while other statistical tests were performed using JMP 4.01 (SAS, 2000). We performed community analyses of RFLP types using nonmetric multidimensional scaling (NMDS), a robust nonparametric analytical technique that is applied to the dissimilarity matrix calculated among RFLP types using the Bray–Curtis dissimilarity coefficient (Faith et al., 1987). We tested the significance of differences in the EMF communities among treatment groups using analysis of similarity (ANOSIM) which is analogous to an F-test where distances or similarities within groups are compared between group differences. The ANOSIM r values measure the strength of these differences and scale between −1 and +1. We determined subsequent P values through a randomization procedure (Clarke, 1993; Clarke & Warwick, 2001). These procedures have been successfully used for the analysis of community composition (Dungey et al., 2000; Wimp et al., 2005) and provide results that can be interpreted ecologically (Faith et al., 1987). We tested for differences in EMF composition among moth-resistant and moth-susceptible trees and moth removal trees in each of the 2 yr sampled. We compared the mean EMF species richness per tree, and per cent EMF classified in the division Ascomycota among treatment groups in both years using a one-way ANOVA, followed by a Tukey’s HSD test to locate treatment differences.

In addition, we examined the trees that were sampled in both years and used MANOVA in JMP 4.01 to test for changes in EMF species richness and percent Ascomycota among treatments across time, including the interaction effect of treatment by time. Also, using a nested ANOSIM in PRIMER 5 we tested for changes in EMF community composition across time.

**Results**

**Summary of EMF taxa**

When samples from all trees (18 moth resistant, 19 moth susceptible and 19 susceptible moth removal) from both years were combined, we observed a total of 21 EMF taxa (Table 2). In 1994, we observed a total of 18 EMF taxa, including three that we observed only on resistant trees and five that we observed only on susceptible/removal trees. In 2004, we observed a total of 12 EMF taxa, including three that we did not observe in 1994. Nine EMF taxa from 1994 were not observed in 2004. In 2004, we observed three taxa only on resistant trees and four only on susceptible/removal trees. Of these 21 total taxa, we were able to assign some level of taxonomic identification to 20 (Table 2). Two EMF sequences yielded only division level information (Basidiomycota) and sequence quality was too poor to allow identification for one taxon (unknown EMF). These last three species were uncommon, making up only 3.25% of the total community. The relatively low EMF species richness observed is typical for the study site and nearby areas (e.g., Gehring et al., 1998).
Table 2  Restriction fragment length polymorphism (RFLP) identification of ectomycorrhizal fungi (EMF) observed in this study based on matches with sporocarp RFLPs or ITS sequence data

<table>
<thead>
<tr>
<th>RFLP ID</th>
<th>Nearest BLAST match</th>
<th>Accession #</th>
<th>Percentage</th>
<th>Bit score</th>
<th>Asco/Basidio</th>
<th>Frequency of RFLP type</th>
<th>1994</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>AF266709</td>
<td>96</td>
<td>984</td>
<td>Asco</td>
<td></td>
<td>7</td>
<td>10</td>
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<td>94</td>
<td>839</td>
<td>Asco</td>
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<td>6</td>
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<td>Pezizales 3</td>
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<td>92</td>
<td>844</td>
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<td>AY351625</td>
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<td>3</td>
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<td>Geopora cooperi</td>
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<td>DQ822805</td>
<td>93</td>
<td>577</td>
<td>Asco</td>
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<td>1</td>
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<td>Unknown ascomycete</td>
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<td>564</td>
<td>Asco</td>
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<td>Uncultured basidiomycete</td>
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<tr>
<td>Unknown basidiomycete 2</td>
<td>Unknown basidiomycete</td>
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<td>Unknown EMF</td>
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</tr>
</tbody>
</table>

1Percent similarity of query and reference sequences.
2Bit score is an evaluation of sequence alignment that takes into account the number of gaps and substitutions and the length of the query and reference sequences. Because bit scores are normalized, they allow for comparison among searches.
3Frequency of RFLP types in both sampling years. R, moth resistant; S, moth susceptible; RE, moth removal. Value represents the number of trees on which a given RFLP type was found. Numbers in parentheses are the number of trees in each treatment group.
4RFLP patterns were identified to species based on a match with an identified sporocarp collected from the study site.
5This species was identified using both sequence data and an RFLP match with a sporocarp collected from the study site.
EMF community structure of moth-resistant and moth-susceptible trees

The abundance and distribution of EMF species differed significantly between moth-resistant and moth-susceptible trees (ANOSIM $r = 0.370$, $P < 0.0001$; Fig. 1). These community composition differences were associated with a more than threefold higher percentage of ascomycete EMF in susceptible trees than in resistant trees in 1994 (mean ± 1 SE percentage ascomycete fungi = 81.1% ± 4.5 for susceptible trees, $n = 10$ and 28.8% ± 7.29 for resistant trees, $n = 10$, $F = 37.26$, $P < 0.0001$).

