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Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system

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Summary

• We investigated the effects of arbuscular mycorrhizal fungal (AMF) species richness and composition on plant community productivity and diversity, and whether AMF mediate plant species coexistence by promoting niche differentiation in phosphorus use.

• Our experiment manipulated AMF species richness and identity across a range of P conditions in tallgrass prairie mesocosms.

• We showed that increasing AMF richness promoted plant diversity and productivity, but that this AMF richness effect was small relative to the effects of individual AMF species. We found little support for AMF-facilitated complementarity in P use. Rather, the AMF richness effect appeared to be caused by the inclusion of particular diversity- and productivity-promoting AMF (a sampling effect). Furthermore, the identity of the diversity-promoting fungi changed with P environment, as did the relationship between the diversity-promoting and productivity-promoting benefits of AMF.

• Our results suggest that plant diversity and productivity are more responsive to AMF identity than to AMF diversity *per se*, and that AMF identity and P environment can interact in complex ways to alter community-level properties.

Key words: arbuscular mycorrhizas, species richness, diversity, productivity, composition, phosphorus.

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Introduction

Identifying the determinants of productivity and diversity has been a central focus of ecology (Naeem *et al.*, 1994; Tilman *et al.*, 1996; Hooper & Vitousek, 1997; Hector *et al.*, 1999). Recent work has brought attention to the role of soil organisms in promoting plant productivity and mediating plant–plant interactions (van der Heijden *et al.*, 1998b; Packer & Clay, 2000; Bever, 2003; Reynolds *et al.*, 2003). Arbuscular mycorrhizal fungi (AMF) are ubiquitous root symbionts that form obligate associations with approx. 80% of all terrestrial plants (Smith & Read, 1997). The symbiosis between plants and AMF is ancient (Brundrett, 2002) and ranges from mutualistic to antagonistic depending on a variety of factors, including soil nutrients (Johnson, 1993), identity of plant host and/or symbiont (Bever, 2002; Klironomos, 2003; Reynolds *et al.*, 2005), and their interactions (Reynolds *et al.*, 2006). As mutualists, AMF can increase plant access to low-mobility soil minerals such as phosphorus in exchange for carbon photosynthesized from their hosts, thus promoting host plant growth (Smith & Read, 1997). At the plant community level, suppressing AMF with fungicide does not appear to change overall productivity (Hartnett & Wilson, 1999; O'Connor *et al.*, 2002). However, AMF can promote diversity when subdominant plants benefit most from the mutualism (Grime *et al.*, 1987; van der Heijden, 2002). Conversely, when dominant plant species derive strong benefits from AMF, plant community diversity can be reduced in the presence of AMF (O'Connor *et al.*, 2002).

While the simple presence vs absence of AMF can clearly have strong effects on plant community structure, substantial

local diversity of AMF can exist in soil (Johnson *et al.*, 1991; Bever *et al.*, 1996), and one study has demonstrated a positive relationship between AMF species richness and both the diversity and productivity of experimental North American old-field plant communities (van der Heijden *et al.*, 1998b). It is challenging to manipulate microbial diversity, particularly in a belowground system, and perhaps this explains why, nearly 10 yr later, the generality of the relationship between AMF diversity and plant diversity or productivity is untested. Likewise, the mechanism(s) behind such relationships remain obscure (van der Heijden, 2002).

Mechanisms underlying biodiversity–ecosystem functioning relationships fall into two major classes: sampling effects and effects of diversity *per se* (Hector *et al.*, 1999). In the context of relationships between AMF richness and plant community diversity or productivity, sampling effects would reflect the greater probability of including fungal isolates that are particularly good at promoting plant diversity or growth (van der Heijden *et al.*, 1999; Wardle, 1999). Sampling effects are indicated when individual fungal isolates generate similar or greater responses of plant communities in diversity, productivity, or other community properties than do mixed AMF inoculum treatments. Effects of diversity *per se* are indicated when diversity, productivity or other plant community responses of mixed inoculum treatments outperform those from any single inoculum treatment.

Effects of diversity *per se* can arise via niche differentiation, facilitation (Tilman *et al.*, 1997), or negative feedback (Bever, 2002; Bever *et al.*, 2002). For example, it has been hypothesized that intense competition for P under limiting soil resource conditions could favor AMF-mediated P-niche partitioning, whereby specialization in phosphatase enzymes or other mechanisms allows different plant host–AMF species combinations to access different sources of P (Reynolds *et al.*, 2003). AMF have been shown to promote plant access to various sources of inorganic and organic P in single-plant culture (Koide & Kabir, 2000; Reynolds *et al.*, 2006), but the hypothesis of niche partitioning on different P sources has not been tested for competitive plant species mixtures.

