Mycorrhizal species identity affects plant community structure and invasion: a microcosm study

Elizabeth D. Stampe and Curtis C. Daehler

Previous studies have shown that arbuscular mycorrhizal fungi (AMF) can mediate plant interactions, thereby affecting plant community structure. Little is known, however, about whether the presence of different AMF species leads to differences in plant community structure or invasion success by introduced species. To investigate the effects of AMF species on community structure and invasion, we created replicate microcosms containing soil inoculated with one of three different AMF species (Glomus sp., Scutellospora erythropa Koske & Walker Walker & Sanders, or Scutellospora verrucosa (Koske & Walker) Walker & Sanders) or a mixture of all three AMF species. Seeds of seven naturally co-occurring plant species (Ageratum conyzoides L., Cyperus compressus L., Chamaecrista nictitans (L.), Crotalaria incana L., Hyptis pectinata (L.) Poit., Sida rhombifolia L., Melinis repens (Willd.) Zizka) in Hawai'i were sown equally into these microcosms, which were placed on outdoor benches. Plant community development was monitored over a season. Mid-way through the experiment, an invader (Bidens pilosa L.) was added to the established communities to determine whether mycorrhizal species identity affected invasion success. Final aboveground and belowground phytomass were used to assess plant community differences among treatments. Although the identity of the dominant plant species (Melinis repens) remained the same in all treatments, community dominance, community productivity, plant species richness, Shannon index of diversity, and invasion success all varied with AMF species identity. Invasion success was not inversely related to species richness or diversity. Instead, increased richness, diversity, and invasion success all appeared to be related to decreased dominance by Melinis repens in the presence of certain AMF species. These results indicate that the composition of the AMF community belowground can influence the structure of the plant community aboveground, and may play a role in facilitating or repelling invasion.

Arbuscular mycorrhizal fungi (AMF) are symbiotic fungi that take up resources, primarily phosphorus, from the soil, and supply them to host plants in exchange for photosynthate (Sylvia 1998). AMF can also confer increased nitrogen uptake, drought and heavy metal tolerance, and pathogen resistance to their host plants (Ames et al. 1983, Busse and Ellis 1985, Newsham et al. 1995, Weissenhorn et al. 1995). Ninety-five percent of the world’s plant species belong to families that are typically mycorrhizal (Smith and Read 1997). AMF can have strong effects on plant growth (Fitter 1989, Allen 1991). These effects can be positive or occasionally negative, and the degree of these effects differs among plant species (Buwalda and Goh 1982, Plenchette et al. 1983, Allen et al. 1989, Wilson and Hartnett 1998). By affecting different plant species in different ways, AMF can mediate interactions between plants. Many studies have found that AMF can affect competition between two plant species by differentially benefiting the weaker competitor, allowing the weaker
competitor to persist, or even reversing the outcome of competition (Hall 1978, Allen and Allen 1984, Hetrick et al. 1988). This mediation of plant-plant interactions has implications for plant community structure and dynamics; AMF have the potential to affect plant species richness, diversity and productivity (Grime et al. 1987, van der Heijden et al. 1998b, Hartnett and Wilson 1999, Klironomos et al. 2000).

AMF may also influence whether a community can be invaded by new plant species. If AMF effects vary among plant species, both in type and degree, these varying effects in turn may mediate the competitive interactions between the native and invasive plant species. Although several studies have examined the role of AMF in succession (Allen and Allen 1984, 1988, Gange et al. 1990, 1993), few studies have explicitly addressed effects of AMF on invasion by non-native species (Hoffman and Mitchell 1986, Halvorson and Koske 1987, Goodwin 1992). In competition experiments between a native bunchgrass and an invasive weed, Marker et al. (1999) found that the presence of AMF conferred a strong competitive advantage on the invader even though AMF had little effect on either plant species when grown alone. Emergent effects like these, which could not have been predicted from the single-species inoculation trials, illustrate the need for further experiments on AMF mediation of plant-plant interactions.

Although AMF are not generally characterized as being host-specific, AMF species can display preferences for certain host species (Johnson et al. 1992, Bever et al. 1996), and the effects of AMF species on their hosts can vary (Jensen 1984, Streitwolf-Engel et al. 1997). van der Heijden et al. (1998a) created microcosm communities composed of plants from European calcareous grasslands and inoculated them with each of four different species of AMF. The aboveground biomass of each plant species varied depending on which AMF species was in the soil. van der Heijden et al. (1998b) also created microcosm plant communities using North American old-field species, in which AMF species diversity varied from two to 14 AMF species. Plant community diversity and ecosystem productivity increased with increasing AMF species diversity, regardless of which AMF species were present.

