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Evidence for ecological matching of whole AM fungal communities to the local plant–soil environment

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Abstract. The range of ecological roles exhibited by arbuscular mycorrhizal (AM) fungi depends on functional differences among naturally occurring local assemblages of AM species. While functional differences have been demonstrated among AM fungal species and among geographic isolates of the same species, almost nothing is known about functional differences among whole communities of naturally occurring AM fungi. In the greenhouse, we reciprocally transplanted whole AM fungal communities between plant–soil systems representing a serpentine grassland and a tallgrass prairie, using as hosts two grasses common to both systems. For *Sorghastrum nutans*, native fungi consistently enhanced plant growth more than fungi switched from the alternate system. For *Schizachyrium scoparium*, foreign and native fungi promoted plant growth similarly in both the serpentine and prairie systems. Thus, the use of foreign inoculum in restoration could change the relative performance, and potentially the competitive abilities, of co-occurring plant species. Moving AM fungal inocula into foreign environments also caused changes in the taxonomic composition of the resultant spore communities, demonstrating their response to environmental influences. These results provide strong evidence for functional differences among naturally occurring AM communities and suggest that a particular AM fungal community may be better matched ecologically to its local habitat than communities taken from other locations.

Key words: *arbuscular mycorrhizal fungi; plant growth; plant–soil; reciprocal inoculation;* Schizachyrium scoparium; *serpentine grassland;* Sorghastrum nutans; *tallgrass prairie; whole community*.

Introduction

By forming symbiotic relationships with plant roots, arbuscular mycorrhizal (AM) fungi provide a variety of ecological functions. In exchange for carbon, they can enhance their host's uptake of nutrients, especially phosphorus (Smith et al. 2001), improve plant water relations (Augé 2001) and tolerance to herbivory (Kula et al. 2005, Bennett and Bever 2007), and afford protection from soil-borne pathogens (Borowicz 2001). AM fungi can impact ecosystem processes by affecting plant productivity and species diversity (van der Heijden et al. 1998*b*, Vogelsang et al. 2006), soil structure (Rillig and Mummey 2006), and even flowering, with consequent changes to the pollinator community (Cahill et al. 2008). Variation in how effective AM fungal species are at promoting plant growth or in the particular services they provide can have important ramifications at different levels within the ecosystem (Fitter 2005, van der Heijden and Scheublin 2007).

Functional differences occur both among AM fungal species (Streitwolf-Engel et al. 1997, van der Heijden et al. 1998*a*) and among different isolates of the same species (Klironomos 2003, Munkvold et al. 2004, Koch et al. 2006). Such differences are sometimes measured simply by disparity in host plant growth, but in other cases variation is identified in the particular nutritional or physiological benefits provided the host (Stahl and Smith 1984, Fidelibus et al. 2001, Klugh and Cumming 2007). Interspecific differences in morphological characters, such as extra-radical hyphal structure, and in phenology or other life history traits may also translate into differences in function (van der Heijden and Scheublin 2007).

Because AM fungi do not normally exist as isolated species, functional variation needs to be examined at the whole community level in order to better understand its ecological significance. Multiple AM fungal species typically exist as fungal assemblages (Morton et al. 1995) and simultaneously infect the roots of a single plant (Vandenkoornhuyse et al. 2002, Öpik et al. 2006). Plant growth can be greatly enhanced when infected with a mixture of AM fungal species compared to a single species (van der Heijden et al. 1998*b*, Gustafson and Casper 2006, Jansa et al. 2008), suggesting the fungi may provide complementary services to the plant or engage in other synergistic relationships. On the other hand, plant growth may be no greater with a mixture of fungal partners than with the single most effective partner alone (van der Heijden et al. 1998*b*, Bennett and

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Bever 2007). Thus, it is difficult to predict the action of whole AM fungal communities from the action of constituent AM fungal species examined separately. To date, only a limited number of studies have used the whole community of AM fungi as inoculum for experimentation (e.g., Johnson 1993, Kiers et al. 2000, Moora et al. 2004, de la Peña et al. 2006).

Patterns of AM fungal species distributions suggest that ecological factors impact AM fungal community structure in nature. The taxonomic composition of AM fungal communities is known to vary across such abiotic gradients as nutrients, clay vs. sand, pH, and aridity (Anderson et al. 1984, Porter et al. 1987, Egerton-Warburton and Allen 2000) and with the introduction of exotic plants (Hawkes et al. 2006). Interspecific competition between AM fungal species can also affect their abundance and vary with soil type (Lekberg et al. 2007). Of particular relevance to our study, involving one serpentine site, is recent evidence that nearby serpentine and non-serpentine ecotypes of *Collinsia sparsiflora* harbor different AM fungal communities (Schechter and Bruns 2008).

We ask whether naturally occurring whole AM fungal communities differ functionally, and if so, whether those differences suggest AM fungal communities are ecologically well matched to their local host-soil environment. The idea was suggested by Klironomos (2003) who demonstrated that plants from an old-field community more often grew better with native vs. foreign isolates of several AM fungal species examined individually. Native fungal isolates can also be more effective in promoting plant growth when applied in species mixture (Shah et al. 2008). A critical test, however, must involve reciprocally transplanting AM fungi between ecological systems. Such an approach would distinguish between the possibility that AM fungal communities are ecologically matched to their respective plant–soil environments and the possibility that fungi from a particular area are uniformly more robust or effective in promoting plant growth than fungi from another.

In a greenhouse experiment, we provide a direct test of functional differences between naturally occurring AM fungal communities by reciprocally transplanting whole communities between two grassland systems with the same dominant grasses but disparate soil chemistry, one prairie and one Eastern U.S. serpentine grassland, and determining the consequences for plant growth. By examining the spores produced when the same AM fungal inoculum was placed in the two systems, we could ascertain environmental influences on the composition of the spore community. We are aware of only two other studies involving reciprocal transplantation of AM fungal communities between ecological systems (Weinbaum et al. 1996, Johnson et al. 2010), and one of them (Weinbaum et al. 1996) focused on the temporal persistence of foreign fungi and not on plant performance.

