

LETTER

Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism

James D. Bever,* Sarah C. Richardson,[†] Brandy M. Lawrence, Jonathan Holmes and Maxine Watson

Department of Biology, Indiana University, Bloomington, IN 47405, USA

*Correspondence: E-mail: jbever@indiana.edu

[†]Present address: Environmental Sciences Program, DePaul University, Chicago, IL 60614, USA.

Abstract

Mutualisms, beneficial interactions between species, are expected to be unstable because delivery of benefit likely involves fitness costs and selection should favour partners that deliver less benefit. Yet, mutualisms are common and persistent, even in the largely promiscuous associations between plants and soil microorganisms such as arbuscular mycorrhizal fungi. In two different systems, we demonstrate preferential allocation of photosynthate by host plants to the more beneficial of two AM fungal symbionts. This preferential allocation could allow the persistence of the mutualism if it confers sufficient advantage to the beneficial symbiont that it overcomes the cost of mutualism. We find that the beneficial fungus does increase in biomass when the fungi are spatially separated within the root system. However, in well-mixed fungal communities, non-beneficial fungi proliferate as expected from their reduced cost of mutualism. Our findings suggest that preferential allocation within spatially structured microbial communities can stabilize mutualisms between plants and root symbionts.

Keywords

Cheaters, mutualism, mycorrhizae, partner choice, plant–microbe interaction, preferential allocation, spatial structure, symbionts.

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INTRODUCTION

Symbioses have played critical roles in the history of life including the origin of eukaryotes (Margulis 1981; Smith & Szathmari 2002) and the colonization of land by plants (Pirozynski & Malloch 1975; Redecker *et al.* 2000). While symbiotic mutualisms can be stabilized by vertical transmission (Jeon 1972; Frank 1996; Smith & Szathmari 2002; Weeks *et al.* 2007), the stability of horizontally transmitted symbioses remains an unexplained problem. In these interactions, the fitness costs to the symbiont incurred during the delivery of benefit to hosts should theoretically favour those symbionts that deliver less benefit (Ferriere *et al.* 2002). For example, arbuscular mycorrhizal (AM) fungi, root symbionts that play critical roles in terrestrial ecosystems (Van der Heijden *et al.* 1998; Vogelsang *et al.* 2006), are transmitted horizontally. Also, individual plants simultaneously associate with multiple species of AM fungi that are known to vary in their delivery of benefit (Bever 2002b). Given this promiscuity and the cost of mutualism, these mutualisms are expected to decay because less effective partners will have a competitive advantage over partners that reciprocate (Bronstein 2001; Denison *et al.*

2003). Consistent with this expectation, the composition of AM fungi within plant root systems in well-mixed laboratory mesocosms changed in a manner that reduced the benefit to hosts (Bever 2002b).

Partner choice is one factor that may contribute to the stability of the mutualisms between plants and root symbionts (Noë & Hammerstein 1994; Denison 2000; Simms & Taylor 2002; West *et al.* 2002; Sachs *et al.* 2004). Observations consistent with partner choice have been demonstrated in the interactions between legumes and nitrogen-fixing bacteria, including host sanctions against ineffective nodules by legumes (Kiers *et al.* 2003), and patterns of nodule growth consistent with preferential allocation toward the most beneficial rhizobia (Simms *et al.* 2006). However, population increase in the proportion of beneficial symbionts, while logical extensions of partner choice, remains to be demonstrated. Spatial structure of symbionts within plant root systems is a second factor that has been suggested to be important for the evolution of mutualism in root associated symbionts (Bever & Simms 2000; Hoeksema & Kummel 2003). General models indicate that spatial structure can facilitate the evolution of beneficial interactions by separating patches of mutualists from less

beneficial or pathogenic counterparts (Doebeli & Knowlton 1998; Wilson *et al.* 2003; Lion & van Baalen 2008). However, as of yet, there is no empirical support for partner choice or spatial structure in contributing to the maintenance of mutualisms involving highly promiscuous root symbionts such as AM fungi (Denison *et al.* 2003; Kiers & van der Heijden 2006).