It is likely that AMF species identity and diversity play important roles in structuring other plant community types as well, but studies from a variety of systems are necessary to determine the general importance of AMF species identity. This study utilized co-occurring species from a disturbed, low elevation, mesic habitat in subtropical Hawai‘i to examine whether AMF species identity or diversity could affect community structure and invasion success. We inoculated soil in replicated microcosms with one of three different species of AMF, with a combination of all three, or with no AMF. We monitored plant community development after sowing seeds of seven naturally co-occurring herbaceous plant species in each microcosm. The microcosms were then subjected to disturbance and invasion treatments, in which aboveground biomass was reduced by clipping, and the seeds of an additional plant species (the invader) were added, to determine whether community recovery and invasion depended on AMF species identity.

**Methods**

**Site description**

This experiment was conducted at the Hawai‘i Agriculture Research Center’s Maunawili Breeding Station, located on the windward side of Oahu, Hawai‘i, United States (21°22′ N, 157°46′ W, 120 m elevation). This site receives between 1.3 and 1.5 m of rain per year, and is in a subtropical climate, with average temperatures ranging between 21.5°C and 27.5°C for the timeline of the experiment (National Weather Service data for Oahu). At this site, seeds were collected from eight plant species. These species were naturally occurring on abandoned agricultural fields (Table 2).

**Field mycorrhizal assessment**

Roots were collected from three individuals of each plant species to assess whether these species were mycorrhizal in the field. The roots were stained with trypan blue using Koske and Gemma’s (1989) procedure. This entailed washing the roots, clearing them in 2.5% KOH at 90°C, rinsing them, soaking them in 1% HCl Table 1. Definitions of plant community variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community dominance</td>
<td>Dominant species’ aboveground phytomass</td>
</tr>
<tr>
<td>Plant species richness</td>
<td>Number of species present at final harvest</td>
</tr>
<tr>
<td>Plant species diversity</td>
<td>Shannon index calculated from aboveground phytomass of each species</td>
</tr>
<tr>
<td>Invasion success</td>
<td>Invader phytomass</td>
</tr>
<tr>
<td></td>
<td>Total community phytomass</td>
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</tbody>
</table>
overnight, then staining them in a mixture of acidic glycerol and trypan blue at 90°C. Root fragments were then examined using the slide method (Giovannetti and Mosse 1980); ten randomly-selected 1-cm root fragments per microcosm were laid on a glass slide and examined under a compound microscope at 100 × and 400 × magnification. A species was classified as mycorrhizal if AMF spores, vesicles, or arbuscules were found in the roots of at least one individual.

AMF inoculum

Pure culture inocula (250 ml each) of three species of AMF that occur naturally in Hawai’i were obtained from INVAM (International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi, West Virginia, USA). The AMF species were *Glomus sparcum* Pfeiffer, Walker & Bloss (INVAM accession NB106A-5), *Scutellospora erythropa* (Koske & Walker) Walker & Sanders (INVAM accession HA150B-6), and *Scutellospora verrucosa* (Koske & Walker) Walker & Sanders (INVAM accession VA150A-6).

Each inoculum was bulked on roots of Bermuda grass (*Cynodon dactylon* (L.) Pers.) grown in sterile basalt sand in plastic tubs. The sand was sterilized by autoclaving at 120°C for 45 minutes. In three tubs, inoculum of one AMF species was mixed with sand in a 37.7 g/l inoculum:sand ratio to make a roughly 1:30 volume ratio. Another tub was set up identically, with equal amounts of inoculum of all three AMF species to create the mixed-species inoculum. A final tub was set up identically, with no AMF inoculum, to create non-mycorrhizal control “inoculum” that would include other soil microflora and Bermuda grass roots. The grass was regularly watered and fertilized with low-phosphorus (1.15 µg P/ml) Hoagland’s solution (Mohr and Schopfer 1995). Colonization was confirmed after 4 months by examining roots, and the grass was then allowed to dry slowly over a period of a month. The roots were chopped into 2-cm pieces, and the sand substrate and roots from each tub were evenly divided into 12 samples of 550 g each, one for each microcosm. This amount was approximately equivalent to 380 ml of inoculum, to result in a final volume ratio of 1:30 inoculum:soil in each microcosm.