We tested for richness and composition effects of AMF on plant community productivity and diversity, as well as the specific hypothesis of AMF-facilitated complementarity in P use, by manipulating AMF species richness and composition and the diversity of P sources experienced by initially identical experimental North American prairie mesocosms. Under the hypothesis of AMF-facilitated complementarity in P use, unique plant–AMF species combinations allow plants to specialize in acquiring different P sources, and the extent of P-niche partitioning increases as AMF species richness and P-source diversity increase. Consistent with AMF-facilitated complementarity in P use, we predicted finding the highest levels of plant community diversity and productivity in mesocosms experiencing both high AMF richness and high P-source diversity.

Materials and Methods

Study system

We modeled our system using perennial tallgrass prairie plants native to the low-fertility sand prairies of north-western Indiana, USA. This region is characterized by a mesic continental climate, with 88 cm of average annual precipitation distributed predominantly over the growing season (Midwest Regional Climate Center database, Champaign, IL, USA). We generated a plant species list from site visits and published floras. We constrained candidate species to herbaceous perennial natives that would germinate from commercially available seed, then randomly selected 20 species that included a mix of C_3 and C_4 grasses, legumes, composites and other forbs, all from families known to be mycorrhizal (Newman & Reddell, 1987). Our mix of species included *Andropogon gerardii* Vitman and *Panicum virgatum* L. (C_4 grasses); *Koeleria cristata* Pers. and *Sporobolis heterolepis* (A. Gray) A. Gray (C_3 grasses); *Aster laevis* L., *Echinacea pallida* (Nutt.) Nutt., *Helianthus occidentalis* Riddell, *Liatris aspera* Michx., *Rudbeckia hirta* L., *Silphium laciniatum* L., *Solidago nemoralis* Aiton and *Solidago rigida* L. (composites); *Desmodium illinoense* A. Gray and *Lupinus perennis* L. (legumes); and *Allium cernuum* Roth., *Anemone cylindrica* A. Gray, *Asclepias tuberosa* L., *Monarda fistulosa* L., *Penstemon digitalis* Nutt. Ex Sims and *Potentilla arguta* Pursh (other forbs).

We included six AMF isolates in our study, all from tallgrass prairies in the southern portion of the Chicago region. AMF isolates include *Entrophospora infrequens*, *Glomus claroideum* 1, *Glomus claroideum* 2, *Glomus mosseae*, *Scutellospora fulgida* and an undescribed *Scutellospora* that we designate here as *Scutellospora* sp. 1. Fungal species were identified by spore morphology and cultured on *Sorghum bicolor* (L.) Moench in an Indiana University glasshouse for 8 months before being used as inocula in the mesocosm.

Experimental design

Fungal and P-source identity of these mesocosms were manipulated in a factorial design involving zero, one or six AMF species and zero, one or five P sources. Each of the six AMF species was equally and fully represented within the single-species AMF treatment; and each of the five P sources was equally and fully represented within the single-source P treatment. As a result, the mean of the single-species AMF treatment forms the expectation for the six-species AMF effect under the assumption that the AMF species affect the community in proportion to their initial representation. The actual infection by individual fungal species that plants experienced within the six-species AMF treatment may vary depending on the fungal community dynamic, and this is an essential component of a fair test of the dynamic (Bever, 2002; Bever *et al.*, 2002). Under this design, the operation of AMF-facilitated complementarity in P use would be supported by a significant AMF

Table 1 Experimental design of the mesocosm prairie communities

Phosphorus treatments		AMF treatments							
		0	1						6
P number	Added P type	No AMF	EI	GC1	GC2	GM	SF	Ssp1	AMF mix
0	None	4	2	2	2	2	2	2	6
1	Rock	2	1	1	1	1	1	1	3
	$Ca(H_2PO_4)_2$	2	1	1	1	1	1	1	3
	RNA	2	1	1	1	1	1	1	3
	Lecithin	2	1	1	1	1	1	1	3
	Phytin	2	1	1	1	1	1	1	3
5	All	6	3	3	3	3	3	3	12

We maximized the power to test for an arbuscular mycorrhizal fungal (AMF) richness × phosphorus-source diversity interaction to investigate P-niche partitioning facilitated by AMF. Under minimal replication, the effects of added P type and AMF species can also be tested for all possible treatment combinations. The replicates of these combinations are listed in this matrix. AMF species appear individually under AMF richness treatment 1: EI, *Entrophospora infrequens*; GC1, *Glomus claroideum* 1; GC2, *Glomus claroideum* 2; GM, *Glomus mosseae*; SF, *Scutellospora fulgida*; Ssp1, *Scutellospora* sp. 1. All six AMF species are included in the AMF mix.

richness × P-source diversity interaction for plant community productivity and diversity (synergistically higher responses in the treatment combination with all six AMF species and all five P sources compared with other treatment combinations). With eight levels of AMF and seven levels of P, there was a very high number of treatment combinations (56) in the full factorial design. Extensive replication of the full design was untenable and we therefore implemented a limited design (Table 1) that maximized our power to test the AMF species richness × P-source diversity interaction while still giving us the ability to test for individual AMF species and individual P-source effects.