Methods

The study systems and field material collection

Soil, plant, and fungal materials were collected from one serpentine grassland, Nottingham County Park, Chester County, Pennsylvania (39°44′ N, 76°02′ W), and one prairie grassland, Hayden Prairie, Howard County, Iowa (43°26′ N, 92°23′ W) with vastly different soil chemistry (Ji 2007). Serpentine soils are characteristically high in Ni, Cr, Fe, and Mg. The prairie site has higher levels of P (21.6 ± 0.8 mg/kg soil), K (130.3 ± 6.1 mg/kg soil), and Ca (3333.1 ± 84.7 mg/kg soil) in comparison to Nottingham (4.9 ± 0.4 mg P/kg soil; 74.5 ± 4.0 mg K/kg soil; 570.5 ± 26.8 mg Ca/kg soil). The two sites have similar levels of extractable NH_4-N, but extractable NO_3-N is higher at Nottingham (11.4 ± 0.7 mg/kg soil at Nottingham; 3.9 ± 0.3 mg/kg soil at Hayden). Mean annual precipitation (1988–2007) at Nottingham and Hayden is 890 and 1290 mm, respectively (data from the nearest weather stations [for Nottingham, Coatesville 2 W, COOP ID 361591; for Hayden, Cresco 1 NE, COOP ID 131954] on the National Climatic Data Center web site are *available online*).[4]

Despite edaphic differences, the sites share the same dominant C_4 grasses, *Andropogon gerardii* (Vitm.), *Sorghastrum nutans* [(L.) Nash], *Schizachyrium scoparium* [(Michx.) Nash], and *Sporobolus heterolepis* [(A. Gray) A. Gray], although the prairie plant community is more diverse. Seeds of *Schizachyrium scoparium* and *Sorghastrum nutans* were collected from both sites in October 2005 for our experiment.

The AM fungal communities in the two sites are also similar. Based on previously conducted field surveys and two generations of trap cultures (Ji 2007), Hayden and Nottingham share nine AM fungal morphospecies. Hayden has slightly higher diversity within the Glomeraceae and lacks *Gigaspora gigantea* found at Nottingham.

Soils were collected in June 2006. Soils used as sources of AM fungal spores came from 15 widely separated (by >30 m) collection points per site. At each point location, approximately 1.0 L of soil was collected from the root zone of one individual each of *Schizachyrium* and *Sorghastrum*, transported in coolers and stored at 4°C until used for spore extraction within four weeks. Bulked soils to be used as growth media were taken from six other arbitrary locations per site, thoroughly homogenized, and passed through a 2-cm sieve to remove large roots and rocks. They were then steamed at 100°C for 2 h to kill resident soil microbes, mixed with autoclaved (121°C) sand (in a 6:1 ratio) to promote drainage, and placed in 10 cm deep, 500-mL pots.

Preparation of AM fungal spore inocula

We chose to use AM fungal spores as our inoculum for two reasons: (1) it allowed us good control over both

[4] ⟨http://www.ncdc.noaa.gov⟩

the quantity and species composition of the inoculum and (2) the use of whole-soil inoculum or infected roots would introduce non-AM organisms and potentially abiotic factors differentially associated with serpentine and prairie systems. Our goal was to manipulate only the AM fungal communities while maintaining natural combinations of soils, plants, and non-AM microbes.

To obtain the AM fungal spores representative of the two distinct, naturally occurring communities to use as inocula, we homogenized the soil collected under a particular individual plant and extracted spores from six separate 50-mL quantities using the modified wet sieve method (McKenney and Lindsey 1987). All spores were visually inspected under a dissecting microscope and those appearing fresh and healthy were picked and cleaned by vigorous rinsing with diH_2O. Cleaned spores were soaked in a solution of 500 mg/L streptomycin and 500 mg/L penicillin overnight for surface sterilization (Castelli and Casper 2003). Spores from the same host species and site of origin were then pooled together and sterile diH_2O added to bring the volume to 30 mL. These spore mixtures were shaken to maintain the spores in suspension during the removal of 30 1-mL aliquots, which were stored at 4°C until used to inoculate plants.

Experimental design

We conducted separate, replicated experiments for *Sorghastrum* and *Schizachyrium*. Plants were grown in their native soil and inoculated with the AM fungal community collected in association with the same host grass species either at the same site (native AM fungal community) or with the AM fungal community collected in association with the same grass species but at the alternate site (foreign AM fungal community). AM fungi were not switched between the two grasses in case there were host-specific intraspecific (Castelli and Casper 2003) or community-level differences in fungi. Seedlings were grown in a sterilized mixture of sand and vermiculite and transplanted, two per pot, at two weeks of age. The two plants were inoculated with an AM fungal spore community by the application of a single 1-mL aliquot to their bare roots. A solution containing non-AM microbes native to the particular soil was added to each AM fungal treatment. The solution was obtained by stirring a 3-L quantity of freshly collected soil into 6-L diH_2O and then filtering through a 20-μm sieve (Klironomos 2002). Pots were watered with 50 mL of this solution one day after transplanting. In addition, there were two treatments without AM fungi for each plant–soil combination: one with native non-AM microbes only (non-AM treatment) and the other with neither group of microbes (control treatment); the latter was watered with 50 mL of diH_2O.

Plants were grown in the greenhouse at the University of Pennsylvania for 13 weeks (mid-July to late October 2006). Greenhouse temperature was kept at 25°C on average between 06:00 and 18:00 and 21°C otherwise. Light was maintained above photosynthetically active radiation (PAR) of 430 $\mu mol \cdot m^{-2} \cdot s^{-1}$ by either artificial light or ambient sunlight for at least 12 h/d. Because space was limited, pots from the different treatments were segregated in rows with at least 40 cm spacing between rows in order to minimize potential cross contamination among treatments. Pots were rotated within rows and the rows rotated between two greenhouse benches every two weeks. All plants were fertilized with 5 mL of one-quarter strength Hoagland's solution roughly once per week beginning in late August except that a 10-mL quantity was used for two successive applications in October.

Plant harvest

All plants survived, and the sum aboveground biomass of the two seedlings per pot, after oven drying at 70°C for 96 h, was used in the statistical analysis. Approximately 0.5 g (wet mass) of roots was harvested from each pot that had been inoculated with AM fungal spores and from a subset of pots (five of 15) in both the non-AM and control treatments in order to quantify the degree of AM root colonization. Root samples were cleared in heated 10% KOH solution then stained with 0.1% trypan blue (Koske and Gemma 1989). Mycorrhizal colonization was scored using the magnified intersect method (McGonigle et al. 1990). Percentage of root colonization by AM fungi was based on the presence or absence of hyphae, arbuscules, or vesicles on at least 100 root intersections per sample examined at 200× using a compound microscope.