Sampling 10 yr later, in 2004, produced the same pattern in community composition where moth-resistant and moth-susceptible trees had significantly different EMF communities (ANOSIM $r = 0.256$, $P = 0.015$; Fig. 1). The EMF community of susceptible trees had a significantly higher percentage of ascomycete fungi than the resistant trees, as in 1994, but the magnitude of the difference was greatly reduced (mean ± 1 SE percentage ascomycete fungi = 92.3% ± 2.92 for susceptible trees, $n = 9$, and 63.3 ± 14.54 for resistant trees, $n = 8$, $F = 4.28$, $P = 0.05$). However, EMF species richness did not differ between moth-resistant and moth-susceptible trees in 1994 or 2004 (1994 mean ± 1 SE species richness = 3.90 species ± 0.62 for susceptible trees and 3.70 ± 0.52 for resistant trees, $F = 0.68$, $P = 0.78$; 2004 mean ± 1 SE species richness = 2.22 species ± 0.32 for susceptible trees and 2.38 ± 0.18 for resistant trees, $F = 0.54$, $P = 0.70$).

EMF of moth removal trees: herbivory vs genetics

The EMF community composition of moth removal trees was similar to that of moth-susceptible trees but different from that of moth-resistant trees, supporting the hypothesis that the genetic differences between resistant and susceptible trees directly influenced the composition of associated EMF communities, independently of moth herbivory (Fig. 1). The EMF communities of moth removal trees were significantly different from the EMF communities of moth-resistant trees in 1994 (ANOSIM $r = 0.543$, $P < 0.0001$) and 2004 (ANOSIM $r = 0.409$, $P < 0.0001$). However, the communities of moth removal trees did not differ significantly from the EMF communities of moth-susceptible trees 10 (ANOSIM $r = 0.050$, $P = 0.182$) and 20 yr (ANOSIM $r = 0.093$, $P = 0.132$) following the initiation of experimental moth removal.

The percentage of ascomycete fungi on moth removal trees also did not differ from that on moth-susceptible trees in either 1994 or 2004, but was significantly higher than moth-resistant trees in both years (mean ± 1 SE percentage ascomycete fungi for removal trees in 1994 = 89.4% ± 2.78, $F = 39.96$, $P < 0.0001$; mean ± 1 SE percentage ascomycete fungi for removal trees in 2004 = 91.7% ± 10.95, $F = 3.90$, $P = 0.035$). Removal of the moths from susceptible trees did not result in an increase in EMF species richness in either year (mean ± 1 SE species richness for removal trees in 1994 = 4.40 ± 0.62, $P = 0.514$; mean ± 1 SE species richness for removal trees in 2004 = 2.67 ± 0.37, $P = 0.589$).

Temporal changes in EMF communities and individual species

Owing to mortality associated with a severe drought from 2001 to 2004 (Mueller et al., 2005a; Shultz et al., 2009)
not all of the trees that we sampled in 1994 could be sampled in 2004. Therefore, we examined those trees that were sampled in both years (resistant \( n = 8 \), susceptible \( n = 8 \), removal \( n = 6 \)) for changes in EMF communities across time. We found that, although there no differences in mean EMF species richness among treatment groups, mean EMF species richness declined significantly from 1994 to 2004 (Table 3). There was also a significant treatment by time interaction, with susceptible trees showing a somewhat larger decrease in EMF species richness with time than the other two groups of trees. By contrast, the percentage of ascomycete EMF was not significantly different between the 2 yr, and there was no significant interaction (Table 3).

The overall EMF community composition also shifted from 1994 to 2004 (ANOSIM \( r = 0.173 \), \( P = 0.030 \)). However, this change was driven by changes in EMF associated with resistant trees (ANOSIM \( r = 0.309 \), \( P = 0.011 \)). Susceptible trees (ANOSIM \( r = 0.044 \), \( P = 0.226 \)) and removal trees (ANOSIM \( r = 0.158 \), \( P = 0.074 \)) showed no significant change in EMF community composition between the two sample times.

When we examined individual species responses (Fig. 2), the two species that were fairly common on resistant trees in 1994 (Tricholoma terreum and an unknown basidiomycete) were not observed on the same resistant trees in 2004. One member of the Basidiomycota not present in the 1994 sample was observed in 2004 (Suillus sp.). However, resistant trees experienced increases in two members of the Ascomycota (Pezizales 2 and 3), and a small increase in another species of ascomycete that was found only on moth-susceptible trees and moth removal trees in 1994 (Pezizales 4). These changes resulted in a shift in dominant species on resistant trees from members of the Basidiomycota in 1994 to members of the Ascomycota in 2004. The dominant species of EMF on moth-susceptible trees and moth removal trees did not change from 1994 to 2004, though a new ascomycete was observed in 2004 (Geopora sp.).

