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Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses

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Abstract

Plant invasions have the potential to significantly alter soil microbial communities, given their often considerable aboveground effects. We examined how plant invasions altered the arbuscular mycorrhizal fungi of native plant roots in a grassland site in California and one in Utah. In the California site, we used experimentally created plant communities composed of exotic (*Avena barbata*, *Bromus hordeaceus*) and native (*Nassella pulchra*, *Lupinus bicolor*) monocultures and mixtures. In the Utah semi-arid grassland, we took advantage of invasion by *Bromus tectorum* into long-term plots dominated by either of two native grasses, *Hilaria jamesii* or *Stipa hymenoides*. Arbuscular mycorrhizal fungi colonizing roots were characterized with PCR amplification of the ITS region, cloning, and sequencing. We saw a significant effect of the presence of exotic grasses on the diversity of mycorrhizal fungi colonizing native plant roots. In the three native grasses, richness of mycorrhizal fungi decreased; in the native forb at the California site, the number of fungal RFLP patterns increased in the presence of exotics. The exotic grasses also caused the composition of the mycorrhizal community in native roots to shift dramatically both in California, with turnover of *Glomus* spp., and Utah, with replacement of *Glomus* spp. by apparently non-mycorrhizal fungi. Invading plants may be able to influence the network of mycorrhizal fungi in soil that is available to natives through either earlier root activity or differential carbon provision compared to natives. Alteration of the soil microbial community by plant invasion can provide a mechanism for both successful invasion and the resulting effects of invaders on the ecosystem.

Introduction

Plant invasions often dramatically alter the aboveground plant community, but we know little about what happens to the soil biota (Belnap and Phillips, 2001). Soil microbial communities have the potential to determine both the success of invasion and the ultimate effects of invaders both on the resident plant community and associated ecosystem functioning (Callaway et al., 2004; Mitchell and Power, 2003). Because different plant species can harbor different root-associated microbial communities, the invasion of new plant species is likely to impact soil microbial composition (Kourtev et al., 2002; 2003). This may be particularly true of microbes that are closely associated with plant roots such as mycorrhizal fungi.

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Arbuscular mycorrhizal (AM) fungi are an important component of the belowground microbial community. AM fungi depend on carbon from plant roots, exhibit differential stimulation by, and confer differential benefits to different plant species (Bever, 2002; Bever et al., 1996; Klironomos, 2003; Sanders and Fitter, 1992). Such selectivity can lead to distinct communities of AM fungi in plant roots of neighboring species (Vandenkoornhuyse et al., 2002; Vandenkoornhuyse et al., 2003) and can cause shifts in aboveground plant community composition (van der Heijden et al., 1998). This level of plant-AM fungal association may not be sufficient to act as a barrier to plant invasions (Richardson et al., 2000), but is likely to be an important pathway through which invading plants can affect the performance of native species.

Changes in the community of AM fungi in soil can also cause changes at the ecosystem level (Rillig, 2004). Because AM fungi are functionally quite diverse, individual combinations of AM fungal and host plant species can have very different effects on ecosystem function (Munkvold et al., 2004). A change in the species of AM fungi that colonize a host plant has the potential to impact plant carbon exudation (Schwab et al., 1984), rates of decomposition (Hodge et al., 2001), addition of carbon to soil through hyphal turnover (Staddon et al., 2003), retention of carbon and nutrients in recalcitrant fungal tissues (Rillig et al., 2001), and plant nutrient uptake (Cavagnaro et al., 2005). Thus, alteration of the mycorrhizal fungal community may also provide a mechanism through which plant invaders can affect ecosystem function.

We compared associations of mycorrhizal fungi with several native plants and with exotic annual grasses. Exotic grasses are found throughout landscapes of the western United States (D'Antonio and Vitousek, 1992), can effectively compete with and displace native grasses, and are able to alter ecosystem nitrogen cycling (D'Antonio and Vitousek, 1992; Mack and D'Antonio, 2003; Mack et al., 2001; Pickart et al., 1998; Wedin and Tilman, 1990). Most grasses have AM fungal symbionts that may affect their ability to invade ecosystems and their interactions with other grasses, herbs, and shrubs (Callaway et al., 2004). In this study, we asked whether the non-native annual grasses could alter the composition of AM fungi that colonize roots of native plants at two grassland sites where dramatic annual grass invasions have occurred. Experimental plots were constructed in the California site to mimic native and invaded plant communities, while at the Utah site we took advantage of the natural invasion of an exotic annual grass into long-term native perennial grass demography plots.

Materials and methods

We sampled plant roots from grasslands in two locations: University of California Hopland Research and Extension Center in Mendocino County, California and Virginia Park, Needles District of Canyonlands National Park, SE Utah. All samples were collected between April and May 2001, the time of peak growth in California and typically also in Utah depending on rainfall.