We first evaluated the ability of plants to preferentially allocate their carbon resources to more beneficial mutualists and then we tested the consequence of this allocation on fungal population dynamics as a function of spatial structure within root systems. We did these tests using a split root system (Fig. 1) that took advantage of the phenology and vascular architecture of wild onion (*Allium*). Because *Allium* are monocots, internal resource movement is not constrained by vascular architecture (Watson & Casper 1984); which means that locally derived resources have the potential to move throughout the plant. Because of this, if concentration of label is detected in particular organs, it cannot be attributed to vascular constraints. Moreover, *Allium* are cool season perennials whose roots die back at the end of the growing season, forcing associated AM fungi within our experiments to sporulate, thereby ensuring that sporulation occurs. Other experiments have found that sporulation represents a good measure of fungal fitness (Bever 2002b). Using two species of *Allium* and their co-occurring fungi and native soil, we confirmed that preferential allocation can overcome the cost of mutualism in AM fungi.

METHODS

Experiments 1 and 2

We developed a split root system in which roots of individual *Allium vineale* plants were divided equally between two pots. Growth of *A. vineale* is responsive to mycorrhizal fungi (Ronsheim & Anderson 2001) and it is a cool season perennial whose roots die back at the end of the growing season, forcing associated AM fungi to sporulate. Each of these pots was inoculated with an undescribed *Glomus* (*Gl*), *Gigaspora margarita* (*Gig*), a mixture of both (*Gl-Gig*) or no inoculum (Con). The first experiment tested for short-term preferential allocation of carbon to the more mutualistic mycorrhizal fungus, and the second experiment assessed the effect of this allocation on the population dynamics of the AM fungi. In both of these experiments, the plants, fungi and soil were derived from the same field in North Carolina, USA (Bever *et al.* 1996, 2001). The undescribed *Glomus* [*Gl. d1* in (Bever *et al.* 1996)], which resembles *Gl. clarum* and is called *Gl.* 'white' hereafter, and *Gi. margarita* have been deposited in INVAM (NC172 and NC175, respectively). The soil at this site is a sandy loam, containing

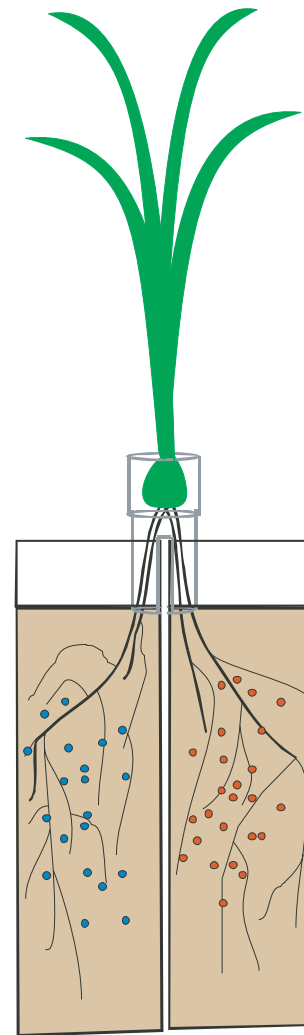


Figure 1 Schematic of experimental split root chambers. The two sides of the root systems were inoculated independently. The grey lines represent the PVC pipes, which were filled with sterile sand.

113 ppm nitrate, 7 ppm ammonia and 29 ppm phosphorus at the time it was collected for this experiment (Reynolds *et al.* 2006).

Experiment 1: testing preferential allocation

We directly measured allocation of photosynthetically fixed carbon by exposing plants to radioactive carbon in the form of $^{14}\text{CO}_2$, and monitored the distribution of labelled carbon to roots split between *Glomus* and *Gigaspora* (*Gl/Gl*) inoculated soil. We set up 24 sets of split root pots divided equally (six replicates each) among four chase periods: 2 h, 1 day, 2 days, and 4 days following exposure to $^{14}\text{CO}_2$, at which point the plants were harvested. In addition, to test plants' growth response to the two fungal species, we

included six split root replicates of *Glomus* and *Gigaspora* paired with themselves (*Gl/Gl* and *Gi/Gi*, respectively), as well as uninoculated controls.