Mesocosm set-up and AMF treatments

We established mesocosms of our prairie plant communities in 23.1-l nursery pots, with target densities of 80 individuals per pot (approx. 1100 seedlings m^{-2}, within the range of competitive field densities). Seeding densities were adjusted according to pretested germination rates to promote initially even distribution of individuals among the 20 species.

The AMF treatments included a mixed culture control autoclaved at 121°C over two consecutive days for 90 min each day (zero AMF species); each of the six single-species AM cultures (one AMF species); and one mixed culture of the six AM species equally represented by volume (six AMF species). Mesocosms were filled with a mix of local clay loam topsoil amended with sand (1 : 1 by volume) to promote reliance on P. To kill existing seeds and microbes, we steam-sterilized this mix at 82°C for 4 h, allowed the mix to cool for 1 d, then applied steam again for 4 h. Each pot received 16.5 l of this soil : sand mix, which we irrigated before implementing our treatments to settle the soil. The bulk density of the soil mix was 1.2 g ml^{-1}. We then added 600 ml of the appropriate AMF treatment inoculum; buried this inoculum with an additional 1.0 l autoclaved soil : sand mix; distributed the seeds, which were mixed into 100 ml sterile sand to aid dispersal; and buried the seeds with an additional 1.0 l autoclaved soil : sand mix. We stirred the surface soil lightly to distribute seeds throughout the top 2 cm. The AMF inoculum consisted of chopped roots and soil from *S. bicolor* cultures, the mycorrhizal inoculation potential (MIP) of which we verified by staining and inspecting roots from a separate growth assay with 4 × replication. Roots from this MIP were prepared and scored according to standard methods (McGonigle *et al.*, 1990). All mesocosms received a microbial filtrate of soil from the live AMF cultures processed through a 38-μm sieve and then through 20-μm filter paper to exclude fungal spores and hyphae, but allow smaller microbes such as bacteria. We randomized the mesocosms and allowed the communities to grow outdoors at the Indiana University Botany Research Station (39°10.52′ N, 86°30.34′ W).

Phosphorus treatments

Our P-source diversity treatments were: no P addition; each of five inorganic or organic P sources individually; and the mixture of all five P sources. The clay loam soil we used tested at 9.5 ppm (Bray-1 extractable P; Michigan State University, Soil and Plant Nutrient Laboratory, East Lansing, MI, USA), a moderate level of available P (Binkley & Vitousek, 1989). We cut this soil 1 : 1 with sand to further reduce P availability, promoting mycorrhizal dependence and reliance on use of P from our treatment sources. During the first 12 wk of growth, we applied P treatments on three separate occasions to deliver a total of 81 mg or approx. 4 mg kg^{-1} soil of elemental P per pot. This modest amount was intended to supply variable sources of P without overwhelming the system with P fertilizer, thereby

promoting plant dependence on mycorrhizas. All P treatments were applied as pH-adjusted aqueous solutions. We used calcium phosphate [$Ca(H_2PO_4)_2$ (Sigma-Aldrich, St. Louis, MO, USA)] and rock phosphate [$Ca_3(PO_4)_2{\cdot}CaF_2$ (North County Organics, Bradford, VT, USA)] as two common inorganic sources of P, and three common organic P sources used were ribonucleic acid (RNA from torula yeast, Sigma-Aldrich); lecithin (L-α-phosphatidylcholine, obtained in water-dispersible form from Sigma-Aldrich); and phytin (inositol hexaphosphoric acid, Sigma-Aldrich). These sources of P vary in solubility (and thus plant availability), occur naturally in soils (Paul & Clark, 1996), and stimulate phosphatase activity in the rhizosphere (Tarafdar & Jungk, 1987; Yadav & Tarafdar, 2001). As such, these sources of P were suitable for our test of AMF-facilitated complementarity in P use. We applied a half-strength modified Hoagland's solution (lacking P) each week to provide a 7 : 1 N : P ratio and all other nutrients in proportion.