In California, we grew plants for 4 years in circular plots constructed of steel drums 0.114 m^2 in area, 0.63 m deep, and plumbed at the bottom to allow free drainage. These were filled with Laughlin soil (fine-loamy, mixed, mesic Ultic Haploxeroll) collected from nearby field sites.. Plots were planted with either exotic annual grasses (*Avena barbata* Link, *Bromus hordeaceus* L.), the native perennial grass *Nassella pulchra* (Hitchc.) Barkworth, the native annual dicot *Lupinus bicolor* Lind, or the following mixtures: *N. pulchra* + exotic annual grasses, *L. bicolor* + exotic annual grasses ($n = 5$ plots per treatment). The treatments were maintained by seeding and weeding for four years prior to sampling. Seed additions to the plots were based on seed production per unit area in the field (Eviner, 2001), resulting in the following weights (g) of seed added to monoculture and mixture plots: *N. pulchra* 8.3, 5.5; *A. barbata* 23.0, 7.7; *B. hordeaceus* 3.8,1.9; and *L. bicolor* 14.0, 8.0. Roots of species in each treatment were collected from three of the five replicate plots by digging up entire plants with a trowel to a depth of 20 cm. The samples were kept on ice for transport to the lab, where roots were kept on ice overnight, then separated from the plant, washed with distilled water, wiped with towels, oven-dried, and either stored at −80 °C or fixed in ethanol. To be certain of identity, only fine roots attached to the plant were used. Aboveground plant biomass

(from 2 to 3 plants per plot) was dried and weighed.

In Utah, plants were sampled from long-term plots set up and monitored by J. Belnap. The Virginia Park site has never been grazed by livestock and biological soil crusts remain intact. Soils are classified as part of the Begay series (coarse-loamy, mixed, mesic Ustollic Camborthids). Collections were made from twelve plots that were dominated by one of two native grasses, *Stipa hymenoides* R. & S. or *Hilaria jamseii* (Torr.) Benth., and have either been invaded by the exotic annual, *Bromus tectorum* L., or not ($n=3$ plots per treatment, a total of 12 plots). Two individuals of the dominant native grass were collected from every plot and two individuals of the exotic grass were additionally collected from invaded plots. To collect roots, holes 40 cm deep and 20 cm diameter were dug around the grasses, loose soil was shaken off, and the sample was placed in a plastic bag. Collections were put on ice within four hours and sent overnight to the lab in Berkeley, CA. Upon arrival, they were treated as above. Again, only fine roots still attached to the plant were used.

From both sites, two to three individual plants were sampled from each plot and the roots were lumped together into a single composite sample. These roots were then divided into subsets for DNA-based characterization and measurement of root colonization with staining and microscopy. A total of 3 g root from each plot were stored at −80 °C for molecular analysis. DNA extraction from roots was accomplished by grinding 1 g of washed root tissue in liquid nitrogen, followed by processing with a Qiagen DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA). Two replicate extractions were combined. Before grinding in liquid nitrogen, we attempted several methods of DNA extraction from roots that were not as destructive. None of the PCR reactions containing the alternate method extractions were successful in amplifying fungal DNA. The subsequent success of grinding the roots in liquid nitrogen suggests that fungal DNA amplified in this study had colonized the interior of the root rather than simply the root surface.

All remaining root biomass was fixed in ethanol for measurement of colonization. Because of limited root tissue and the unexpected need for repeated DNA extractions, we were only able to quantify the percentage of root length colonized by AM fungi in grass roots at the California site (*N. pulchra*, *A. barbata*, *B. hordeaceus*); all root tissue from the Utah site and L. *bicolor* plants from the California site were used for DNA-based methods. We also know from other studies that *B. tectorum* and *S. hymenoides* are colonized by AM fungi in the Moab region (Harper and Pendleton, 1993, Belnap, unpublished data). The roots were stained with trypan blue (Koske and Gemma, 1989) and AM fungus colonization was quantified with microscopy using the magnified intersections method (McGonigle et al., 1990). Colonization was quantified for roots from nine plants per species, with 100 fields of view counted per slide at 200× magnification. ANOVA was used to test for differences in colonization rates among the three species in the California site only, with the percentage data arc sine transformed to achieve normality.