Single species fungal cultures were grown on sorghum and the infected soil and root mixture was air dried and chopped for use as inoculum. *Allium* plants were grown from bulbils in sterile Metromix for 3 weeks. Next, each plant was placed in a 'split root pot' in sterile conditions in order for the plant to interact with both fungi while the fungi were spatially isolated from each other. Split root pots were constructed by connecting two adjacent pots (two duct-taped 2 3/8" × 5" 'short tree bands' from Anderson Die Co., Portland, OR, USA). PVC piping with a slit was clipped over adjoining pots (Fig. 1). The roots of each plant were divided into approximately equal halves; each half was guided into one of the two paired pots. The bulb of the plant was centred and secured above the pots using fishing line, which prevented *Allium's* contractile roots from pulling the bulbs into an individual side of PVC pipe. The PVC piping was then filled with sterilized sand. Pots were filled with three layers: a bottom layer of 75 mL of a sterile 1 : 1 soil : sand mix, then a layer of 200 mL of a 1 : 9 mixture of inoculum to sterile soil : sand mix, which came up to the bottom of the PVC pipes, and 75 mL of sterile soil : sand mix on top. Sterile inoculum included 10% autoclaved AM fungal culture. Ten millilitres of pooled bacterial wash from both inocula was added to all pots. Both fungi had similar inoculum potential at this density (38 ± 6 and $34 \pm 6\%$ AM root infection, for *Gigaspora* and *Glomus*, respectively) as measured using a standard infection assay on sorghum.

After 9 weeks of growth in split root pots, plants were covered and exposed to radioactive carbon in the form of $^{14}\text{CO}_2$. By 9 weeks, we expected the roots to be infected by mycorrhizal fungi and the association to be physiologically active (Miller *et al.* 2002). $^{14}\text{CO}_2$ was released into bags containing the *Allium* shoots by mixing 5 μCi of ^{14}C -sodium bicarbonate (ICN177441H05, 55 mCi mmol^{-1}) with a drop of 42% lactic acid into a cuvette within the bag. Plants were pulsed with $^{14}\text{CO}_2$ for 30 min before bags were removed. There were four chase durations: 2 h, and 1, 2, and 4 days. Plants were harvested, divided into six components: (1) shoot, (2) bulb, (3 and 4) roots within the PVC tube in side A and B (which lack AM fungi), and (5 and 6) roots within the two pots A and B, (which contain AM fungi). Each component was oven dried and weighed. To analyse the ^{14}C content, subsamples were removed from each component, weighed and oxidized (Harvey Biological Materials Oxidizer, OX-400; Harvey Biological, Hillsdale, NJ, USA). The released $^{14}\text{CO}_2$ was trapped in Carbon-14 Cocktail (Harvey Biological) and analysed by liquid scintillation counting. External fungal hyphae were extracted from a subsample of the soil and collected on filter paper through a modification of the procedure of Miller (Miller *et al.* 1995). Samples were

vigorously mixed with Triton-X into water. The solution was passed through sieves with the hyphae being caught on a 20-micron sieve. After rinsing with water, these hyphae were resuspended in water and decanted through filter paper. The hyphae on filter papers were then dried, oxidized and radioactivity was counted as above.

Statistical analysis

Plant mass was log transformed to equalize variance and analysed with an ANOVA using initial leaf length as a covariate. Differences in allocation of labelled carbon in the roots from each pair of pots in the split root chambers were measured using metric of a ratio minus one. The ratio is the concentration of ^{14}C in roots associated with *Gl*. 'white' over the concentration of ^{14}C in roots associated with *Gigaspora*. With the subtraction of one from this ratio, the metric will be zero when the allocation is unbiased. If allocation was biased toward *Gl*. 'white', the metric would be positive; if the bias was toward *Gigaspora*, it would be negative. For the ANOVA, the metrics were log-transformed and the total label in the plant and the ratio of the weight of roots in the sides of the tube were used as covariates. Measures of external hyphae were analysed similarly.

Experiment 2: testing consequences of preferential allocation in an old field system

We evaluated the response of fungal populations to plant allocation in the second experiment. This experiment included combinations of the two fungi and the uninoculated control [*Gl/Gl*, *Gig/Gig*, *Gig/Gl*, *Gl/con*, *Gig/con*, and *con/con*, as well as each pot with both fungal species mixed (*Gig-Gl/Gig-Gl*)]. The pots were arranged in 13 randomized blocks with the *Gig/Gl* treatment being represented twice within each block and other treatments represented once. 10 mL pooled bacterial wash was added to all pots.