AM fungal communities at harvest

The composition of the AM fungal spore community was determined at the time of harvest for each pot receiving AM fungal spore inoculum. A subset of pots (five of 15) from both the non-AM and control treatments was also checked for spore presence. Spores were extracted from a 50-mL subsample of well-mixed soil per pot, and those appearing fresh and healthy were identified to species under dissecting and compound microscopes based on their color, size, surface ornamentation, hyphal attachment, Melzer's reaction, and wall structure (Schenck and Pérez 1990). Abundance of each spore morphospecies was estimated based on a 0–4 scale: 0, none; 1, <10 spores; 2, 11–50 spores; 3, 51–100 spores; 4, >100 spores. Five AM fungal morphospecies were found in treatments without AM fungal inoculum added (*Glomus aggregatum*, *G. etunicatum*, *G. geosporum*, and *Entrophospora infrequens* in both Hayden and Nottingham soil; *G. constrictum* in Hayden soil only). These spores appeared healthy, but plant roots in those same pots were not colonized. Therefore, the spores were likely nonviable residual spores from the field collections and not actively involved in mycorrhizal establishment or sporulation during the experiment. Since the same residual spores were most likely present in the inoculated pots, for these five species we subtracted the mean abundance score of control pots

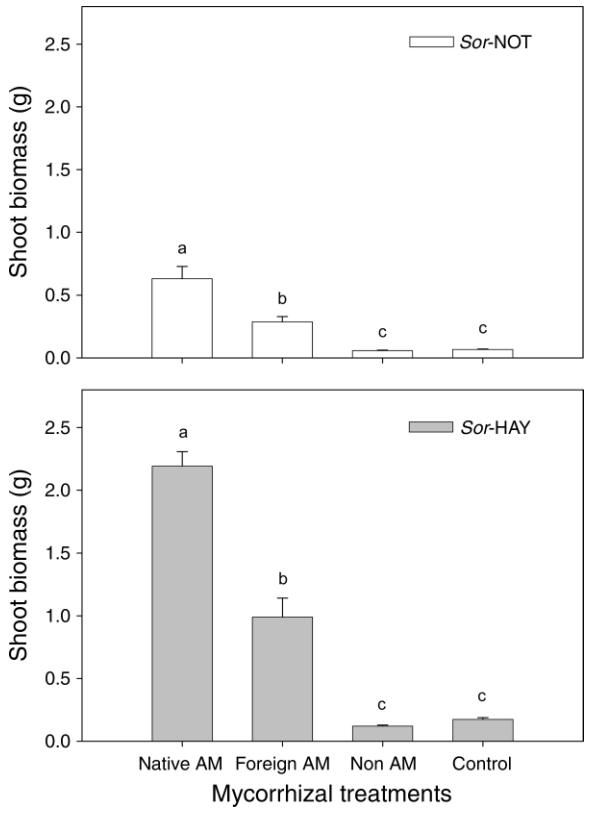

FIG. 1. Shoot biomass (mean and SE) of *Sorghastrum* in the Nottingham serpentine soil (*Sor*-NOT; $F_{3,56} = 29.66$; $P < 0.001$) and *Sorghastrum* in the Hayden prairie soil (*Sor*-HAY; $F_{3,56} = 112.16$; $P < 0.001$) as a function of microbial treatment. Different lowercase letters within a plant–soil combination indicate significant differences at $P < 0.05$. AM stands for arbuscular mycorrhizal fungi.

from the raw abundance scores of inoculated pots and used adjusted scores in analyses.

Data analyses

Within each plant–soil combination, shoot biomass was compared among the four microbial treatments (native AM, foreign AM, non-AM microbes only, or control) using a one-way ANOVA and Tukey's hsd for post-hoc comparisons ($P < 0.05$). Shoot biomass and mycorrhizal root colonization (log-transformed to meet assumptions of normality) were also examined for each plant species as a function of plant–soil origin and as function of foreign or native AM fungal origin in a two-way ANOVA. In additional analyses, root colonization was separately compared between Hayden and Nottingham plants/soils and between *Sorghastrum* and *Schizachyrium*. We calculated responsiveness of the plants to mycorrhizae according to Wilson and Hartnett (1998): ([mean dry mass with native AM fungi and non-AM microbes] – [mean dry mass with non-AM microbes only])/(mean dry mass with native AM fungi and non-AM microbes). Statistical analyses were conducted in JMP Version 5.1 (SAS Institute 2005).

For ease in visualizing results, the frequency of each AM fungal species is reported as the number of pots in each plant–soil inoculum combination (out of 15 replicates) in which a particular AM fungal species was observed. Comparisons of AM fungal spore communities between plant-soil combinations based on the actual spore abundance scores for each AM fungal species with pots as sampling points were made using canonical analysis of principal coordinates (CAP) as described by Anderson and Willis (2003). The first two canonical variables, accounting for the most variation, show potential separation between plant-soil combinations and the contribution of each fungal species to the multivariate pattern. CAP analyses were performed using a computer program from Anderson and Willis (2003: Supplement 1).

RESULTS

Plant growth

Plant growth response to native vs. foreign AM fungi differed between the two host species. For *Sorghastrum*,

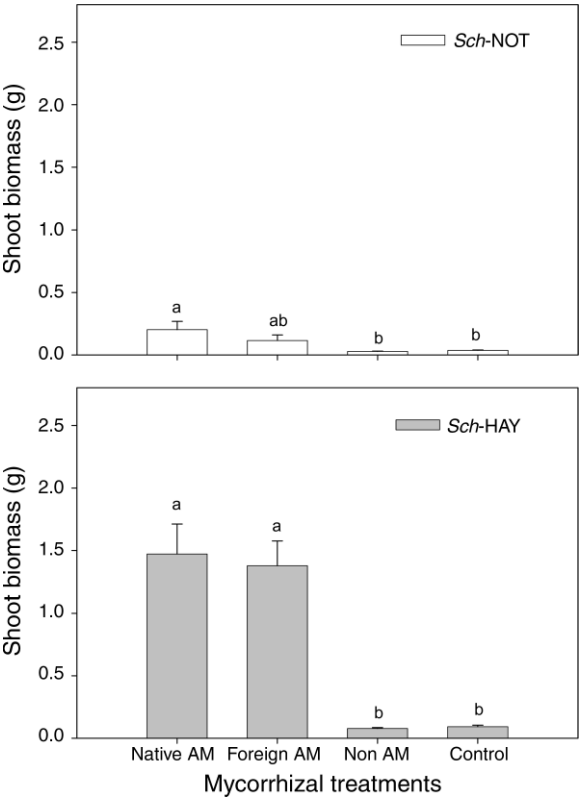

FIG. 2. Shoot biomass (mean and SE) of *Schizachyrium* in the Nottingham serpentine soil (*Sch*-NOT; $F_{3,56} = 4.31$; $P < 0.01$) and *Schizachyrium* in the Hayden prairie soil (*Sch*-HAY; $F_{3,56} = 34.90$; $P < 0.001$) as a function of microbial treatment. Different lowercase letters within a plant–soil combination indicate significant differences at $P < 0.05$.