Because initial attempts at direct amplification of AM fungal DNA from roots were unsuccessful, we used nested PCR following Redecker (2000). Initial amplifications were with universal ITS primers NS5 and ITS4 (white et al., 1990). Amplification success was checked with electrophoresis gels and PCR products were diluted 1:50, 1:100, or 1:1000 depending on band strength. The second round of amplification was performed with (1) GLOM5.8R, GIGA5.8R, and ITS IF designed to amplify the *Glomus mossae*/*intraradices* group and the Gigasporaceae, and (2) ARCH 1311, LETC1670, ACAU1660, and ITS4i targeting the A *gerdemannii*/*A. trappei* group, the *Glomus occultumlG. brasilianicm* group, the *G. etunicatum*/*claroideum* group, and the Acaulosporaceae *sensu stricto* (Redecker, 2000, Redecker et al., 2003). PCR amplification was performed on a Bio-Rad iCycler (Bio-Rad Laboratories Inc., Hercules, CA, USA) with a total volume of 25 μl containing 1 μl of DNA extract or initial PCR product, 0.5 μM of each primer, 2 μg BSA, 3 mM $MgCl_2$, 2.5 μl 10× buffer, 2.5 U Taq DNA polymerase (Promega, Madison, WI, USA), and ddH_2O to make a final reaction volume of 25 μl. In this study, only the reactions containing GLOM5.8R and GIGA5.8R were successful in amplifying AM fungal DNA from both native and exotic plant roots and some root samples had no successful amplification. Products

ranged from 200 to 700 bp in size. PCR products were cloned into the Invitrogen pCR®. 4-TOPO® vector (Invitrogen, Carlsbad, CA, USA), according to the manufacturers instructions.

Groups of samples with common sequences were identified by restriction fragment length polymorphism (RFLP) analysis (Redecker et al., 1997). Inserts were reamplified and restriction digests were performed with a combination of the enzymes Mspl and Alul (Promega, Madison, WI, USA). Digests contained 15 μl of PCR product, lU MspI,1U Alul, 0.1 μg/μl BSA, and 1.83 μl of 10× buffer.

To examine community-level patterns, we characterized clones by their RFLP patterns. Different RFLP patterns were identified using Kodak's Digital Science 1D version 3.0.0 imaging software (Kodak Scientific Imaging Systems, New Haven, CT). RFLP patterns are reproducible and can be matched to sequences to reduce the need for sequencing (Redecker et al., 2003). The RFLP pattern data were summarized in a presence-absence matrix and analyzed with non-metric multidimensional scaling (NMS) in PC Ord (McCune and Mefford, 1999). Percent shift in composition was also calculated from the RFLP patterns.

Representatives of each RFLP pattern that occurred more than once were sequenced (forward and reverse). PCR products to be sequenced were purified with QIAquick PCR purification Kit (Qiagen Inc., Valencia, CA, USA) and labeled for sequencing with the Big Dye Terminantor Cycle Sequencing Kit (ABI, Foster City, CA, USA). Sequencing was performed on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). For the California samples, RFLP patterns were analyzed for 233 clones and 46 clones were successfully sequenced. For the Utah samples, RFLP patterns were analyzed for 237 clones and 32 were successfully sequenced. Pairwise alignments and consensus sequences for forward and reverse sequences were performed using the alignment and consensus functions in BioEdit Sequence. Alignment Editor (Hall, 1999), The similarity of obtained sequences to those submitted to the NCBI GenBank database was determined with the NCBI BLAST Sequence Similarity Search (Altschul et al., 1990). All sequences were submitted to GenBank and were assigned the accession numbers listed in Tables 3 and 4.

Results

Individual plant biomass aboveground was not significantly different ($P > 0.05$) among species at the time of sampling in California (*N. pulchra* = 0.21 ± 0.33 g; *L. bicolor* = 0.24 ± 0.26 g; *A. barbata* = 0.21 ± 0.15 g; *B. hordeaceus* = 0.21 ± 0.12 g) (average ± 1 SD). In Utah, the native grasses were significantly larger than the invading *B. tectorum* (*S. hymenoides* = 17.88 ± 9.04 g; *H. jamesii* = 7.74 ± 2.61 g; *B. tectorum* = 1.40 ± 0.72 g).

AM fungal colonization of roots in the California site did not differ significantly among the three grass species ($P > 0.05$), with colonization only slightly higher in the native, *N. pulchra*, at 46.3 ± 15.6 % compared to either exotic grass (*A. barbata* = 41.3 ± 14.9%; *B. hordeaceus* = 36.6 ± 12.2 %).

A total of 29 and 34 RFLP patterns were found in the California and Utah sites, respectively (Tables 1, 2). Exotic grasses changed the richness of AM fungi in native plant roots, decreasing their number in the three native grasses across both sites and increasing it in the single native dicot at the California site (Tables 1, 2). In California, the number of AM fungal RFLP patterns in roots of the California native grass, *N. pulchra*, decreased by five while in the native *L. bicolor* AM richness increased by four in the presence of exotic grasses relative to when the natives were grown alone (Table 1). The number of AM fungal RFLP patterns in the Utah site decreased by six in *H. jamesii* roots and decreased by five in *S. hymenoides* roots in the plots invaded by *B. tectorum* (Table 2).