The plants and pots were set up as in Experiment 1 and grown in a growth chamber on a 12/12 h light (22 °C)/dark (10 °C) schedule (60% humidity) for 6 months. Plants were watered through the central tube to prevent root allocation based on soil moisture. Fertilization was not necessary given the small stature of the plant. The bulb and leaves were separated at harvest, dried and weighed. Roots from each pot were chopped and mixed into the soil. Fifty millilitres subsamples of the soil-root mixture were taken for measurement of root mass and extraction of spores. The density of spores within each pot was determined as in (Bever *et al.* 1996). Spore diameter was measured from 20 to 30 spores chosen at random from a subsample of the pots. Spore counts and measurements were made without regard to treatment. Spore volumes were estimated from spore diameters assuming a spherical shape. Spore volume is likely

directly related to AM fungal fitness, as individual spores can produce abundant hyphae in search of roots to colonize, with the amount of hyphae, and therefore volume of soil explored, being dependent on spore reserves (Siqueira *et al.* 1982). We estimate the total spore volume produced as the product of the spore number and average spore size within each treatment. Total spore volume has previously been shown to be a good measure of fungal population growth rates in this system (Bever 2002a,b). We did not find evidence of contamination across the split root chambers.

Statistical analysis

Plant mass was analysed as in Experiment 1. As amount of inoculum of individual fungal species was manipulated, with the plants with *Glomus* on both sides (*Gl/Gl*), for example, having twice as much *Glomus* in the plant root system as the plants with *Glomus* on one side (*Gig/Gl* or *Gl/con*) or the mixture on both sides (*Gig-Gl/Gig-Gl*), spore production was scaled according to the initial inoculum density of each species. Spore production was log-transformed to improve homogeneity of variance and analysed with ANOVA using the GLM procedure of SAS.

Experiment 3: testing consequences of preferential allocation in a prairie system

This experiment was similar to Experiment 2, but used plants, AM fungi, and soil derived from the tallgrass prairie of Northern Indiana (USA). *Allium cernuum*, nodding onion, is a native prairie forb that is typical of prairie remnants in the eastern tallgrass prairie. We used two fungi, *Glomus claroideum* and *Scutellospora fulgida*, derived from prairies near the Kankakee Sands Restoration Area in Northern Indiana (INVAM IN214 and IN212, respectively). *Scutellospora fulgida* has been shown to be a better growth promoter of prairie plants than *Glomus claroideum* (Vogelsang *et al.* 2006). The soil was collected from Kankakee Sands Restoration area and is a sandy loam containing 26 ppm nitrate, 28 ppm ammonia, and 226 ppm phosphorus. Seeds of *Allium cernuum* were collected by Spence Restoration Nursery (Muncie, IN, USA) from native prairie remnants in northern and central Indiana. We cold-moist stratified seeds in flats with sterile Metromix for 2 weeks and then germinated them in a growth chamber on a 12/12 h light (22 °C)/dark (10 °C) schedule (60% humidity).

Experiment 3a: test of A. cernuum growth response to inoculation

We tested for differences between species of fungi in growth promotion of *A. cernuum* by growing seedlings of *A. cernuum* with each of the two fungal species as well as a sterile control. Twenty replicates of the fungal treatments

and 10 replicates of the control treatments were divided among four randomized blocks. Pots (500 mL) were filled with double sterilized 1 : 1 mixture of sand and soil, inoculated with AM fungal cultures and microbial wash as described above. An analysis of mycorrhizal infection potential indicated that there were not significant differences in the density of these inocula ($F_{1,21} = 2.8$, ns). Plants were grown in a greenhouse with additional light that increased daylength to 12 h for 15 weeks, and then harvested, dried and weighed. Plant mass was analysed using analysis of variance with initial leaf length used as a covariate to control for variation in plant size at the start of the experiment.