Mesocosm maintenance and harvest

For the first week after mesocosm set-up, we covered each pot with perforated plastic film to retain soil moisture, which promoted robust germination. We observed caterpillars and signs of herbivory on young seedlings, and so quantified the extent of early-season (< 8-wk growth) herbivory for use as a covariate in later analyses of community productivity. Herbivory was assessed as specific to monocots or dicots, as seedlings could not be identified beyond these broad categories, and quantified as a percentage of the total number of individuals exhibiting grazed leaves. We controlled Lepidopteran herbivory with applications of *Bacillus thuringiensis* as needed. Plant communities were harvested after growing for 23 wk. We clipped all aboveground biomass and sorted plants by species. Plant materials were dried at 65°C for 72 h, then weighed to provide estimates of total aboveground net community productivity (in g) and species diversity. We used the Shannon–Wiener index to calculate H' using the function:

$$H' = -\sum(p_i)(\log_e p_i) \qquad \text{Eqn 1}$$

where $p_i = m_i/M$; m_i is the mass of all shoots for a given species; and M is the total shoot mass for the community. Following harvest, we collected soil samples to verify that our control pots remained nonmycorrhizal relative to our AMF-inoculated pots. We did this by staining and examining roots recovered from these samples, using the techniques described above for the MIP.

Statistical analyses

For all percentage mycorrhizal colonization data (MIP and final colonization), we used a one-way ANOVA with linear contrasts between the controls and the live inocula grouped by AMF isolate. These data were arcsine square-root transformed to improve normality. For comparing MIP and final colonization means, we used the Tukey–Kramer mean separation test. We used a two-way ANOVA to test for AMF and P-treatment effects on the aboveground net primary productivity and diversity of our plant communities. Early-season herbivory on grasses (the only monocot present) was a significant covariate ($P < 0.10$) in all two-way tests except for the productivity response to AMF richness and P identity, where $P = 0.11$. Excluding the herbivory covariate from the effects of AMF richness and P identity on productivity slightly increased the AMF richness effect, but did not alter the interpretation of the analysis, and we therefore retained the covariate as a conservative measure. Our design (Table 1) allowed us to test for both diversity and identity effects of AMF and P treatments, and all interactions except for AMF identity × P identity. We increased the generality of our analysis by treating the different P sources within the single P-source treatment as random effects for the AMF species × P-source diversity test. We natural log-transformed the productivity data [$Y' = \log_e(1 + Y)$] to satisfy the variance assumptions of the ANOVA.

We computed the community diversity-promoting and community productivity-promoting differences of each AMF isolate relative to their respective uninoculated controls, and used these data to test hypotheses of zero slopes for each treatment of P-source diversity. To assess changes in the relative abundance of individual plant species, we generated proportional abundance curves for each P-source diversity treatment, and ranked the plant species by their biomass representation in the uninoculated no-P treatment. *Panicum virgatum* emerged as the community dominant in all treatments, and we constructed a statistical model with linear contrasts to test for the effects of community productivity-promoting AMF (*E. infrequens*, *G. claroideum* 1, *G. mosseae*, *S. fulgida*) vs community productivity-inhibiting AMF (*G. claroideum* 2, *Scutellospora* sp. 1) on the relative abundance of *P. virgatum* under P-source diversity levels 0 and 5. We used SAS ver. 9.1 for all statistical analyses (SAS, 2003).

Results

Effectiveness of AMF inoculations

The roots from the MIP assay exhibited large differences in percentage mycorrhizal colonization (hyphae, coiled hyphae, arbuscules and vesicles) between live cultures and sterile control ($F_{1,21} = 48.13$, $P < 0.0001$) indicating the viability of the live inoculum added to the mesocosms. The mean separation test revealed no differences in percentage mycorrhizal colonization among the six live cultures, but all cultures differed significantly from the control (Table 2). We also found our AMF control pots to be relatively free of colonized roots at the end of the season (Table 2), with a mean colonization of 1.7% compared with 30.8% in the AMF-inoculated pots ($F_{1,105} = 307.77$, $P < 0.0001$).

Table 2 Mean percentage colonization (±SE)

Treatment	MIP (%)	Final colonization (%)
Control	1.5 (± 0.005) a	1.7 (± 0.007) a
Scutellospora sp. 1	15.5 (± 0.049) b	24.8 (± 0.027) bc
Entrophospora infrequens	21.0 (± 0.010) b	36.9 (± 0.020) bc
Scutellospora fulgida	12.5 (± 0.021) b	30.3 (± 0.032) bc
Glomus claroideum 1	22.0 (± 0.012) b	33.8 (± 0.026) bc
Glomus mosseae	11.0 (± 0.019) b	29.7 (± 0.028) bc
Glomus claroideum 2	20.0 (± 0.052) b	24.9 (± 0.035) b
Mix	–	35.5 (± 0.020) c

Mycorrhizal inoculum potential (MIP) used *Sorghum bicolor* to test the viability of AMF-treatment inoculum before use in the mesocosm. Final colonization used roots sampled from each pot after harvest. Within each column, means with the same letters do not differ significantly from each other.