The presence of exotics also dramatically altered the composition of AM fungal communities in native root systems at both sites (Table 1, 2). Ordination analysis clearly showed distinct AM fungal communities in the presence vs. absence of exotic grasses in California (Figure 1) and Utah (Figure 2). In the presence of the exotic annuals (*Avena barbata*, *Bromus hordeaceus*), AM fungal communities of both California natives (*N. pulchra*, *L. bicolor*) shifted toward the community found in exotic roots rather than the community seen when the native species were grown alone (Figure 1). A similar transformation was found in the AM fungus community of native Utah grasses in invaded plots; the

Table 1. AM fungal RFLP patterns as distributed among the species and treatments in the California site

Plant species		RFLP patterns only in monocultures*	RFLP patterns only in mixtures	RFLP patterns in both treatments
Nassella pulchra	Individual patterns	3, 4, 8, 9, 12, 14, 18, 26	13, 16, 20, 25	19, 21, 23, 27
	Total	8	4	4
Lupinus bicolor	Individual patterns	18, 22, 23, 24, 26	4, 5, 6, 10, 11, 15, 25, 27, 29	2, 16, 20
	Total	5	9	3
Avena barbata	Individual patterns	1, 10	17	2, 4, 5, 6, 7,11,13, 15, 16, 19, 20, 21, 23, 25, 28
	Total	2	1	15
Brornus hordeaceus	Individual patterns	7, 10	5, 29	2, 4, 6, 11, 13, 15, 16, 17, 19, 20, 21, 23, 25, 28
	Total	2	2	14

* In the case of the exotic grasses, the plots contained both *A. barbata* and *B. hordeaceus*.
RFLP patterns are listed by number and total numbers of RFLP patterns are given below each list.

Table 2. AM fungal RFLP patterns as distributed among the species and plot types in the Utah-site

Plant species		RFLP patterns only in uninvaded plots	RFLP patterns only in invaded plots	RFLP patterns in both plot types	RFLP patterns in *Bromus* roots
Stipa hymenoides	Individual patterns	11, 14, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31	2, 3, 5, 9, 10, 12, 16	15, 17, 32, 34	6, 9, 15, 16, 17, 34
	Total	12	7	4	6
Hilaria jamesii	Individual patterns	4, 8, 14, 15, 17, 19, 20, 22, 23, 24, 25, 27, 28, 31	3, 5, 7, 10, 13, 16, 18, 33	30, 32, 34	1, 9, 10, 14, 15, 16, 17, 18, 21, 33, 34
	Total	14	8	3	11

RFLP patterns are listed by number and total numbers of RFLP patterns are given below each list.

composition of AM fungi was more similar to the community found on the exotic grass than the community found on native grasses when growing without the exotic grass (Figure 2). In the Utah site, however, we do not have plots where the invader is growing alone. In California, where the experimental design allowed us to examine the change in the AM community of exotic grass roots as well, this was clearly an asymmetric interaction. The AM fungi in *A. barbata* and *B. hordeaceus* roots were relatively unaffected by the presence of natives, with at least 88% of the RFLP patterns observed in exotic grass only treatments maintained in mixture with natives (Table 1).

Most of the sequenced RFLP patterns in California were matched to uncultured species of *Glomus* that had been identified primarily from plant roots in German grasslands (Table 3). It is interesting to note that the best matches for AM fungal sequences found in *Lupinus* roots came primarily from other nitrogen-fixers (*Trifolium* spp., *Glycine max*). A few samples were also most similar to *Gigaspora* sp. or *Scutellospora* sp., sister genera in the Gigasporaceae that do not form vesicles inside roots. Four clones from two exotic grass root samples were most similar to a saprophytic basidiomycete (*Mycena* aff. *murina*) (Table 3).

The sequences from the Utah site were most similar to uncultured *Glomus* species from German grassland plant roots and *Glomus intraradices* (Table 4). Some of the sequences were most similar to non-mycorrhizal Ascomycota or Basidiomycota in the database, particularly in plots invaded by *B. tectorum*. Eight cloned root samples (16 clones total) were most similar to sequences of non-mycorrhizal fungi in the database. These

Table 3. Best matches for sequenced samples at the California site based on NCBI MegaBlast searches (Altschul et al., 1990)