Experiment 3b: test of consequences of preferential allocation

We evaluated the response of fungal populations to plant allocation in the prairie system using a design similar to Experiment 2. Treatments were replicated in 12 blocks with two extra replicates per block of *Sf/Gl* pots. Split root pots were constructed of two 2 7/8" × 9" 'deep' tree bands from Anderson Die Co. (Portland, OR, USA), duct-taped together, each holding a 6.5 cm segment of 3/4" PVC pipe topped by a coupling tube. Roots of each plant were divided between the pots. Plants were secured with fishing line within the PVC pipes, which were filled with sterilized pool sand. Each split pot contained 394 mL of sterilized 1 : 1 soil with 10% inoculum or 10% sterilized inoculum, covered by a 118 mL layer of sterile 1 : 1 mixture of sand and soil. All pots were inoculated with 10 mL microbial wash per pot. Plants were watered through the central PVC pipes. Plants were grown for nine months in a greenhouse with additional light that increased daylength to 12 h. Spores and spore size was measured as above. Again there was no evidence of contamination across split root chambers. The reversal of the competitive dominance with spatial structure was tested using multivariate profile analysis in GLM of SAS (Bever *et al.* 1996).

RESULTS

Experiment 1

We tested preferential allocation of carbon resources using a split root system (Fig. 1) in which a single plant's roots were split into separate pots with different symbionts. In the first two experiments, we used the host plant *Allium vineale* and two of its co-occurring AM fungal symbionts, all collected from the same site in North Carolina (Bever *et al.* 2001). *Glomus* 'white' was much more beneficial, i.e. successful in promoting plant growth, than *Gigaspora* (Reynolds *et al.* 2005, 2006). In this study, as expected, *A. vineale* growth was promoted by *Gl. 'white'*, but not by *Gl. margarita* (Fig. 2a,b).

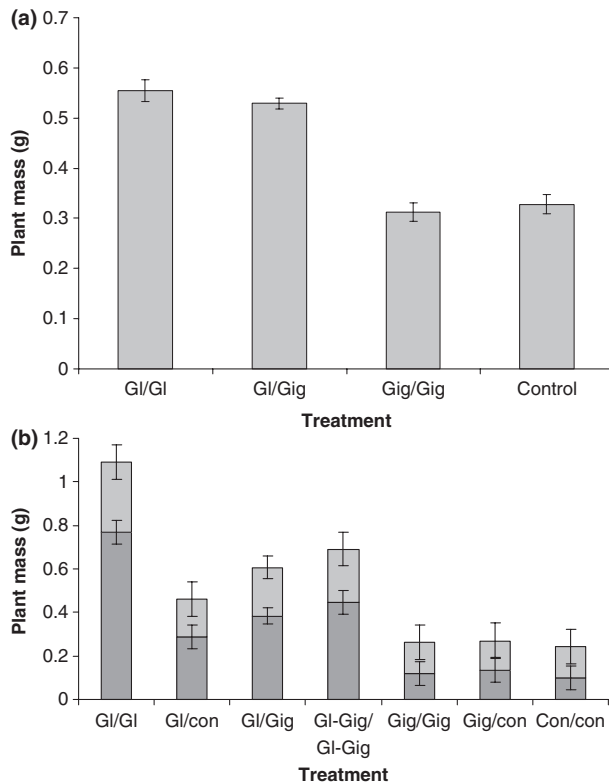


Figure 2 *Allium vineale* grew largest with *Glomus* 'white' (represented by 'Gl') while growth with *Gigaspora margarita* (represented by 'Gig') was not significantly different from the sterile control. These patterns held for growth over 3 months in Experiment 1 (a, $F_{3,37} = 13.3$, $P < 0.0001$) and over an entire growing season in Experiment 2 [b, for total plant mass (total size of bar): $F_{6,89} = 15.5$, $P < 0.0001$; for bulb mass (bottom part of bar): $F_{6,89} = 19.7$, $P < 0.0001$]. The two species in separate chambers and the two species inoculated in mixture into both chambers are represented as Gl/Gig and Gl-Gig/Gl-Gig, respectively. Error bars represent standard errors.