Table 3

<table>
<thead>
<tr>
<th>Richness</th>
<th>1994 (wet)</th>
<th>2004 (dry)</th>
<th>Repeated measures MANOVA</th>
<th>( P )-value(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moth resistant ( n = 8 )</td>
<td>3.250 (0.250)(^2)</td>
<td>2.375 (0.183)</td>
<td>Treatment</td>
<td>0.6567</td>
</tr>
<tr>
<td>Moth susceptible ( n = 8 )</td>
<td>4.25 (0.526)</td>
<td>2.25 (0.366)</td>
<td>Time</td>
<td>\textless 0.0001</td>
</tr>
<tr>
<td>Moth removal ( n = 6 )</td>
<td>3.5 (0.428)</td>
<td>2.67 (0.494)</td>
<td>Treatment ( \times ) time</td>
<td>0.0233</td>
</tr>
<tr>
<td>Ascomycota (%)(^3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moth resistant ( n = 8 )(^a)</td>
<td>35.9 (12.5)</td>
<td>63.3 (14.5)</td>
<td>Treatment</td>
<td>\textless 0.0001</td>
</tr>
<tr>
<td>Moth susceptible ( n = 8 )(^b)</td>
<td>76.3 (4.1)</td>
<td>93.4 (3.1)</td>
<td>Time</td>
<td>0.2180</td>
</tr>
<tr>
<td>Moth removal ( n = 6 )(^b)</td>
<td>96.5 (2.3)</td>
<td>87.5 (4.6)</td>
<td>Treatment ( \times ) time</td>
<td>0.3072</td>
</tr>
</tbody>
</table>

\(^1\)\( P \)-values for treatment, time and interaction effects of the repeated measures ANOVA. Bold values indicate a significant difference.

\(^2\)Value in parenthesis represents the standard error of the mean.

\(^3\)Different letters indicate significant differences among groups in the treatment factor of the repeated measures MANOVA.
Discussion

Herbivore vs genetic influences on EMF communities

In contrast to predictions of the model proposed by Gange (2007), resistant and susceptible trees did not differ in EMF species richness. Gange (2007) proposed that mycorrhizal fungal species richness would be maximized under moderate levels of herbivory because of stimulation of photosynthesis and increased carbon allocation below ground. High levels of herbivory, such as those experienced by moth-resistant trees, were predicted to lead to the survival of fewer species of mycorrhizal fungi because of low tolerance of reduced carbon supply in some species (Gange, 2007). We found that EMF species richness was very similar, and very low, in resistant and susceptible trees. The low levels of species richness and lack of difference in this measure between resistant and susceptible trees could be result from significant carbon limitation in this system, even in the absence of herbivory. Pinyon pines growing at a site with similar climate to our study site experienced significant periods of carbon starvation owing to water stress from 1994 to 2003, culminating in extensive tree mortality following 10 months without significant carbon assimilation in 2001–2002 (Breshears et al., 2009). Only a small subset of EMF species may be able to persist with such an intermittent supply of carbon from the host plant, resulting in consistently low species richness.

Although EMF community composition of moth-resistant and moth-susceptible trees differed substantially, no significant differences were detected between susceptible and moth removal trees. The fact that susceptible and moth removal trees failed to differ even after 20 yr of moth removal suggests that these community differences resulted from innate genetic differences between resistant and susceptible trees rather than the indirect effects of moth herbivory on carbon allocation to EMF. While some experiments using artificial defoliation have provided evidence that herbivory can alter the community composition of associated EMF (Saikkonen et al., 1999; Cullings et al., 2001; Markkola et al., 2004), the few that have examined the effects of natural herbivores on EMF composition have produced mixed results (Gehring & Whitham, 2002; Kosola et al., 2004). More studies of the impacts of herbivory on EMF communities are clearly needed. Given that the relationships between herbivores and their host plants frequently have a genetic basis (Fritz & Simms, 1992; O’Reilly-Wapstra et al., 2004), further comparisons of the EMF communities of plant populations that are resistant vs susceptible to herbivores may be particularly important. Our findings also clearly demonstrate the importance of long-term herbivore removal experiments in unravelling these complex relationships.