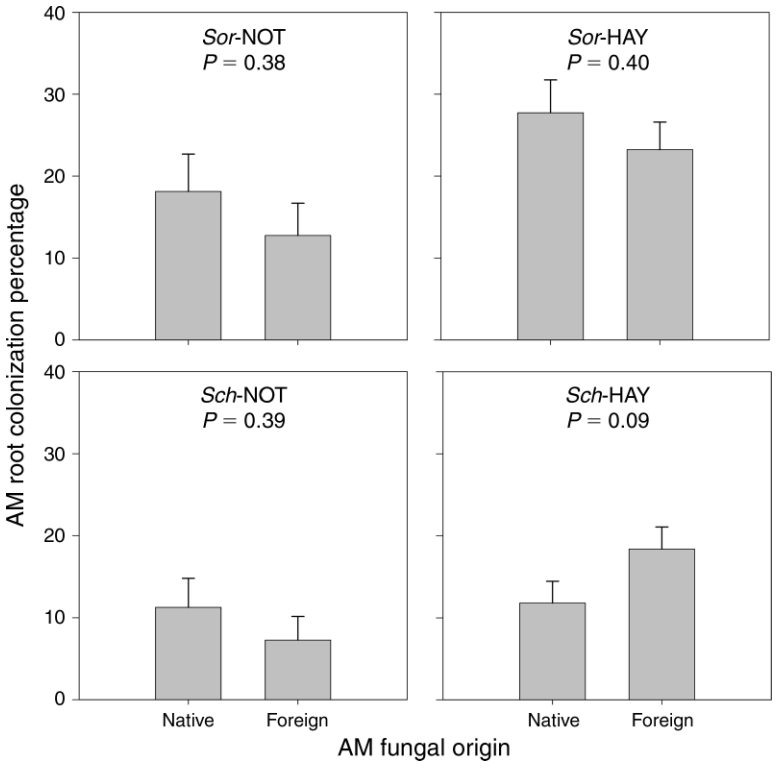

FIG. 3. Root AM colonization percentage (mean and SE) for each of the four plant–soil combinations. *P* values refer to comparisons of native vs. foreign AM fungal origins within each plant–soil combination.

reciprocally transplanting AM fungal communities between the Nottingham serpentine and Hayden prairie systems resulted in greater shoot growth with native AM fungi in both systems ($F_{1,56} = 6.44$, $P < 0.01$; Fig. 1). *Sorghastrum* in the Hayden prairie soil (*Sorghastrum*-HAY) responded slightly more strongly to native fungi (significant plant–soil × fungal source interaction; $F_{1,56} = 39.81$, $P < 0.001$). The same results did not occur for *Schizachyrium* for which shoot biomass did not differ between native and foreign mycorrhizae for either the Hayden or Nottingham system ($F_{1,56} = 0.09$, $P = 0.76$; Fig. 2).

The overall importance of AM fungi to the performance of these plants is also apparent. Plants grew much larger with any AM fungal inoculum, regardless of its origin, than without AM fungi, with one exception (Figs. 1 and 2). *Schizachyrium* in the Nottingham serpentine soil (*Schizachyrium*-NOT) receiving foreign AM fungi from Hayden prairie (HAY AM_{Sch}) did not produce significantly greater biomass than plants without AM fungi added.

The less favorable soil chemistry of serpentine most likely explains the difference in shoot biomass as a main effect of plant–soil origin. Shoot biomass was greater for *Sorghastrum*-HAY than for *Sorghastrum*-NOT ($F_{1,56} = 89.41$, $P < 0.001$) and greater for *Schizachyrium*-HAY than for *Schizachyrium*-NOT ($F_{1,56} = 77.23$, $P < 0.001$).

Evaluation of the plant/fungal relationship

The percentage root length colonized, a measure of infection by AM fungi, does not explain why *Sorghastrum* responded to fungal origin while *Schizachyrium* did not. For both hosts, colonization levels were not affected by whether the AM fungi were native or foreign (*Sorghastrum*, $F_{1,56} = 0.02$, $P = 0.88$; *Schizachyrium*: $F_{1,56} = 3.23$, $P = 0.08$). Root colonization was greater in the Hayden plants/soils than in Nottingham plants/soils ($F_{1,118} = 10.75$; $P < 0.01$) and was slightly greater for *Sorghastrum* than for *Schizachyrium* overall ($F_{1,118} = 10.02$; $P < 0.01$; Fig. 3).

Similarly, differences between the two host species in growth response to inoculum source cannot be explained by differences in how much they rely on mycorrhizae. Calculations of mycorrhizal responsiveness (Wilson and Hartnett 1998, Janos 2007) yielded high values of 0.91 and 0.94 for *Sorghastrum*-NOT and *Sorghastrum*-HAY, respectively. A similar value, 0.95, was obtained for *Schizachyrium*-HAY. The lower value of 0.86 for *Schizachyrium*-NOT seems to reflect overall poor growth for *Schizachyrium* in serpentine soil.

AM fungal spore communities at harvest

Placing AM fungal inocula in novel plant–soil environments mainly altered spore numbers per morphospecies, but there were changes in the presence/absence of others (Table 1). Some cases involved a

TABLE 1. Presence of the various arbuscular mycorrhizal (AM) fungal species at harvest as a function of origin of the fungal inoculum (NOT AM_{Sor}, NOT AM_{Sch}, HAY AM_{Sor}, or HAY AM_{Sch}) and the plant–soil combination (*Sor*-NOT, *Sor*-HAY, *Sch*-NOT, or *Sch*-HAY) onto which the inoculum was placed.