We found that *A. vineale* allocated significantly more carbon resources toward the mutualistic *Gl. 'white'* than the non-beneficial *Gi. margarita* when the fungi were spatially separated. In Experiment 1, we monitored carbon allocation toward roots infected with the two fungi following exposure of the plants to $^{14}\text{CO}_2$. More than 80% of the label remained in the leaves 2 h after exposure and there was no difference in allocation at this time. However, over the next 4 days a greater amount of the label was delivered to roots infected with *Gl. 'white'* than roots infected with *Gi. margarita* (Fig. 3a). More labelled carbon was also found in external *Gl. 'white'* hyphae than external *Gi. margarita* hyphae (Fig. 3b). Total weight of the root masses associated with each of the two fungi did not differ ($t = 0.44$, d.f. = 22, $P = 0.66$), indicating that preferential allocation was not a simple consequence of differential root growth. Although

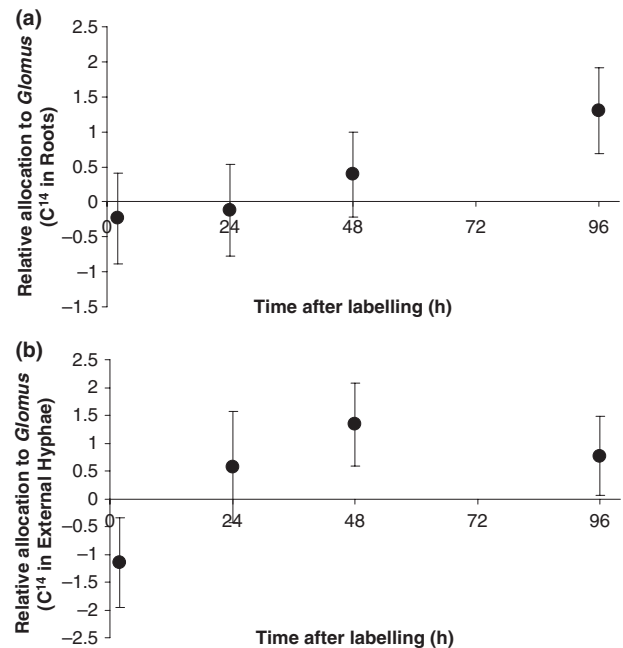


Figure 3 *Allium vineale* allocated more carbon resources to the beneficial fungus, *Glomus* 'white', than to *Gigaspora margarita*. The dependent variable is a metric indicating relative allocation of ^{14}C to the two species, a positive value for which indicates a bias toward *Gl. 'white'*. The proportion of labelled carbon present in the roots infected with *Gl. 'white'* (a) and in *Gl. 'white'* external hyphae (b) increased after time of labelling ($F_{1,20} = 4.4$, $P < 0.05$; $F_{1,20} = 4.6$, $P < 0.05$; for root and external hyphae, respectively). Error bars represent standard errors.

we do not know the molecular mechanism, our experiment showed that photosynthate was allocated preferentially to the more beneficial species when fungal species were separated.

Experiment 2

The preferential allocation of carbon to the roots associated with the more beneficial fungus should lead to an increase in that fungus in the soil community. In contrast, earlier work showed that it was the less beneficial fungi that proliferated, but in these experiments the fungal species were well-mixed (Bever 2002b), rather than spatially separated from each other as in the experiment above. These latter data suggest that under well-mixed conditions beneficial fungi have lower competitive ability – a result consistent with there being a cost of mutualism. We predicted that spatial structure of the AM fungal community within the host root system could reduce competition between AM fungal species, thereby allowing beneficial fungi to translate increased allocation of resources by the host plant into higher fungal fitness. We tested this possibility by taking advantage of a prior

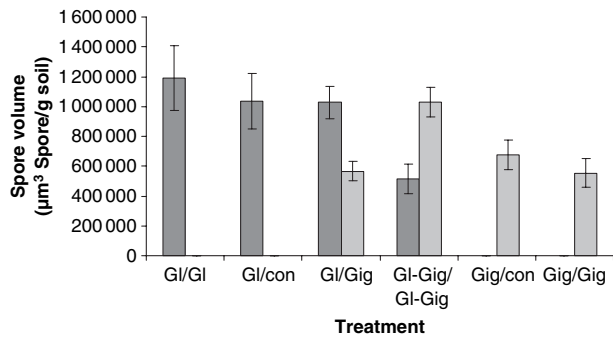


Figure 4 The total spore volume produced by *Glomus 'white'* was greater when separated from *Gigaspora margarita* in adjoining split root chambers than when in mixture with *Gi. margarita* ($F_{1,49} = 6.7$, $P < 0.01$). Conversely, the total spore volume produced by *Gi. margarita* was greater in mixture with *Gl. 'white'* than when separated from *Gl. 'white'* in adjoining split root chambers ($F_{1,50} = 13.4$, $P < 0.0006$). The labels in the x -axis and error bars are as in Fig. 2.

observation that fungal sporulation reflects fungal population growth rates in this system (Bever 2002a,b).