Plant species	Treatment	Clone	Accession (AY929-)	Most similar sequence	Accession	% Sim	S'
N. pulchra	*N. pulchra* Alone	1–1	132	Uncult. *Glomus* 2a25.3	AJ567789	90	179
		2–2	134			87	210
		1–2	133	*Glomus* sp. 4.4	AJ518864	84	245
	N pulchra+ Exotic Grasses	13–8	135	Uncult. *Glomus* 2a23.3	AJ567777	90	270
		14–8	136			91	276
		16–1	138			53	147
		14–9	137	Uncult. *Glomus* 2a25.3	AJ567789	90	179
		16–8	139	Uncult. *Glomus* a43d8	AJ56773 1	89	228
L. bicolor	*L. bicolor* Alone	16–3	085	*Glomus intraradices* a40.9	AJ567732	89	287
		16–5	086	Uncult. *Glomus* 2a23.3	AJ567777	92	337
		17–1	088			92	337
		17–2	089			92	337
		17–3	090			91	313
		18–3	091			89	234
		16–6	087	*Uncult. Glomus* 2a25,3	AJ567789	91	280
		21–10	092	*Gigaspora margarita* JJ56	AY035661	56	42
	L. bicolor + Exotic Grasses	9–2	073	*Uncult. Glomus* 2a23.3	AJ567777	91	313
		9–6	074			92	321
		9–10	075			92	321
		10–2	076			91	299
		10–4	077			92	321
		11–5	081			92	321
		10–4	078	*Uncult. Glomus* a41.5	AJ567752	91	287
		11–2	080			92	295
		12–1	082			91	285
		12–9	083	*Uncult. Glomus* 2a25.4	AJ567791	90	248
		12–11	084	*Glomus mosseae*	X96828	54	50
		11–1	079	*Uncult. myco fungus*	AY656972	53	46
A. barbata	Exotic Grasses Alone	41–1	149	*Uncult. Glomus* Pa019	AY236234	75	174
		41–5	152			76	174
		41–8	153			76	174
		41–2	150	*Scutellospora heterogama*	U36593	26	52
		41–4	151	*Uncult. Glomus* 2a23.3	AJ567777	91	313
	N. pulchra + Exotic Grasses	22–1	140	*Uncult. Glomus* 2a23.3	AJ567777	91	266
		22–2	141			91	266
		22–3	142			91	280
		23–1	143	*Uncult. Glomus* a43 d8	AJ5 67731	91	311
		23–2	144			89	293
		23–9	145	*Mycena* aff. *murina*	AF335444	68	202
		25–6	146			68	194
		25–7	147			67	184
		25–8	148			68	202
	L. bicolor+ Exotic Grasses	3–4	070	*Gigaspora rosea*	AJ504639	53	46
		8–5	071	*Uncult. Glomus* 2a23.3	AJ567777	91	313
		8–12	072			92	329

Sequences from this study were deposited in the NCBI GenBank database and assigned accession numbers AY929070–AY929092 and AY929132–AY929153. These are listed in the table by the last three digits. We report the pairwise similarity (%) of the obtained sequences to the best match from the database and the S' (bit score) P-values were less than 0.001 in all cases except the following clones: 21–10, $P = 0.131$; 11–1, $P = 0.016$; and 3–4, $P = 0.029$. The P values were calculated from E values as $P = 1-e^{-E}$ where the E-value (calculated by the BLASTn program) describes the number of hits expected by chance based on the database size. 'Uncult.' is used as an abbreviation for uncultured.

Table 4. Best matches for sequenced samples at the Utah site based on NCBI MegaBlast (Altschul et al., 1990)

Species & treatment	Clone	Accession (AY929-)	Most similar sequence	Accession	% Sim	*S′*
H. jamesii Not Invaded	1–5	093	*Glomus intraradices*	AY842570	97	228
	1–8	094			95	236
	1–12	095	*Glomus intraradices* 1a30.3	AJ567764	96	291
	7–2	101	*Glomus intraradices* 1v33.2	AJ567768	86	262
	7–8	103			86	262
	7–4	102	*Glomus intraradices* 1a31.1	AJ567761	85	228
	3–1	096	*Glomus sp*. 0514	AY174698	95	303
	3–5	097			95	303
	3–7	098			95	303
	6–1	099	*Psilocybe calongei*	AJ519794	86	509
	6–2	100			86	1140
S. hymenoides Not Invaded	2–1	110	*Glomus sp*. 028	AY174716	58	133
	2–2	.111	*Glomus* sp. 0518	AY 174699	87	176
	8–3	114			90	264
	8–2	113	*Glomus intraradices* 1a31.1	AJ567761	89	293
	12–3	116			89	293
	12–2	115	*Glomus sp*. N16.4	AJ518863	84	251
	14–1	117			85	252
	14–3	119			85	252
	15–1	120			85	252
	8–1	112	Uncult. endophytic fungus	AJ879659	91	319
	14–2	118	*Phaeosphaeria pontiformis*	AJ496632	93	1051
	15–2	121	*Marchandiomyces corallinus*	AY583327	65	355
	15–3	122	*Cryptococcus magnus*	AF190009	77	839
H. jamesii Invaded	9–1	104	*Psilocybe calongei*	AJ519794	100	100
	9–2	105			98	934
	9–4	106			98	305
	11–12	109			86	797
	11–8	107	*Phoma herbarum**	AY864822	87	505
	11–10	108			86	446
S. hymenoides Invaded	21–1	123	*Glomus* lv1.2/1	AJ504638	84	258
	21–2	124			84	252
	22–2	125	*Glomus* sp. 4.4	AJ518864	69	100
	25–1	126	*Cryptococcus magnus*	AF190009	92	1169
	25–3	128	*Cryptococcus magnus*	AB032680	89	614
	25–2	127	*Alternaria alternate* *	DQ023279	97	1110
	26–2	129	*Alternaria* IA202*	AY154681	83	692
	26–3	130	*Sporobolomyces gracilis*	AB178481	81	583
B. tectorurn in S. hymenoides	30–1	131	*Alternaria* IA202*	AY154681	88	850