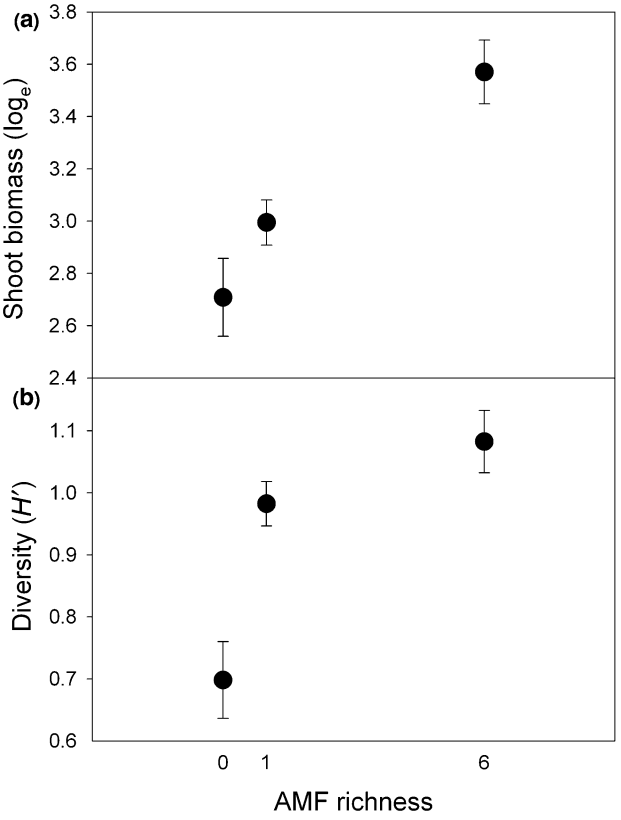

Fig. 1 Plant community productivity (a) (in g) and diversity (b) response to arbuscular mycorrhizal fungal (AMF) richness. AMF effects were evident for both response variables, although species diversity was much more responsive to AMF inoculation than to AMF richness *per se*. Error bars, ±1 SE.

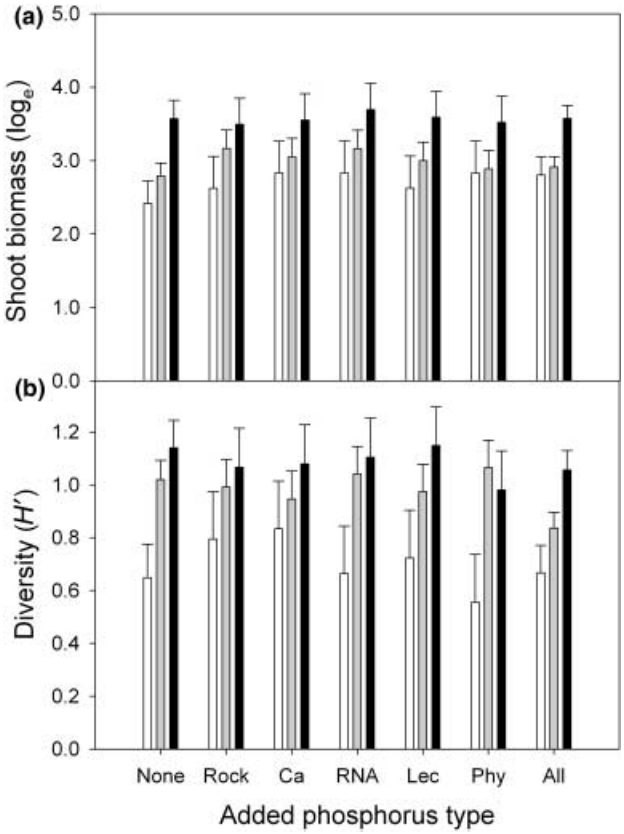

Fig. 2 Plant community productivity (a) (in g) and diversity (b) response to phosphorus identity. Open bars, no added arbuscular mycorrhizal fungi (AMF); grey bars, one AMF species; black bars, six AMF species. Phosphorus additions include (from left to right) none, rock phosphate, $Ca(H_2PO_4)_2$, RNA, lecithin, phytin, and all P types mixed in equivalent portions of PO_4^{3-}. AMF richness effects were similar across many added P types. No interaction with AMF richness and P type suggests a general AMF effect on plant diversity and productivity. There is a trend toward increasing plant diversity when all P types and AMF are present. Error bars, ±1 SE.

Plant productivity and diversity

Plant community productivity and diversity increased with AMF richness (Fig. 1; $F_{2,91} = 11.69$, $P < 0.0001$ for productivity; $F_{2,91} = 12.14$, $P < 0.0001$ for diversity). While plant productivity increased substantially from one to six fungal isolates (Fig. 1a), most of the effects of AMF richness on plant diversity were associated with the change from zero to one AMF species, rather than the change from one to six species (Fig. 1b).