	NOT AM_{Sor}		NOT AM_{Sch}		HAY AM_{Sor}		HAY AM_{Sch}	
Species	*Sor*-NOT	*Sor*-HAY	*Sch*-NOT	*Sch*-HAY	*Sor*-NOT	*Sor*-HAY	*Sch*-NOT	*Sch*-HAY
Amo	8	0	8	5	2	6	10	2
Gag	6	9	7	0	5	14	3	7
Gcl	0	0	0	0	0	5	1	2
Gco	0	3	2	6	2	9	6	9
Get	13	0	14	2	15	0	13	14
Gge	0	15	1	4	1	14	6	14
Gmo	0	0	0	0	0	15	2	9
Gru	0	2	0	0	2	0	2	0
Ein	0	7	5	6	1	14	5	13
Gig	9	10	3	4	0	0	0	0
Sca	6	0	4	4	10	0	4	0
Spe	8	4	5	3	0	0	2	0

Note: Values reported are the species frequencies measured as the total number of pots (out of 15 replicate pots) in which a particular AM fungal species was found at harvest. Abbreviations: NOT, Nottingham; HAY, Hayden; *Sor*, *Sorghastrum nutans*; *Sch*, *Schizachyrium scoparium*; *Amo*, *Acaulospora morrowiae*; *Gag*, *Glomus aggregatum*; *Gcl*, *Glomus claroideum*; *Gco*, *Glomus constrictum*; *Get*, *Glomus etunicatum*; *Gge*, *Glomus geosporum*; *Gmo*, *Glomus mosseae*; *Gru*, *Glomus rubiforme*; *Ein*, *Entrophospora infrequens*; *Gig*, *Gigaspora gigantea*; *Sca*, *Scutellospora calospora*; *Spe*. *Scutellospora pellucida*.

species that was present when the inoculum was placed with its native plants/soils but was absent with foreign plants/soils, i.e., *G. claroideum* and *G. mosseae* from HAY AM_{Sor} and *Acaulospora morrowiae* and *G. etunicatum* from NOT AM_{Sor} inoculum. But there were also cases when an inoculum did not yield a particular AM fungal species with its native plants/soils but did with foreign plants/soils. For example, *Scutellospora calospora*, occurred in 10 replicates of *Sorghastrum*-NOT inoculated with HAY AM_{Sor} but no replicates of *Sorghastrum*-HAY with the same inoculum. Such species must have been present in native soil in low natural abundance but sporulated in greater numbers in the novel environment.

Some variation in the composition of AM fungal spore communities at harvest can also be directly attributed to the source of inoculum (Table 1). Two AM fungal species (*G. claroideum* and *G. mosseae*) sporulated from inocula collected at Hayden but did not sporulate from inocula collected at Nottingham. Likewise, one AM fungal species (*Gigaspora gigantea*) was produced by inocula collected at Nottingham but never by inocula collected at Hayden.

CAP analysis quantified environmental effects on the composition of the AM fungal community at the time of harvest. For three of the four different fungal inocula—i.e., collected on *Sorghastrum* or *Schizachyrium* at either Hayden or Nottingham—the species composition of the fungal community diverged when placed in the prairie vs. serpentine systems (Figs. 4a and 5a). The one exception occurred for the AM fungal community collected on *Schizachyrium* at Nottingham (NOT AM_{Sch}), which did not differ when placed with *Schizachyrium*-HAY compared to *Schizachyrium*-NOT (Fig. 4a).

CAP analysis was also useful for identifying the fungal species primarily responsible for the divergence in the spore communities. Inoculated with the NOT AM_{Sor} spore community, *Sorghastrum*-HAY pots had greater numbers of *Gi. gigantea* spores than did *Sorghastrum*-NOT pots, while the latter had higher abundance of *A. morrowiae* spores (Fig. 4b). The separation among plant–soil groups receiving Hayden-derived inocula was driven by the higher abundance of *G. mosseae*, *G. aggregatum* and *G. claroideum* in pots with Hayden plants/soils and the lower abundance of *G. etunicatum* and complete absence of *S. calospora*, *S. pellucida* and *G. rubiforme* in those same pots (Fig. 5b). CAP analyses showed some differences in the AM fungal communities associated with the two host plant species within a soil type. AM fungal communities harvested from *Sorghastrum*-HAY inoculated with HAY AM_{Sor} and AM fungal communities from *Schizachyrium*-HAY inoculated with HAY AM_{Sch} differed significantly (Fig. 5a). There were much smaller differences between the fungal communities on *Sorghastrum*-NOT and *Schizachyrium*-NOT inoculated with fungi originating from Nottingham (Fig. 4b).

DISCUSSION

Our research using a reciprocal transplant approach provides direct evidence for functional differences between naturally occurring AM fungal communities that are ecologically meaningful with respect to native combinations of plants, soils, and non-AM microbes. *Sorghastrum* exhibited reduced growth benefit when fungi were switched from the native community to the foreign community in both the prairie and serpentine systems. Our initial expectation that the serpentine inoculum would be more effective than the prairie inoculum in promoting plant growth in serpentine was based on a number of studies demonstrating increased metal tolerance in AM fungi in soils polluted by heavy metals (Weissenhorn et al. 1994, Hildebrandt et al. 1999,

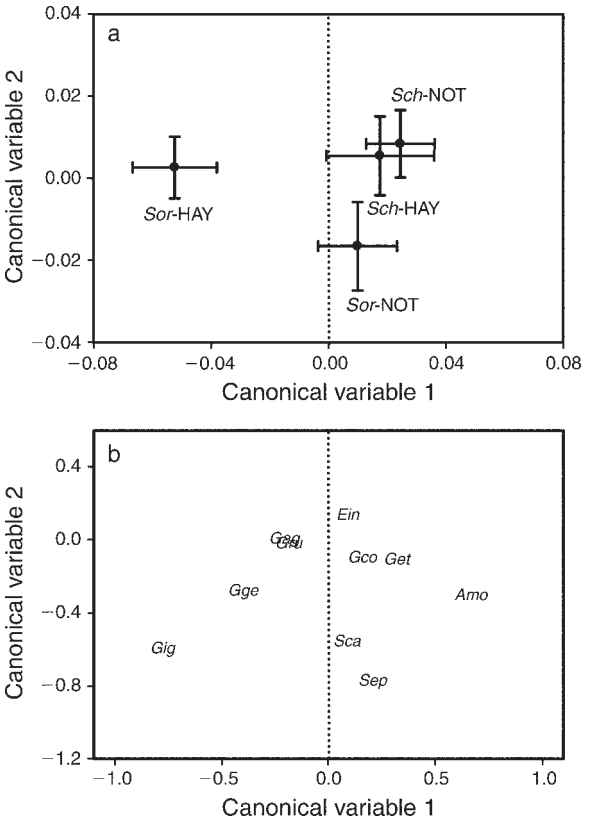

FIG. 4. Canonical discriminant (CAP) plots of (a) AM fungal spore communities produced by inoculum from Nottingham as a function of the plant–soil environment into which the inoculum was placed and (b) the relative placement of AM fungal species with respect to those community differences, i.e., *Sor*-HAY and *Sor*-NOT inoculated with NOT AM_{Sor} and *Sch*-HAY and *Sch*-NOT inoculated with NOT AM_{Sch}. Error bars represent 95% confidence intervals of the group mean. Fungal abbreviations are given in Table 1.