Accession numbers are AY929093–AY929131. An asterisk indicates fungal genera also cultured from soil and plant tissue at the Utah site (Belnap and Phillips, 2001). Column headings and calculations follow Table 3. For all sequence matches, $P < 0.001$.

sequences were most similar to genera that contain both saprophytic and pathogenic fungi including *Alternaria*, *Phaeosphaeria*, *Phoma*, and *Sporobolomyces*.

The similarity of sequences obtained in this study to non-mycorrhizal fungi in the GenBank database was as low as 65% with only a single case of a 100% match. Thus, their classification as non-mycorrhizal may in some cases reflect that no similar sequences of mycorrhizal fungi have yet been identified. This was the case for several sequences that matched non-mycorrhizal fungi in initial database searches, but in more recent searches were more similar to newly

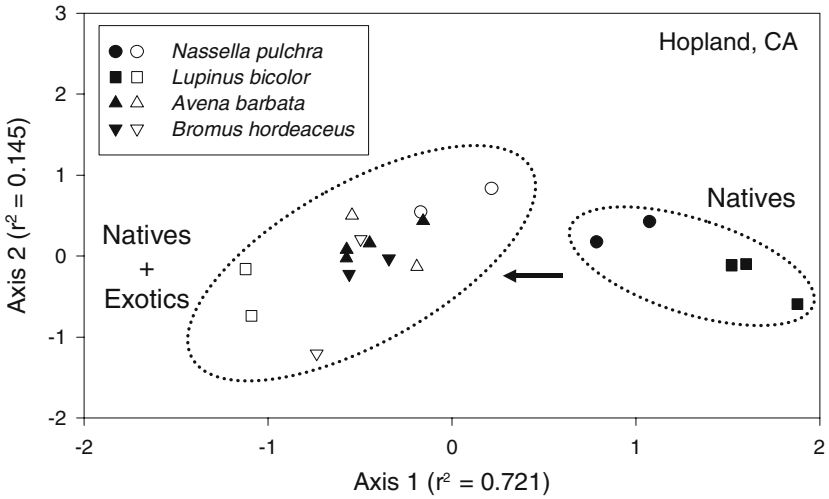

Figure 1. Non-metric multidimensional scaling ordination of AM fungal RFLP pattern profiles from the California site. The AM communities in *roots of* the native grass, *N pulchra*, and the native forb, *L. bicolor*, growing on their own are clearly separated from the AM communities that occur in both native and exotic roots once exotic grasses (*A. barbata*, *B. hordeaceus*) are present. Closed symbols represent species grown in monocultures; open symbols are species grown in the treatment mixtures. Note that sample sizes are unbalanced because amplification was not successful with all root samples.

submitted sequences of uncultured AM fungi. We assume, however, that if these fungi are in fact non-mycorrhizal, that there was no methodological bias in the amplification of AM or non-AM fungi across the planting treatments. Based on compositional analysis including all RFLP data, we conclude that the AM compositional change in California was primarily driven by a shift in AM fungi (Table 3, Figure 1). In Utah, however, the invasion by *B. tectorum* may have caused a more pronounced shift from AM fungi to what are apparently saprophytic and pathogenic fungi (Table 4, Figure 2).

Methodological considerations

We chose to characterize AM fungi with a DNA-based approach which permits the direct identification of fungi that inhabit plant roots. All methods for the identification of AM fungi from field roots have generally been lacking in one way or another. Historically, AM fungi most commonly have been identified based on spore morphology, but spore production in soil does not necessarily reflect the community of AM fungi in roots (Clapp et al., 1995; Merryweather and Fitter, 1998). Identification based on AM fungal structures inside the root is limited to the level of family (Merryweather and Fitter, 1998) and fungal staining of intraradical hyphae with standard dyes does not work for some newly described groups (Redecker, 2000). Molecular methods also are not without potential pitfalls. Most primers developed for the amplification of AM fungi (18S rDNA, 28S rDNA, or the ITS regions) are not conserved across all Glomeromycota or they may amplify other fungi (for detailed discussion, see Redecker et al., 2003). We chose to use a nested primer approach following Redecker (2000) which has proven successful with field roots in several studies (Bidartondo et al., 2002; Redecker, 2000; Redecker et al., 2003), though one caveat of using specific primers is that new sequences might not be detected.