Plant community productivity and diversity were not affected by the main effect of P treatment ($F_{6,91} = 0.30$, $P = 0.93$ for productivity; $F_{6,91} = 0.45$, $P = 0.84$ for diversity). There was also no significant interaction between P-source diversity and AMF richness on plant community productivity or diversity ($F_{4,103} = 0.30$, $P = 0.88$ for productivity; $F_{4,103} = 0.62$, $P = 0.65$ for diversity), contrary to the predicted effect if AMF were facilitating P-niche partitioning. However, there was a trend towards increased plant community diversity from one to six AMF species when all forms of P were provided (Fig. 2b; $t_{28} = 2.30$, $P = 0.029$). Phosphorus-source diversity also interacted with AMF species in complex ways, which we describe below.

AMF species identity effects were evident on plant productivity. Across all P-source diversity treatments, a number of

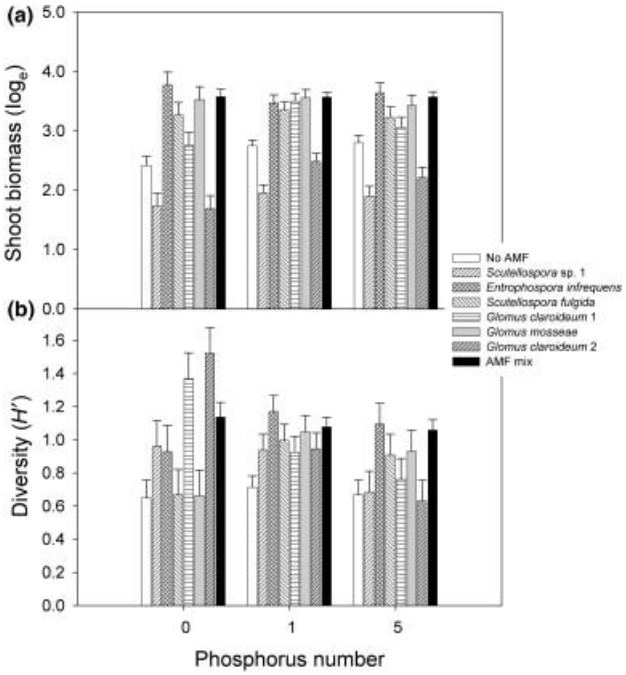

Fig. 3 Plant community productivity (a) (in g) and diversity (b) response to phosphorus-source diversity. Across all P treatments, at least one fungal isolate is at least equivalent to the high fungal diversity treatment in its promotion of plant productivity and diversity. For plant diversity, arbuscular mycorrhizal fungal (AMF) species identity interacts with P-source diversity (b). In particular, *Glomus claroideum* promotes species diversity under environments lacking added P, while *Entrophospora infrequens* emerges as the best diversity promoter under P-amended environments. Error bars, ±1 SE.