Microcosm and mycorrhizal treatment setup

Sixty microcosms were set up in 26 × 31 × 18 cm (14.8 l capacity) plastic tubs with drainage holes. These were filled with 8.5 l of sterile growth medium consisting of a 3:1:1 mixture of sterilized local topsoil, vermiculite, and perlite. The appropriate AMF inoculum, spread as an even layer, was then added to each microcosm. Finally, the inoculum was topped with a thin layer of sterile medium (< 1 cm) to hold it in place. The soil used in the microcosms was local brown topsoil, sterilized by autoclaving for 45 minutes at 120°C and stored for one month to dissipate any phytotoxic effects of autoclaving (R. Koske, pers. comm.). The soil pH was 7.33 and the approximate soil nutrient levels were: 40 µg g⁻¹ phosphorus, 2870 µg g⁻¹ potassium, 2830 µg g⁻¹ calcium, 490 µg g⁻¹ magnesium, and 0.28% nitrogen (Univ. of Hawai’i Agricultural Diagnostic Service Center). The values given are for the soil prior to mixing with vermiculite and perlite.

Twelve replicate microcosms were established for each of four treatments. These included: *Glomus sparcum* inoculum (GS treatment), *Scutellospora erythropa* inoculum (SE treatment), *Scutellospora verrucosa* inoculum (SV treatment), and mixed-species inoculum (GS + SE + SV treatment). In the fourth treatment, the twelve microcosms contained sand and Bermuda grass roots, identical to the other inocula, but lacked AMF (no AMF treatment).

The microcosms were placed in the field at the Maunawili breeding station on 1-meter-high benches in full sun. Microcosms were elevated 3 cm off the benches and separated by 46-cm-high clear plastic barriers to reduce the risk of AMF cross-contamination and reduce natural seed dispersal into the microcosms. The treatments were arranged in a random block design.

Seeds of seven plant species (all species except *Bidens pilosa*; Table 2) were added to the microcosms on April 18, 2000 (day 1). Each microcosm was divided into four quadrants and one-fourth of the seeds of each species were added to each quadrant to minimize spatial differences in starting conditions for each community. Based on germination trails, seed viability was known to vary substantially among species. The number of seeds added for each species was varied accordingly (ranging from 8 to 56) to obtain a projected yield of 6 seedlings per species (525 total seedlings per m²).

This density assured that competition would occur among species, and is within the range of seedling densities observed naturally on recently abandoned Table 2. Plant species in microcosm communities.

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Life history</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ageratum conyzoides</em> L.</td>
<td>Asteraceae</td>
<td>A (P) Herb</td>
</tr>
<tr>
<td><em>Bidens pilosa</em> L.²</td>
<td>Asteraceae</td>
<td>A Herb</td>
</tr>
<tr>
<td><em>Cyperus compressus</em> L.</td>
<td>Cyperaceae</td>
<td>P Sedge</td>
</tr>
<tr>
<td><em>Chamaecrista nictitans</em> (L.)</td>
<td>Fabaceae</td>
<td>A (P) Herb</td>
</tr>
<tr>
<td><em>Crotalaria incana</em> L.</td>
<td>Fabaceae</td>
<td>A/P Herb</td>
</tr>
<tr>
<td><em>Hyptis pectinata</em> (L.) Poit.</td>
<td>Lamiaece</td>
<td>P Herb/Sub-shrub</td>
</tr>
<tr>
<td><em>Sida rhombifolia</em> L.</td>
<td>Malvaceae</td>
<td>P Herb/Sub-shrub</td>
</tr>
<tr>
<td><em>Melinis repens</em> (Willd.)</td>
<td>Poaceae</td>
<td>A/P Grass</td>
</tr>
<tr>
<td><em>Zizka</em></td>
<td></td>
<td></td>
</tr>
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1 A = annual, P = perennial, (P) = short-lived/weakly perennial (Wagner et al. 1999).
2 Species designated as invader.
fields. Six seed-free control microcosms were also set up to check whether seeds of the plant species used in the experiment were dispersing naturally into the microcosms.