Of the primers used in this study, only one nested set (GLOM5.8R, GIGA5.8R, and ITS1F) amplified fungi from the root DNA extracts and this set also may have amplified some non-mycorrhizal fungi. The lack of amplification by ARCH 1311, LETC 1670, ACAU 1660, and ITS4i may reflect the absence of the *A gerdemannii*/*A. trappei*, *Glomus occultum*/*G. brasilianum*, *G. etunicatum*/*claroideum*, and Acaulosporaceae groups in the plant roots sampled. Alternatively, the primers may not detect all AM fungal species in these groups or we may simply have been unable to successfully optimize the PCR reactions. Other

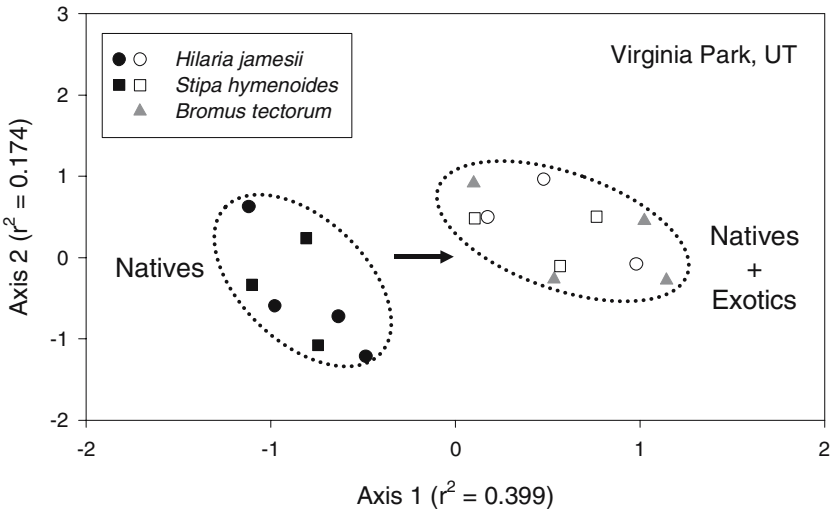

Figure 2. Non-metric multidimensional scaling ordination of AM fungal RFLP pattern profiles from the Utah site. There is a distinct shift in the AM communities in roots of the native *S. hymenoides* and *H. jamesii* grasses growing on their own to those occurring in plots where the exotic *B. tectorum* has invaded. Closed symbols represent samples from uninvaded plots; open symbols are from invaded plots. Recall that *B. tectorum* was only sampled in plots it had invaded, and thus is represented by a single shaded symbol. Sample sizes are unbalanced because amplification was not successful with all root samples.

studies which used these primers have not reported the amplification of non-mycorrhizal fungi (Bidartondo et al., 2002; Redecker, 2000; Redecker et al., 2003). Redecker (2000) noted that when template concentrations were too high in the second round of amplification, primer specificity could decrease resulting in non-specific amplification. The products of our initial round of PCR were diluted prior to use in the second PCR reactions by a factor of 1:50,1:100, or 1:1000; further dilutions did not result in successful amplification. Based on the low similarity of some sequences to non-mycorrhizal fungi in the GenBank database, it is likely that some of these will be better matched to AM fungi as the database grows.

Discussion

The ability of a plant invader to use or change the existing soil microbial community is a potential mechanism for the community and ecosystem effects of invaders (Callaway et al., 2004). Here we demonstrate that invading plants are capable of altering AM fungal communities both in terms of the fungi present in their own roots and those that colonize native plant roots after invasion. Although this is the first demonstration that the specific composition of mycorrhizal fungus communities can be altered by plant invasion, broad changes in soil microbial communities have also been found after other plant invasions. These include shifted PLFA profiles in soil of a New Jersey pine forest invaded by *Berberis thunbergii* and *Microstegium vimineum* (Kourtev et al., 2002) and increased functional diversity of soil bacteria based on BIOLOG measurements in a cold desert community invaded by *Halogeton glomeratus* (Duda et al., 2003). Moreover, we know that other components of the soil communities at our study sites change with invasion. Exotic grasses increased the abundance and changed the composition of ammonia-oxidizing bacteria at the California site (Hawkes et al., 2005). In Utah, other studies have shown that fungal abundance decreased, cultured fungi shifted from specialists to generalists, and entire soil food webs were altered when *B. tectorum* invaded the native perennial grassland (Belnap and Phillips, 2001; Belnap et al., 2005; Evans and Schaeffer, 2003). Thus plant invasions can have significant impacts on both aboveground and belowground diversity.