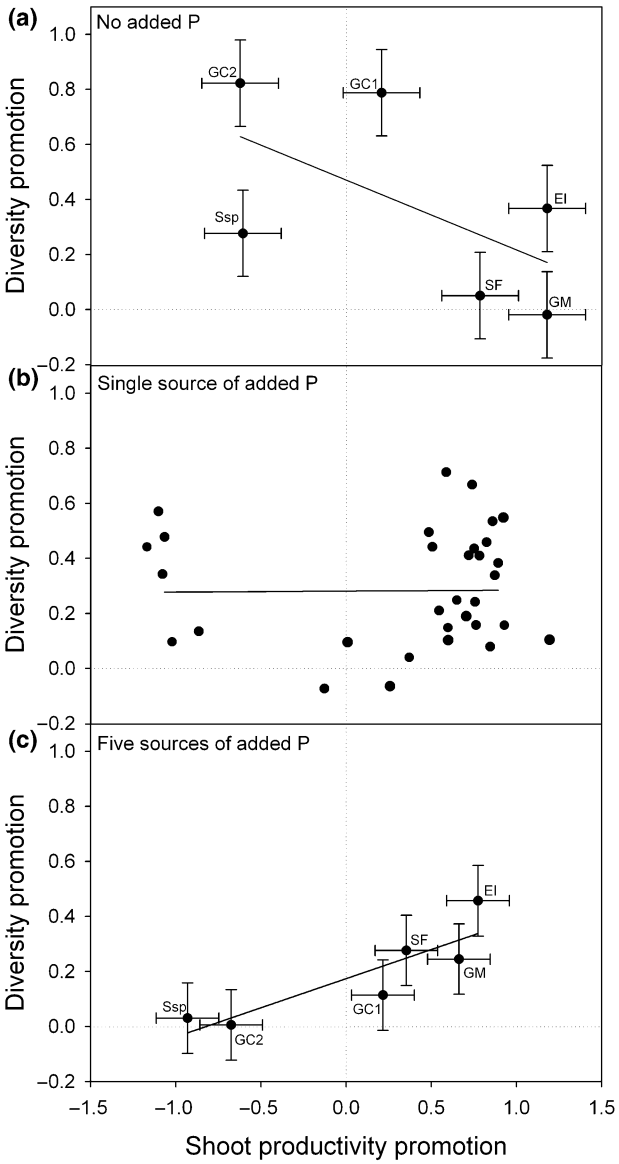

Fig. 4 Phosphorus environment changes the diversity-promoting to productivity-promoting relationships of arbuscular mycorrhizal fungi (AMF). The community diversity-promoting and community productivity-promoting differences of each AMF isolate were calculated relative to their respective uninoculated controls, and these points were used to test hypotheses of zero slopes for each treatment of P-source diversity. Of these treatments, only the five-P treatment (c) was significantly different from zero ($P = 0.0225$). However, this relationship becomes increasingly positive as P is added to the system, and the positive slope in (c) differs significantly from the negative slope in (a). Error bars, ±1 SE [not included in (b) for clarity]. AMF species names are coded according to Table 1.

individual fungal isolates are equivalent to the AMF community treatment in the promotion of plant productivity (Fig. 3a). Furthermore, plant community productivity varied more in response to individual fungi than between the six-species AMF mixture and the uninoculated control (Fig. 3a). Consistently across all three treatments of P number, two isolates inhibited community productivity (*Scutellospora* sp. 1 and *G. claroideum* 2), while the other four isolates promoted community productivity (main effect of AMF species identity: $F_{5,88} = 52.98$, $P < 0.0001$). The productivity response to the mixture of these fungi was similar to that of the response to the best productivity promoters.

As for productivity, plant diversity responses to the mixture of these fungi were generally determined more by the best diversity-promoting AMF than the worst. However, for the plant diversity response, we observed a significant interaction between AMF species identity and P-source diversity ($F_{14,88} = 2.67$, $P = 0.003$). In particular, *G. claroideum* promoted species diversity under environments low in P, but not with amended P (Fig. 3b). In contrast, *E. infrequens* emerges as the best diversity promoter under P-amended environments (Fig. 3b).

We also found that the relationship between the community diversity-promoting and community productivity-promoting effects of AMF reverses with P-source addition. Without P addition, diversity promotion declines with the productivity promotion of the fungal isolates (Fig. 4a), while diversity

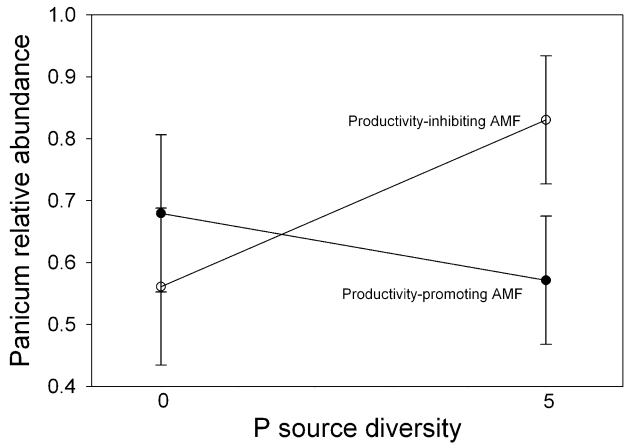

Fig. 5 Dominant grass (*Panicum virgatum*) interacts with the changing phosphorus environment and the arbuscular mycorrhizal fungal (AMF) groups that differentially influence plant community productivity. The interaction of *P. virgatum* with the community productivity-promoting fungi (●) vs the community productivity-inhibiting fungi (○) and P-source diversity contributed to the relationship between diversity promotion and productivity promotion illustrated in Fig. 4. Error bars, ±1 SE.

promotion increases with productivity promotion in the five-P treatment [Fig. 4c; slope differs from that of Fig. 4a ($F_{1,8} = 5.55$, $P = 0.0463$) and from the combined slope of Fig. 4a,b ($F_{2,36} = 3.32$, $P = 0.0475$)].

The shift in relationship between community productivity promotion and community diversity promotion with P addition may reflect a shift in mycorrhizal dependence of the dominant community. *Panicum virgatum* dominated across each mesocosm, and its relative abundance was increased in association with productivity-promoting fungi (*E. infrequens*, *G. claroideum* 1, *G. mosseae*, *S. fulgida*) without P addition, but was decreased by productivity-promoting fungi with P addition (Fig. 5). There is a significant interaction between the productivity-promoting vs productivity-inhibiting fungi (*G. claroideum* 2, *Scutellospora* sp. 1) and P-source diversity ($F_{1,89} = 6.37$, $P = 0.0134$).

Discussion

We found that increasing AMF richness promoted plant community productivity and had a weak effect on plant community diversity across a range of P conditions. However, these effects were small compared with the variation observed in response to individual fungal species. These results make an important contribution to our understanding of the mechanism of AMF diversity effects.