After 30 days it became apparent that far more than six seedlings of three of the plant species (Melinis, Crotalaria and Sida) were germinating in each microcosm, and this was unrelated to the AMF treatments (GLM: Wilks’ Lambda test, $F_{12,140} = 0.631$, $P = 0.813$; Individual $F$ tests: Melinis: $F_{4,55} = 0.417$, $P = 0.796$; Crotalaria: $F_{4,55} = 0.792$, $P = 0.431$; Sida: $F_{4,55} = 0.554$, $P = 0.697$). To reduce the density of these species to near the desired level, these species were thinned to six individuals per microcosm. After this one-time thinning, no further attempt was made to adjust seedling densities.

The microcosms were each watered with one liter of water between three and five times per month, depending on rainfall. Each microcosm was fertilized with one liter of low-phosphorus Hoagland’s solution (Mohr and Schopfer 1995) four times over the course of the experiment, on days 68, 77, 119 and 128.

### Invasion and disturbance treatments

On day 80, seeds of the designated invader, Bidens pilosa, were introduced into the microcosms. Bidens was chosen as the invader because it has successfully invaded natural Hawaiian ecosystems (Smith 1985), and it can be strongly mycorrhizal, as indicated by preliminary examination of the roots, and as found in a previous study (Koske et al. 1992). On the same day that Bidens seeds were added, each microcosm was divided in half with a clear plastic barrier 44 cm high, inserted 2 cm deep in the soil, in preparation for an aboveground disturbance treatment in half of each microcosm.

On days 82 and 83, the disturbance treatment was applied by clipping off the aboveground biomass of one half of each of the communities to a height of approximately 10 cm. The disturbed side was determined randomly for each microcosm. The disturbance treatment was applied in order to reduce competition for light and give the invader a better chance to establish and respond to the AMF treatments. The aboveground disturbance treatment also provided an opportunity to monitor recovery of aboveground biomass by the established community, as related to AMF species identity.

Community recovery in the aboveground disturbance treatment was monitored using changes in percent cover. Cover was estimated for the entire microcosm with the aid of a wooden frame divided with wire into 20 squares, each representing 5% cover. Cover was estimated just after disturbance, on day 89, and then on days 111, 121, 134, and 154.

### Harvesting and data collection

The experiment was terminated by harvesting the plants on days 161–164. The roots of the largest plant of each species in each microcosm were collected, washed and fixed in a 50% ethanol solution to assess AMF colonization. The aboveground phytomass (live and attached dead plant matter) in each microcosm was collected, sorted by species and disturbance treatment, dried at 45°C to a constant mass, and weighed. Soil from each microcosm was collected, dried at an ambient temperature (22°C), and used for AMF spore isolation and nutrient sampling. Finally, all roots in each microcosm, including those used to assess AMF colonization, were washed thoroughly and dried at 45°C to determine total belowground phytomass of each microcosm.

### Soil nutrient analyses

The soil collected from each microcosm was combined into one sample for each AMF treatment and extractable phosphorus levels were analyzed using the Olsen method (Olsen and Sommers 1982) by the Univ. of Hawai’i Agricultural Diagnostic Service Center. The soil solution phosphorus concentration was also determined using M. Habte’s (pers. comm.) method to find the native soil solution P concentration of the soil. This method involved adding a 0.01 M CaCl2–KH2PO4 solution to soil samples, then shaking the samples for one hour, centrifuging them at 10 000 rpm, and mixing them with acid molybdate reagent. The samples’ resulting color was read by a spectrophotometer and compared to absorbancies of standard phosphorus solutions to find the soil solution phosphorus concentration.

### Confirming AMF status of the treatments

Soil samples from each microcosm were examined for AMF spores to confirm that each microcosm contained the expected AMF species, and to confirm that the no AMF treatment microcosms were not contaminated. The spore isolation procedure was modified from Walker et al. (1982), based on suggestions from R. Koske and J. Gemma (pers. comm 1999). Spores were isolated from a soil solution by sieving with a 10-mesh sieve, centrifuging at 3900 rpm (1950 × g) without, then with sucrose (Clinical Centrifuge, International Equipment Company, Needham Heights, MA), and sieving again with a 200-mesh sieve.