Malcová et al. 2003) or in serpentine soils with naturally high levels of metals (Amir et al. 2008). That the reciprocal was also true—that serpentine inoculum was less effective than the prairie inoculum in promoting the growth of *Sorghastrum* in the prairie system—suggests a possible trade-off between metal tolerance in AM fungi and their performance in non-metalliferous soils.

Functional differences between fungal communities must be viewed in the context of the host plant species as the growth of *Schizachyrium* was indifferent to foreign vs. native AM fungi in both the prairie and serpentine systems. Plant species are known to differ in their responsiveness to changes in AM fungal taxa (van der Heijden et al. 1998*b*, Helgason et al. 2002). One previous study with *Schizachyrium scoparium*, using a single soil type, also showed no difference in biomass with different sources of AM fungal inocula (Anderson and Roberts 1993). In our study, *Schizachyrium* was less colonized by AM fungi than was *Sorghastrum*, but it is difficult to draw a causal link between *Schizachyrium*'s indifference to the source of inoculum and the lower root colonization.

Regardless of why the two grasses exhibited different responses to native vs. foreign fungi, the overall results hold important ecological implications for ecosystem management. Our results suggest that the application of foreign AM fungi can alter the relative performance of coexisting plant species, potentially translating into changes in interspecific competitive ability and plant community structure. Because foreign AM fungi improved the performance of *Schizachyrium* relative to *Sorghastrum*, the competitive balance could be shifted toward *Schizachyrium* if nonnative AM fungal communities were applied to either system.

Klironomos (2003) used the term "local adaptation" to describe the situation when a native AM fungal isolate produces more host plant growth than a foreign isolate. We recognize, as Klironomos did, that host plant growth may not be the appropriate metric in evaluating local adaptation—and similarly, ecological matching—in their fungal partners (Helgason and Fitter 2009). Its usage implies that the performance of the host and the performance of the fungi are positively correlated, which may or may not be the case (Ryan et al. 2005, Bever et al. 2009), but see Johnson et al. (2010).

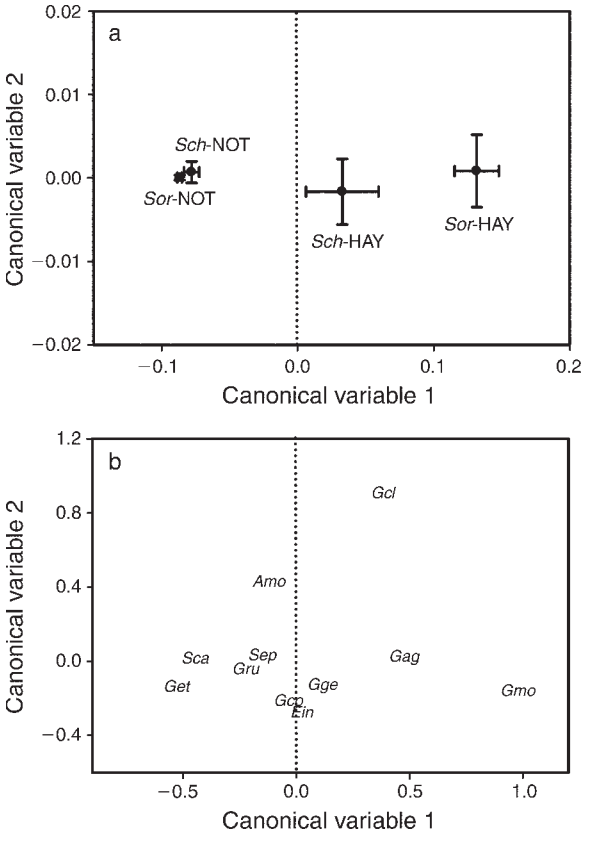

FIG. 5. Canonical discriminant (CAP) plots of AM fungal spore communities providing the same information as in Fig. 4 but for inoculum from Hayden.

Moreover, we cannot know the extent that the functional differences between prairie and serpentine AM fungal communities are attributable to intraspecific variation, which could truly reflect adaptation and which most certainly occurs (Munkvold et al. 2004, Koch et al. 2006), or to differences in the representation of particular AM fungal species.

Divergence in AM fungal spore community composition when the same inoculum was placed in two different environments may reflect interspecific variation in tolerance to the novel environment, expressed either in absolute abundance or in spore production. The fact that such divergence occurred suggests that the structure of the AM fungal community responds to local ecological factors and further supports the idea of ecological matching in the communities. Over time, any AM community placed in a novel environment might become increasingly similar to the AM community native to that environment, but convergence will be constrained by the array of species present in the initial inoculum. In our study, for example, two fungal morphospecies were apparently native only to Hayden and one native only to Nottingham.

Other transplantation studies also support the idea that AM fungal communities function best in their native soils (Lambert et al. 1980, Weinbaum et al. 1996, Johnson et al. 2010). For our system, future experiments should examine the contributions of plant ecotype (Schultz et al. 2001), soil microbes (Vivas et al. 2003, Frey-Klett et al. 2007) and edaphic factors as contributing to environmental effects on AM fungal community composition, but we speculate that the contrasting edaphic characteristics are paramount because of the strong differences between serpentine and prairie soils. The environment might also act indirectly on the communities by affecting competition among the constituent AM fungal species (Lekberg et al. 2007).

That the AM fungal communities differed between the two host-plant species in the same system contrasts with results of one field (Ji 2007) and one greenhouse study (Casper et al. 2008) showing no AM fungal specificity for the host plant species *Sorghastrum* or *Schizachyrium* at either site. We cannot know whether host specific differences in the AM fungal communities detected at harvest were present at the time of field collection or developed during the experiment. Future studies could focus on temporal changes in the composition of the AM fungal community in the novel environment (Weinbaum et al. 1996) by examining spore production, hyphal mass, and root colonization by different species. All plants without AM fungi grew so little that it was difficult to evaluate the effect of native, non-AM microbes, which likely includes soilborne pathogens (Klironomos 2002). A treatment with native AM fungi but in the absence of non-AM microbes—to which plants with native AM fungi and native non-AM microbes could be compared—would prove informative.