Our results do not support the suggestion of AMF-facilitated complementarity in plant P use as the mechanism generating the observed responses of plant community productivity and diversity to AMF. Should AMF species exhibit specificity for both different forms of P and different plant hosts, we would have expected to see the highest levels of plant community diversity and productivity associated with simultaneously high AMF richness and P-source diversity. An experiment conducted using an old-field system also did not detect evidence for AMF-mediated P partitioning (Reynolds *et al.*, 2006). Certainly, the partitioning of different P sources is only one possible form of AMF-facilitated complementarity; other forms include spatial partitioning of total soil P (Smith *et al.*, 2000) or partitioning along seasons (Merryweather & Fitter, 1998; Bever *et al.*, 2001). Positive relationships between AMF richness and plant productivity and/or diversity could also arise via negative feedbacks between plants and AMF (Bever, 2002). Yet both AMF-facilitated complementarity in P use and negative feedbacks predict that the plant diversity/productivity response to AMF richness emerges from AMF diversity *per se*, rather than, as we observed, from the inclusion of particular species with large effects.

While previous work could not differentiate whether the diversity effect of AMF was driven by diversity of the fungal species *per se*, or the probability of including fungal isolates that are particularly good at promoting plant productivity or diversity (van der Heijden *et al.*, 1999; Wardle, 1999), our design allows us to make this distinction. We used a large number of treatment combinations to investigate how P interacts broadly with individual AMF species and AMF species richness on plant communities. While our power to understand specific interactions within individual fungal isolates and added P types was limited, we can nevertheless reject the complementarity and negative-feedback mechanisms of diversity promotion in this system. We find that for both plant community productivity and plant diversity, individual fungal isolates give responses equal to, or even greater than, that of the mixed inocula treatment. This result suggests a 'multiple-species sampling effect' (Reich *et al.*, 2001), where the average diversity or productivity response is high as long as at least one effective AMF species is present in the system.

We identified two distinct ecological groups of AMF species in this experiment: species that inhibited community productivity; and species that promoted community productivity. We note that phylogenetic relatedness bears little correlation with these ecological groups: all three *Glomus* isolates appeared ecologically distinct, as were the two species of *Scutellospora*. Moreover, both genera spanned the range from productivity-promoting to productivity-inhibiting effects. Interestingly, productivity inhibitors were relatively better than productivity promoters at promoting diversity in the absence of added P, while productivity promoters were relatively better than productivity inhibitors at promoting plant community diversity when all five sources of P were added to the mesocosm (Fig. 4).

This shift in the relationship of community diversity promotion and community productivity promotion of the AMF isolates is probably driven by an underlying shift in the fortunes of the dominant plant species. Growth promotion of the dominant

plant species would tend to inhibit subdominant plants, thus depressing plant community diversity and promoting plant community productivity. Conversely, growth inhibition of the dominant plant species would tend to release subdominant plants, thus promoting plant community diversity, but perhaps with less effect on overall plant community productivity. In our experiment, *P. virgatum* was the dominant plant species in all mesocosms, and we observed a shift in its relative response to community productivity-promoting AMF across P environments. Without added P, the relative abundance of *P. virgatum* was enhanced by community productivity-promoting AMF, resulting in decreased plant community diversity with increased plant community productivity. This effect is consistent with the plant community growth patterns observed in systems dominated by strongly mycotrophic plants (Hartnett & Wilson, 1999; O'Connor *et al.*, 2002). With added P, however, the relative abundance of *P. virgatum* was decreased by community productivity-promoting AMF. Furthermore, although not included in our tests here, the relative abundance of several subordinate species, including *S. laciniatum*, *A. tuberosa*, *L. aspera* and *L. perennis*, showed a trend towards opposite dependence on productivity promotion and P addition. This response would be consistent with the plant community patterns observed in systems dominated by weakly mycotrophic plants (Grime *et al.*, 1987; van der Heijden *et al.*, 1998a), where plant community diversity could increase with little effect on community productivity. Our results show how the identity and diversity of AMF can mediate diversity promotion, and that the direction of this effect can shift with the amount of soil P.

Results from biodiversity manipulations have been criticized as being driven by sampling effects (Huston, 1997; Wardle, 1999). We find that plant productivity and diversity are more responsive to AMF identity than diversity *per se*. However, we do not suggest that diversity of the AMF community is unimportant. Rather, given the stochasticity of natural systems and the myriad effects of soil heterogeneity (Hutchings *et al.*, 2003), our observation that the identity of diversity-promoting fungi is environment-dependent suggests that plant communities dependent on the AMF symbiosis would be more susceptible to environmental variability as AMF species are lost from the system. Testing for this possibility would be worthwhile, and may shed light on plant community properties and dynamics where AMF are known to be crucial components of the system.

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