To further confirm AMF status of the treatments, preserved roots of several species from several microcosms were selected randomly and stained with trypan blue using Koske and Gemma’s (1989) procedure.
Plant community variables

Aboveground productivity for the undisturbed treatment was measured as the final harvested phytomass. Aboveground productivity in the disturbance treatment was measured by combining the biomass removed at the time disturbance with the final harvested phytomass. The belowground phytomass was not separated by disturbance treatment because roots from the undisturbed plants likely grew in both sides of the microcosms, making separation of the roots impossible. Total plant community productivity was estimated by adding the aboveground disturbed and undisturbed phytomass and the belowground phytomass for each microcosm.

The community variables other than productivity (Table 1) were calculated using only the aboveground phytomass harvested at the end of the experiment, without including the removed phytomass from the disturbance treatment. This was because the other variables were measures of the community at the time of harvest, not over the course of the experiment. To relate invasion success to the diversity and richness of the established community, diversity and richness were recalculated after omitting the invader and were tested for correlation with invasion success.

Statistical analysis

All statistical analyses were done using SYSTAT 8.0 (SPSS, Inc, Chicago, IL), except chi-squared tests, which were done using StatExact Version 4 (CYTEL, Cambridge, MA).

To determine whether community recovery from disturbance differed among AMF treatments, changes in the total percent cover of all species combined were analyzed over time using a repeated-measures ANOVA. The effects of AMF treatments on community dominance, aboveground phytomass, species richness, diversity, and invasion success were analyzed using a multivariate, random block, split-plot design to incorporate the disturbance treatment. A separate multivariate random blocks ANOVA was used to analyze total phytomass and belowground phytomass, because the roots of disturbed and undisturbed plants were not physically separable by the disturbance treatment. The no AMF treatment was excluded from the above analyses because the purpose was to test the effects of different AMF species on community structure and invasion; however, the results for the no AMF treatment are reported on the graphs. A separate ANOVA, which included the no AMF treatment, was used to test whether the no AMF treatment differed from treatments with AMF present.

The indices of community dominance and invasion success were arcsine square-root transformed before being entered in the model. All other data were normally distributed and did not need to be transformed. A sequential Bonferroni adjustment for multiple comparisons (Wright 1992) was used to assess statistical significance of each univariate F test derived from the MANOVA. Tukey tests were then used to determine which treatments were significantly different from one another for each dependent variable.

Results

Field mycorrhizal assessment

The roots of all field-collected plant species except Cyperus compressus contained AMF structures. This indicates that they are capable of being mycorrhizal in the field.

Soil analysis

The soil phosphorus levels at the end of the experiment were: $\text{GS} = 19 \, \mu g \, g^{-1}$, $\text{SE} = 20 \, \mu g \, g^{-1}$, $\text{SV} = 23 \, \mu g \, g^{-1}$, $\text{GS + SE + SV} = 19 \, \mu g \, g^{-1}$, no AMF = 21 $\mu g \, g^{-1}$ (Univ. of Hawai’i Agricultural Diagnostic Service Center). The soil solution phosphorus concentrations of each AMF treatment were: $\text{GS} = 0.004 \, \text{mg/l}$, $\text{SE} = 0.007 \, \text{mg/l}$, $\text{SV} = 0.008 \, \text{mg/l}$, $\text{GS + SE + SV} = 0.004 \, \text{mg/l}$, no AMF = 0.002 mg/l. Due to lack of replication, statistical differences in phosphorus among treatments could not be assessed; however, these values are considered low, and mycorrhizae are expected to be beneficial at these phosphorus levels (M. Habte, pers. comm.).

Confirmation of mycorrhizal status of microcosms and inclusion in analyses

In three microcosms (one no AMF, one GS, and one SV), the dominant plant species was missing, and the resulting communities were very different from those in the other microcosms. This did not appear to be an effect of AMF treatment, as the missing species was present and dominant in all other microcosms. These anomalous microcosms were excluded from analysis.
Six no AMF treatment microcosms showed no soil or root evidence of contamination with mycorrhizae; these were used for analyses. Spores of *Glomus spurcum* were present in the soil of all twelve GS treatments but spores of another species of AMF species were also found in one GS microcosm; that GS microcosm was excluded from analyses.

Spores of only *Scutellospora verrucosa* were present in the soil of all SV treatments. No spores were found in any of the SE microcosms, so *Sida* roots from ten of the SE microcosms were scanned for AMF (roots of *Sida* plants in two other SE microcosms were not available). AMF structures were present in *Sida* roots in eight of the ten SE treatment microcosms. These eight SE microcosms were considered to contain *Scutellospora erythropa* and only these microcosms were used for analyses. The lack of *S. erythropa* spores in the soil was attributed to the AMF not having sporulated yet.