Counts of fungal spore morphospecies at the termination of the experiment cannot distinguish between environmentally imposed differences in the relative abundance of co-occurring AM species and differences in their sporulation. However, the abundance of freshly produced spores is known to be a good index of fungal population growth rate (Bever 2002), which represents an important aspect of fungal fitness. Clearly, the soil spore community can be different from the fungi actually colonizing plants (Clapp et al. 1995), but the relative abundance of AM species colonizing may not translate into their relative importance to the host plant either (Gustafson and Casper 2006). While DNA-based molecular tools have the potential to provide a more complete picture of the AM fungal community colonizing roots, that method also has its shortcomings. These include a lack of enough specific PCR primers to amplify the whole range of AM fungi and the difficulties in assigning sequences to meaningful taxonomic units (Sanders 2004). Furthermore, typically only small quantities of root material are used, providing a restricted assessment of colonizing fungi. Before molecular methods can provide more satisfying quantitative analyses of AM fungal communities, we believe spore counts remain a valid approach for our purposes.

While our study demonstrates functional differences between communities of AM fungi it also reflects how much is yet to be learned about the abiotic and biotic factors that structure the taxonomic composition (Rosendahl 2008) of AM fungal communities and about differences among naturally occurring communities in their ability to promote plant growth and in other ecological functions (Fitter 2005, Lilleskov and Parrent 2007, van der Heijden and Scheublin 2007). A complete understanding requires more information than we currently have about the fundamental and realized niche space of the different species (Lekberg et al. 2007); yet, this information is crucial to predicting how AM fungi behave in natural ecosystems and in determining how they can be favorably manipulated in managed systems.

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Literature Cited

Amir, H., D. Jasper, and L. Abbott. 2008. Tolerance and induction of tolerance to Ni of arbuscular mycorrhizal fungi from New Caledonian ultramafic soils. Mycorrhiza 19:1–6.

Anderson, M. J., and T. J. Willis. 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84:511–525.

Anderson, R. C., A. E. Liberta, and L. A. Dickman. 1984. Interaction of vascular plants and vesicular–arbuscular mycorrhizal fungi across a soil moisture–nutrient gradient. Oecologia 64:111–117.

Anderson, R. C., and K. J. Roberts. 1993. Mycorrhizae in prairie restoration: response of three Little Bluestem (*Schizachyrium scoparium*) populations to mycorrhizal inoculum from a single source. Restoration Ecology 1:83–87.

Augé, R. M. 2001. Water relations, drought and vesicular–arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42.

Bennett, A. E., and J. D. Bever. 2007. Mycorrhizal species differentially alter plant growth and response to herbivory. Ecology 88:210–218.

Bever, J. D. 2002. Host-specificity of AM fungal population growth rates can generate feedback on plant growth. Plant and Soil 244:281–290.

Bever, J. D., S. C. Richardson, B. M. Lawrence, J. Holmes, and M. Watson. 2009. Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. Ecology Letters 12:13–21.

Borowicz, V. A. 2001. Do arbuscular mycorrhizal fungi alter plant–pathogen relations? Ecology 82:3057–3068.

Cahill, J. F., E. Elle, G. R. Smith, and B. H. Shore. 2008. Disruption of a belowground mutualism alters interactions between plants and their floral visitors. Ecology 89:1791–1801.

Casper, B. B., S. P. Bentivenga, B. Ji, J. H. Doherty, H. M. Edenborn, and D. J. Gustafson. 2008. Plant–soil feedback: testing the generality with the same grasses in serpentine and prairie soils. Ecology 89:2154–2164.

Castelli, J. P., and B. B. Casper. 2003. Intraspecific AM fungal variation contributes to plant–fungal feedback in a serpentine grassland. Ecology 84:323–336.

Clapp, J. P., J. P. W. Young, J. W. Merryweather, and A. H. Fitter. 1995. Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. New Phytologist 130:259–265.

de la Peña, E., S. R. Echeverría, W. H. van der Putten, H. Freitas, and M. Moens. 2006. Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. New Phytologist 169:829–840.

Egerton-Warburton, L. M., and E. B. Allen. 2000. Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. Ecological Applications 10: 484–496.

Fidelibus, M. W., C. A. Martin, and J. C. Stutz. 2001. Geographic isolates of *Glomus* increase root growth and whole-plant transpiration of *Citrus* seedlings grown with high phosphorus. Mycorrhiza 10:231–236.

Fitter, A. H. 2005. Darkness visible: reflections on underground ecology. Journal of Ecology 93:231–243.

Frey-Klett, P., J. Garbaye, and M. Tarkka. 2007. The mycorrhiza helper bacteria revisited. New Phytologist 176: 22–36.

Gustafson, D. J., and B. B. Casper. 2006. Differential host plant performance as a function of soil arbuscular mycorrhizal fungal communities: experimentally manipulating co-occurring *Glomus* species. Plant Ecology 183:257–263.

Hawkes, C., J. Belnap, C. D'Antonio, and M. Firestone. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant and Soil 281:369–380.

Helgason, T., and A. H. Fitter. 2009. Natural selection and the evolutionary ecology of the arbuscular mycorrhizal fungi (Phylum Glomeromycota). Journal of Experimental Botany 60:2465–2480.

Helgason, T., J. W. Merryweather, J. Denison, P. Wilson, J. P. W. Young, and A. H. Fitter. 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. Journal of Ecology 90:371–384.

Hildebrandt, U., M. Kaldorf, and H. Bothe. 1999. The zinc violet and colonization by arbuscular mycorrhizal fungi. Journal of Plant Physiology 154:709–717.

Janos, D. P. 2007. Plant responsiveness to mycorrhizas differs from dependence upon mycorrhizas. Mycorrhiza 17:75–91.

Jansa, J., F. A. Smith, and S. E. Smith. 2008. Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? New Phytologist 177:779–789.

Ji, B. 2007. Taxonomic and functional diversity of AM fungi in serpentine and prairie grasslands. Dissertation. University of Pennsylvania, Philadelphia, Pennsylvania, USA.

Johnson, N. C. 1993. Can fertilization of soil select less mutualistic mycorrhizae? Ecological Applications 3:749–757.

Johnson, N. C., G. W. T. Wilson, M. A. Bowker, J. Wilson, and R. M. Miller. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proceedings of the National Academy of Sciences USA 107:2093–2098.

Kiers, E. T., C. E. Lovelock, E. L. Krueger, and E. A. Herre. 2000. Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. Ecology Letters 3: 106–113.

Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67–70.

Klironomos, J. N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301.

Klugh, K. R., and J. R. Cumming. 2007. Variations in organic acid exudation and aluminum resistance among arbuscular mycorrhizal species colonizing *Liriodendron tulipifera*. Tree Physiology 27:1103–1112.