The capacity of invasive plant species to alter AM fungi may be due in part to differences in plant life history, phenology, and physiology. In both the California and Utah sites, the exotic grasses were annuals while the native grasses

were perennials and the native annual was a nitrogen-fixing forb. The lack of native annual grasses in these sites precludes making comparisons based on life form. Nevertheless, annual invaders are common and widespread throughout invaded regions of the western US and there are several possible mechanisms behind their effects on AM fungi in native plant roots, a number of which are discussed below.

Exotic annual and native perennial herbaceous species typically differ in the timing and extent of their activity during the year. If roots of exotic annual grasses are active earlier in the season than natives, they may associate with and promote specific groups of AM fungi. Once a hyphal network is established, these fungi could have priority access to perennial plant roots and could thus dominate the system. In California, annual plant roots appear to be active earlier than standing perennial roots based on uptake of ^{15}N-labeled ammonium (Hooper and Vitousek, 1998). Moreover, when exotic annuals and native perennials in California grasslands are germinated from seed, the exotics grow faster and have greater root length per unit biomass than the natives (Holmes and Rice, 1996). Phenologies are also different in the Utah site. The exotic annual *B. tectorum* germinates in August or September and is dependent on fall rains. Root growth in *B. tectorum* occurs throughout the winter, with active aboveground growth beginning in early February; the native perennials *S. hymenoides* and *H. jamesii* become active in early March, with an additional active period from August to October (Belnap and Phillips, 2001). Thus the exotic annual grasses at both sites may be active before the native perennials. Pringle and Bever (2002) found that large, seasonal differences in plant phenology helped to maintain different AM fungi in the soil. Differences on a shorter temporal scale, such as within-season, may also be important.

Alternatively, exotic annual grasses may provide more carbon to the AM fungi that colonize their roots, making those fungi more competitive. Changes in soil carbon after invasion, either through exudates or litter inputs, could also help to explain the increase in non-mycorrhizal fungi documented in this study and the switch to generalist saprophytic fungi observed by Belnap and Phillips (2001) at the Utah site. Invaders often have greater CO_2 assimilation, maximal photosynthetic rates, leaf area, litter inputs, and biomass compared to native residents (e.g., Deng et al., 2004; Ewe and Sternberg, 2003; Farnsworth and Meyerson, 2003; Smith and Knapp, 2001; Wilson and Wilson, 2004), all traits which could affect soil carbon and thus fungal communities.

The observed shift in the AM fungus community within roots of native plants in the presence of exotic annual grasses could also have been the result of greater exotic root biomass compared to the native plants. Based on both aboveground biomass data reported here and root biomass data reported elsewhere (Hawkes et al., 2005) from the California plots, the native perennial grass *N. pulchra* biomass was similar to the exotic grasses. In contrast, the native forb, *L. bicolor*, had a similar aboveground biomass, but significantly less root biomass compared to the exotic annual grasses (Hawkes et al., 2005). At the Utah site, it is not surprising that the addition of *B. tectorum* to the system increased the overall biomass of roots in the soil (Belnap and Phillips, 2001). Nevertheless, both *H. jamesii* and *S. hymenoides* are perennial grasses that are far larger with more continuous cover over time than the exotic annual *B. tectorum*. Thus, in three of the four cases studied here, it is likely that the effect of exotic grasses on the AM community in native plant roots were not simply a function of relative biomass.

We know very little about how changes in the fungal community will affect the ecology of the entire ecosystem. A shift from mycorrhizal to more saprophytic or pathogenic fungi may have some predictable consequences, such as decreased nutrient transfer from soil to plants or increased decomposition capacity. Will the replacement, however, of one species of AM fungi by another matter? If different AM species have unique interactions with different members of the plant community (Bever, 2002; Bever et al., 1996; Klironomos, 2003; Sanders and Fitter, 1992), then a change in the community of mycorrhizal fungi can affect the plant community and ecosystem function (Rillig, 2004; van der Heijden et al., 1998). In the Utah site, for example, *B. tectorum* invasion led to a shift from fungal to bacterial utilization of leaf litter that corresponded to increased microbial immobilization of nitrogen

and decreased rates of nitrogen mineralization, apparently due to greater inputs of lower quality *B. tectorum* leaf litter (Evans et al., 2001). Thus, changes in plant inputs and microbial community composition caused by plant invasions can combine to affect ecosystem processes.

Soil microbes, including AM fungi, can be extremely important for individual plant fitness, community composition, and ecosystem processes. Altered soil microbial and fungal communities caused by exotic species invasions may be a mechanism behind many of these effects and one pathway for invasion success. Given the ubiquity of plant-mycorrhizal associations and their potential importance at both the community and ecosystem level, any framework for attacking the problem of biological invasion may need to include them.

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