Spores of *G. spurcum* and *S. verrucosa* were present in all three-species treatments, but as in the SE treatment, no *S. erythropa* spores were found. All GS + SE + SV microcosms were used for analyses under the assumption that *S. erythropa* had not sporulated yet but was present in the roots.

Percent cover after disturbance

Mean cover of disturbed treatments across all AMF treatments was 43% on day 7 after disturbance, 84% on day 29 after disturbance, 80% on day 39, 98% on day 50, and 105% on day 72 (Fig. 1). The repeated-measures analysis showed a significant effect of AMF treatment on cover over time \(F_{3,32} = 3.97, P = 0.016\); the SE treatment had greater cover than the GS treatment (Fig. 1). Prior to disturbance, the SE and GS treatments did not differ significantly in cover (mean = 86% and 89%, respectively). There was also a significant AMF treatment-by-time interaction \(F_{12,128} = 2.01, P = 0.028\), suggesting that the pattern of recovery over time may have differed among AMF treatments, although in general, each AMF treatment showed a similar pattern of increasing cover over time (Fig. 1).

**Final plant community structure**

Multivariate analysis of aboveground productivity, community dominance, species richness, species diversity, and invasion success showed a significant effect of AMF treatment \(F_{15,179} = 4.32, P < 0.0001\). The effect of disturbance was also significant \(F_{5,65} = 5.5, P < 0.0001\), but there was no interaction with AMF treatment \(F_{15,179} = 1.21, P = 0.266\). The multivariate model constructed with total and belowground productivity also showed a significant effect of AMF treatment \(F_{6,62} = 6.85, P < 0.0001\).

**Productivity**

Total productivity varied significantly among AMF treatments with higher productivity in the GS and GS + SE + SV treatments than in the SE and SV treatments \(F_{3,32} = 14.50, P < 0.0001\, \text{Fig. 2a}\). Belowground productivity also varied significantly among AMF treatments, following a similar pattern to that of total productivity \(F_{3,32} = 6.09, P = 0.0021\, \text{Fig. 2b}\). Aboveground phytomass did not vary significantly among AMF treatments (Fig. 2c).

**Community phytomass**

*Melinis repens* was the dominant species in the plant communities (Fig. 3a, 4). The relative dominance of *Melinis* was significantly different across mycorrhizal treatments (Table 3); it was higher in the GS and GS + SE + SV treatments than in the SE treatment.

**Plant species richness and diversity**

Plant species richness varied significantly among AMF treatments (Table 3); it was lower in the GS treatment than in the SE and SV treatments (Fig. 3b).

The Shannon index of diversity was also significantly different among AMF treatments (Table 3, Fig. 3c). Plant species diversity was lowest in the GS and GS + SE + SV treatments and highest in the SE treatment. This pattern was similar to that of plant species richness (Fig. 3b and 3c).

Individual plant species varied in their response to the AMF treatments; some appeared to have little or no variation among treatments and some appeared to respond more strongly to AMF treatments (Fig. 4). For example, the phytomass of both *Bidens* and *Ageratum* was highest in the SE treatment and lowest in the GS treatment, and this held true in both the disturbed and...
Fig. 2a. Plant community total productivity across AMF treatments. b. Plant community belowground phytomass across AMF treatments. Disturbed and undisturbed treatments were combined in both of these analyses. c. Disturbed and undisturbed plant community aboveground phytomass across AMF treatments. See Fig. 1 for abbreviations. Error bars represent 1 SEM. Different letters denote significant differences ($P < 0.05$, Tukey test) between mycorrhizal treatments.

Fig. 3a. Community dominance of Melinis repens in disturbed and undisturbed plant communities across AMF treatments. Community dominance was calculated by dividing Melinis aboveground phytomass by total community aboveground phytomass. b. Species richness of disturbed and undisturbed plant communities across AMF treatments. c. Shannon index of species diversity of disturbed and undisturbed plant communities across AMF treatments. Black bars are the undisturbed treatment, gray bars are the disturbance treatment. See Fig. 1 for abbreviations. Error bars represent 1 SEM. Different letters denote significant differences ($P < 0.05$, Tukey test) between mycorrhizal treatments.