Koch, A. M., D. Croll, and I. R. Sanders. 2006. Genetic variability in a population of arbuscular mycorrhizal fungi causes variation in plant growth. Ecology Letters 9:103–110.

Koske, R. E., and J. N. Gemma. 1989. A modified procedure for staining roots to detect VA-mycorrhizas. Mycological Research 92:486–505.

Kula, A. A. R., D. C. Hartnett, and G. W. T. Wilson. 2005. Effects of mycorrhizal symbiosis on tallgrass prairie plant–herbivore interactions. Ecology Letters 8:61–69.

Lambert, D. H., H. Cole, and D. E. Baker. 1980. Adaptation of vesicular–arbuscular mycorrhizae to edaphic factors. New Phytologist 85:513–520.

Lekberg, Y., R. T. Koide, J. R. Rohr, L. Aldrich-Wolfe, and J. B. Morton. 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. Journal of Ecology 95:95–105.

Lilleskov, E. A., and J. L. Parrent. 2007. Can we develop general predictive models of mycorrhizal fungal community–environment relationships? New Phytologist 174:250–256.

Malcová, R., J. Rydlová, and M. Vosátka. 2003. Metal-free cultivation of *Glomus* sp. BEG 140 isolated from Mn-contaminated soil reduces tolerance to Mn. Mycorrhiza 13: 151–157.

McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and J. A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. New Phytologist 115:495–501.

McKenney, M. C., and D. L. Lindsey. 1987. Improved method for quantifying endomycorrhizal fungal spores from soil. Mycologia 79:779–782.

Moora, M., M. Öpik, R. Sen, and M. Zobel. 2004. Native arbuscular mycorrhizal fungal communities differentially influence the seedling performance of rare and common *Pulsatilla* species. Functional Ecology 18:554–562.

Morton, J. B., S. P. Bentivenga, and J. D. Bever. 1995. Discovery, measurement, and interpretation of diversity in arbuscular endomycorrhizal fungi (*Glomales*, *Zygomycetes*). Canadian Journal of Botany 73:S25–S32.

Munkvold, L., R. Kjøller, M. Vestberg, S. Rosendahl, and I. Jakobsen. 2004. High functional diversity within species of arbuscular mycorrhizal fungi. New Phytologist 164:357–364.

Öpik, M., M. Moora, J. Liira, and M. Zobel. 2006. Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. Journal of Ecology 94:778–790.

Porter, W. M., A. D. Robson, and L. K. Abbott. 1987. Field survey of the distribution of vesicular arbuscular mycorrhizal fungi in relation to soil pH. Journal of Applied Ecology 24: 659–662.

Rillig, M. C., and D. L. Mummey. 2006. Mycorrhizas and soil structure. New Phytologist 171:41–53.

Rosendahl, S. 2008. Communities, populations and individuals of arbuscular mycorrhizal fungi. New Phytologist 178:253–266.

Ryan, M. H., A. F. van Herwaarden, J. F. Angus, and J. A. Kirkegaard. 2005. Reduced growth of autumn-sown wheat in a low-P soil is associated with high colonisation by arbuscular mycorrhizal fungi. Plant and Soil 270:275–286.

Sanders, I. R. 2004. Plant and arbuscular mycorrhizal fungal diversity: are we looking at the relevant levels of diversity and are we using the right techniques? New Phytologist 164:415–418.

SAS Institute. 2005. JMP version 5.1. SAS Institute, Inc., Cary, North Carolina, USA.

Schechter, S. P., and T. D. Bruns. 2008. Serpentine and non-serpentine ecotypes of *Collinsia sparsiflora* associate with distinct arbuscular mycorrhizal fungal assemblages. Molecular Ecology 17:3198–3210.

Schenck, N. C., and Y. Pérez. 1990. Manual for the identification of VA mycorrhizal fungi. Synergistic Publications, Gainesville, Florida, USA.

Schultz, P. A., R. M. Miller, J. D. Jastrow, C. V. Rivetta, and J. D. Bever. 2001. Evidence of a mycorrhizal mechanism for the adaptation of *Andropogon gerardii* (Poaceae) to high- and low-nutrient prairies. American Journal of Botany 88:1650–1656.

Shah, M. A., Z. Reshi, and I. Rashid. 2008. Mycorrhizal source and neighbour identity differently influence *Anthemis cotula* L. invasion in the Kashmir Himalaya, India. Applied Soil Ecology 40:330–337.

Smith, S. E., S. Dickson, and F. A. Smith. 2001. Nutrient transfer in arbuscular mycorrhizas: how are fungal and plant processes integrated? Australian Journal of Plant Physiology 28:685–696.

Stahl, P. D., and W. K. Smith. 1984. Effects of different geographic isolates of *Glomus* on the water relations of *Agropyron smithii*. Mycologia 76:261–267.

Streitwolf-Engel, R., T. Boller, A. Wiemken, and I. R. Sanders. 1997. Clonal growth traits of two *Prunella* species are determined by co-occurring arbuscular fungi from a calcareous grassland. Journal of Ecology 85:181–191.

Vandenkoornhuyse, P., R. Husband, T. J. Daniell, I. J. Watson, J. M. Duck, A. H. Fitter, and J. P. W. Young. 2002. Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. Molecular Ecology 11:1555–1564.

van der Heijden, M. G. A., T. Boller, A. Wiemken, and I. R. Sanders. 1998*a*. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79:2082–2091.

van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998*b*. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72.

van der Heijden, M. G. A., and T. R. Scheublin. 2007. Functional traits in mycorrhizal ecology: their use for predicting the impact of arbuscular mycorrhizal fungal communities on plant growth and ecosystem functioning. New Phytologist 174:244–250.

Vivas, A., A. Marulanda, J. M. Ruiz-Lozano, J. M. Barea, and R. Azcon. 2003. Influence of a *Bacillus* sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG-induced drought stress. Mycorrhiza 13: 249–256.

Vogelsang, K. M., H. L. Reynolds, and J. D. Bever. 2006. Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. New Phytologist 172:554–562.

Weinbaum, B. S., M. F. Allen, and E. B. Allen. 1996. Survival of arbuscular mycorrhizal fungi following reciprocal transplanting across the Great Basin, USA. Ecological Applications 6:1365–1372.

Weissenhorn, I., A. Glashoff, C. Leyval, and J. Berthelin. 1994. Differential tolerance to Cd and Zn of arbuscular mycorrhizal (AM) fungal spores isolated from heavy metal-polluted and unpolluted soils. Plant and Soil 167:189–196.

Wilson, G. W. T., and D. C. Hartnett. 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. American Journal of Botany 85:1732–1738.