While the genetics of plant hosts, including genetically based differences in insect resistance, have been shown to affect EMF development and colonization (Gehring & Whitham, 1991; Tagu et al., 2001, 2005; Van der Heijden & Kuypers, 2001; Khasa et al., 2002), the influence of plant genetics on the community composition of EMF is not well understood. However, our observation that EMF community composition was not influenced by the moth directly, but rather by genetic factors associated with herbivore resistance, adds to a growing body of evidence that genetically based plant traits can have effects that extend beyond the individual or population to affect the associated community (Dungey et al., 2000; Johnson & Agrawal, 2005; Wimp et al., 2005; Crutsinger et al., 2006; Whitham et al., 2006) and ecosystem processes (Treseder & Vitousek, 2001; Crutsinger et al., 2006; Schweitzer et al., 2008) in diverse ecosystems. Our results are consistent with these observations and provide support for a surprising and potentially important finding: genetically based resistance traits to an above-ground herbivore can be strongly associated with soil mutualist community composition. Complex aboveground and belowground linkages are being demonstrated with greater frequency in the literature (Wardle et al., 2004; Callihill et al., 2008), but these recent studies suggest that plant genetics plays a strong, but as yet poorly understood, role in mediating these interactions. In this pinyon study system, reciprocal inoculation studies are necessary to further explore the potential for genetic control of EMF community composition in resistant and susceptible trees. Reciprocal transplant studies provide a powerful approach to separate soil and plant genetic effects (Macel et al., 2007). Further study is also necessary to understand how and why genetically based resistance and susceptibility to herbivory appears to be linked with genetically based EMF community composition in this system.

Dominance by ascomycete fungi and temporal patterns

Moth-resistant and moth-susceptible P. edulis varied significantly in EMF community composition, with extreme dominance by ascomycete EMF in moth-susceptible trees and moth removal trees in both years sampled. Most of these ascomycete fungi could only be identified to the level of taxonomic order (Pezizales) with ITS sequence data. Although basidiomycete fungi were initially thought to dominate ectomycorrhizal associations (Egger, 2006), the importance of ascomycete fungi has recently been recognized in diverse ecosystems (Dickie & Reich, 2005; Tedersoo et al., 2006; Morris et al., 2008). Tedersoo et al. (2006) reported high diversity of EMF in the order Pezizales from coniferous and boreal forests on several types of soil in Denmark and Estonia. In pinyon–juniper woodlands, pezizalean fungi responded positively to environmental stressors such as mistletoe parasitism (Mueller & Gehring, 2006) and interspecific competition (Haskins et al. 2009).
Gehring, 2004). Morphologically, members of the Pezizales tend to produce ectomycorrhizas with thin mantles and few emanating hyphae (Tedersoo et al., 2006), and some genera have rapid growth rates and significant saprotrophic capabilities (Egger, 1986). These characteristics may be highly advantageous to host plants in some environments as more robust basidiomycetous mycorrhizas that produce numerous, large sporocarps may represent an excessive carbon cost (Egger & Hibbett, 2004). Comparative studies on the costs and benefits of ascomycete and basidiomycete fungi in a variety of environments would further elucidate the importance of functional variation among the two groups of EMF to their host plants.

The EMF from trees sampled 10 yr apart revealed constancy in the relationships among tree groups, but significant differences in species richness and community composition with time. Species richness was significantly lower in the very dry year (2004) than in the wet year (1994), while ascomycete abundance on moth-resistant trees showed the opposite pattern. These results are consistent with previous findings (Gehring et al., 1998; Swaty et al., 2004) and with the hypothesis that EMF species richness declines and ascomycete fungi increase in response to drought stress. Ascomycete EMF have been shown to dominate in other drought-prone forests (Danielson & Pruden, 1989; Izzo et al., 2005) and laboratory studies demonstrate that species of EMF vary in their ability to survive drying (Di Pietro et al., 2007). Because EMF communities can vary substantially with time (Koide et al., 2007), we must exercise caution in drawing strong conclusions from a comparison of only one wet and one dry year. However, the fact that the EMF communities of moth-susceptible trees and moth removal trees remained similar in two samples collected 10 yr apart suggests that the changes in EMF of resistant trees may be driven by changes in climate. This shift towards ascomycete EMF could become more pronounced because moth-resistant trees experienced three times higher mortality than moth-susceptible trees during a record drought (Shultz et al., 2009). The marked reduction in the abundance of resistant trees could dramatically alter the distribution of basidiomycete EMF in this system. In addition, the fact that moth-susceptible trees were consistently associated with drought-tolerant members of the Ascomycota suggests the possibility that these species of EMF contributed to the survival of moth-susceptible trees. We believe that it is important to continue to explore the links between plant genetics, herbivore susceptibility and belowground communities to further place microbial community ecology within an evolutionary framework.

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References