Undisturbed treatments. This variation in the response of Bidens and Ageratum to AMF treatment contributed to the higher species richness in the SE treatment and the lower species richness in the GS treatment (Figs. 3b, 4). Chamaecrista was missing from the no AMF treatment (Fig. 4). This could have indicated that Chamaecrista was better able to establish in the presence of AMF. However, Chamaecrista was very rare in all treatments (12 Chamaecrista individuals in total observed in all microcosms), and its absence from the no AMF treatment was not statistically unexpected (chi-squared test = 1.67, df = 1, $P = 0.33$).

Invasion success
Invasion success differed significantly among AMF treatments (Table 3, Fig. 5). The No AMF, SE, and SV

Table 3. Results ($P$-values) of univariate $F$ tests for plant community variables (aboveground productivity, community dominance, species richness, species diversity, and invasion success), with four AMF treatments (three single-species AMF treatments and one three-species AMF treatment) and the split-plot disturbance treatment.

<table>
<thead>
<tr>
<th>AMF</th>
<th>Aboveground productivity</th>
<th>Community dominance</th>
<th>Plant species richness</th>
<th>Plant species diversity</th>
<th>Invasion success</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AMF</td>
<td>0.2089</td>
<td>0.0160</td>
<td>0.0010</td>
<td>0.0135</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Disturbance</td>
<td>0.0004</td>
<td>0.0270</td>
<td>0.2266</td>
<td>0.0093</td>
<td>0.0025</td>
</tr>
<tr>
<td>AMF × Dist.</td>
<td>0.0717</td>
<td>0.9442</td>
<td>0.7643</td>
<td>0.8552</td>
<td>0.3766</td>
</tr>
<tr>
<td>Block</td>
<td>0.9861</td>
<td>0.8876</td>
<td>0.4311</td>
<td>0.8685</td>
<td>0.0338</td>
</tr>
</tbody>
</table>
treatments were significantly more invaded than the GS treatment, and the SE treatment was also significantly more invaded than the GS + SE + SV treatment. In general, the more invaded treatments (Fig. 5) were also the treatments with higher species richness and diversity across AMF treatments (Fig. 3b, 3c).

When diversity was recalculated for each microcosm without including the invader, diversity was not significantly correlated with invasion success (all AMF treatments combined, Pearson's correlation coefficient; $r = 0.1355$, $P = 0.1879$).

**Discussion**

**Mycotrophy and AMF species identity**

In this study system, individual plant species appeared to be less mycotrophic than plant species used in community experiments by van der Heijden et al. (1998b); plants in the no AMF treatment did not show markedly lower phytomass than plants in the AMF treatments (Fig. 4). This may be due to the fact that our study involved a weedy plant community that occurs in disturbed habitats; weedy species are often facultatively mycotrophic (Halvorson and Koske 1987). While facultative mycotrophs can grow without mycorrhizae, their growth responses in the presence of mycorrhizae can vary, as in this study. Most previous studies have focused on comparing these differences in plant performance in the presence and absence of AMF. In this study the identity of the AMF species present apparently mattered as much as whether AMF was present or absent (although differences between community structure in the presence and absence of AMF were relatively small). This result is meaningful because while
there are not many natural habitats that lack AMF entirely, the species composition and diversity of AMF communities can differ widely, and these differences may contribute to significant variation in natural plant communities.

**Melinis dominance and AMF species effects**

AMF species identity significantly affected total and belowground productivity, as measured by phytomass. The aboveground productivity of the community was almost completely determined by *Melinis* (Fig. 3a, 4). Though it was not possible to reliably separate the roots of *Melinis* from those of the rest of the community, when the belowground phytomass was being washed and weighed, it was evident that it was primarily composed of *Melinis* roots. Variation in total community productivity seemed to be largely determined by differences in *Melinis*’ response to the AMF treatments.

The observed differences in community recovery between the GS and SE treatments following disturbance (Fig. 1) could also be explained by differences in *Melinis* dominance (Fig. 3a). The disturbance entailed cutting all aboveground biomass to a height of 10 cm. As a result, the cover of the shorter, subordinate species was less affected by this treatment. The greater abundance of subordinate species in the SE treatment could explain its higher total cover following disturbance, relative to the GS treatment, in which *Melinis* was more dominant.

The degree of dominance by *Melinis* may in turn have affected other community variables. For example, patterns of species richness, diversity, and invasion success across AMF treatments tended to be the opposite of the productivity pattern; they were generally lower in the GS and GS + SE + SV treatments and higher in the SE and SV treatments (Fig. 3b, 3c, 5). Since the growth of *Melinis* largely determined productivity, it is likely that an increased abundance of *Melinis* in the GS treatment led to decreased species richness, diversity and invasion success in that treatment. We suspect that the dominant was influencing the invader and other species, rather than the converse, because of the much greater phytomass attained by the dominant.

Of course, plants grown together in competitive communities potentially respond to both the AMF and to each other. From our experiment, it is not possible to distinguish whether the invader and subordinate species were responding directly to the AMF infection, which may have altered their survival, growth rates, and/or competitive abilities, or if the dominant’s response to AMF treatments led to changes in success of the other species, or whether some combination of these was occurring. Whether the mechanism was direct or indirect, the end result was that AMF species identity affected invasion success. Although the invader’s phytomass was small relative to that of the community, its establishment and persistence varied significantly based on the AMF species present. To our knowledge, this is the first direct experimental evidence that plant community invasion can be affected by the species composition of the AMF community belowground.

**Invasion success and diversity**

Invasion did not show an inverse relationship to diversity and richness, as predicted by some researchers (Elton 1958, MacArthur 1972, Tilman 1997). Some other studies have also failed to observe this pattern; Peart and Foin (1985) found that plots dominated by one grass species were less invasible than mixed species plots of grasses and forbs. Robinson et al. (1995) also found that species-rich plots were more invasible than species-poor plots; dominance by one grass species was negatively related to the invader’s germination and fruit production. If a community’s low diversity results from dominance by one species that attains very high phytomass, then low diversity may also mean low invasibility.

Levine and D’Antonio (1999) suggest that invaders are generally not any different from the established species within a community, and that they benefit from the same factors that the original species do. In this experiment, subordinate species within the established community (such as *Ageratum*) appeared to respond to AMF species identity the same way an invader entering the community did. Both responses may have been mediated by the dominant’s growth. Factors that pro-
mote diversity in communities, like certain AMF species that reduce the success of a dominant, may also promote invasion in the same way. This is contrary to the idea that diversity reduces invasibility (Elton 1958, MacArthur 1972, Tilman 1997).

Feedback between AMF and invaders

In this experiment, invasion success increased with disturbance in most AMF treatments, although even with disturbance the invader certainly did not take over the community (Fig. 5). This suggests a way by which AMF could play an important role in invasion. By mediating the early establishment (young seedling survival) of introduced species, AMF could determine whether an invader survives long enough for a second factor (like disturbance) to weaken native competitors (Hobbs and Huenneke 1992) and allow it to spread. Other factors, such as the arrival of a pollinator (Ramirez and Montero 1988) could act similarly to promote the spread of the invader. A similar idea in which synergistic interactions among invaders accelerate their invasion has been described as “invasional meltdown” (Simberloff and Von Holle 1999).

This kind of acceleration could also occur as a result of positive feedback between the invader and the AMF community. Goodwin (1992) suggested that when non-mycorrhizal alien plant species colonize a site previously dominated by mycorrhizal natives, AMF populations decline. This decline could then reduce the competitive ability of the native species, and so create a positive feedback that facilitates invasion. A mycorrhizal invader could also affect the AMF community belowground. As shown in the present study, an invader can be more successful in the presence of certain AMF species, and this could increase the abundance of those AMF species, possibly to the detriment of native species. The presence of certain plant species has already been shown to cause shifts in AMF community composition (Johnson et al. 1991, 1992, Bever et al. 1996, Johnson and Wedin 1997).

Taking the opposite perspective, in some cases, negative feedback could also occur between an invader and the AMF community. In this experiment, differences in invasion success could have been partly due to the presence of an AMF species that was less favorable to the invader’s establishment.

Conclusions

The composition of the AMF community belowground can affect plant community structure and invasion success aboveground, and this response can apparently occur even in plant communities composed of species that are not strongly dependent on mycorrhizae for growth. This suggests that simple comparisons of plant growth with and without mycorrhizae, without considering the identity of AMF that naturally occur with the plant, may be of limited relevance to plant growth in the field. Further ecological studies that take into account the composition and effects of the local AMF community may lead to an improved understanding of natural variation in plant community structure and invasion success.

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