Using the same system as in Experiment 1, we found evidence that preferential allocation of carbon resources increases the fitness of the more beneficial fungus. Mutualistic *Gl. 'white'* tended to produce fewer spores ($F_{3,49} = 2.3$, $P = 0.09$) and produced significantly smaller spores when in mixture with non-mutualistic *Gi. margarita* than when separated from it ($F_{3,40} = 7.7$, $P < 0.0004$). This is the first demonstration of reduction in the size of spores of an AM fungus in response to competition. Conversely, *Gi. margarita* produced a greater number of spores in mixture than when separated from *Gl. 'white'*. As a consequence, dominance, as reflected by total spore volume, was reversed by spatial separation of the fungi within the host root system (Fig. 4). The beneficial *Gl. 'white'* attained greater volume of spores when spatially separated from *Gi. margarita* than when mixed, as expected from preferential allocation of host resources towards the more beneficial fungus seen in Experiment 1. *Gl. 'white'* spore volume was significantly reduced when *Gl. 'white'* was mixed with *Gi. margarita* (Fig. 4), presumably reflecting low competitive ability due to the better mutualist, *Gl. 'white'*, bearing a greater cost of mutualism. Conversely, non-mutualistic *Gi. margarita* had highest fitness in mixture where it could outcompete *Gl. 'white'* for plant resources and lowest fitness when spatially separated from *Gl. 'white'* (Fig. 4). Thus, these experiments provide evidence that preferential allocation to the better mutualist can overcome the higher cost of mutualism born by the beneficial fungus provided that the fungi are spatially separated within the plant's root system.

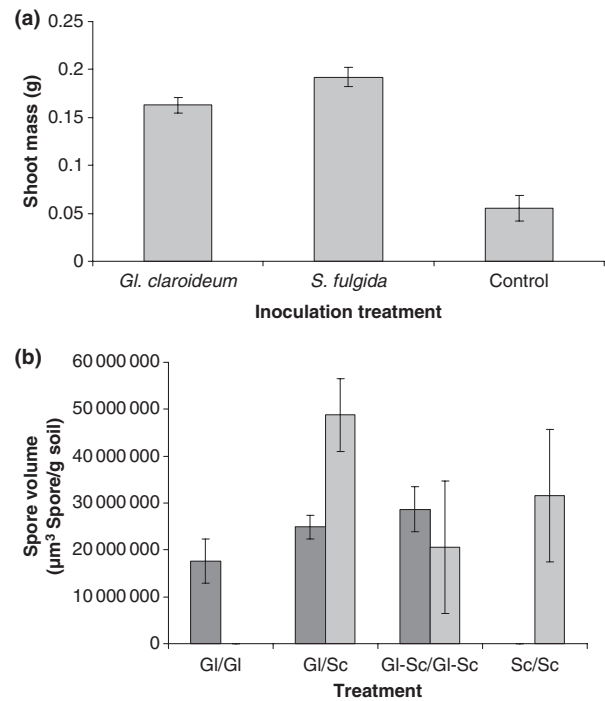


Figure 5 Evidence that a cost of mutualism can be overcome by preferential allocation toward the more effective mycorrhizal fungus in a native forb. *Allium cernuum* grew better in association with *Scutellospora fulgida* than *Glomus claroideum* ($F_{1,25} = 4.59$, $P = 0.04$, a). *S. fulgida* dominated when spatially separated, as is consistent with preferential allocation toward the better mutualist. This dominance was reversed, however, when the two species were in mixture, as is expected by a cost of mutualism. The reversal in rank when with and without spatial structure was significant (Wilkes Lambda $F_{1,37} = 4.6$, $P = 0.04$). Error bars represent standard errors.

Experiment 3

We found similar results in a parallel, but independent, test using plants and fungi native to the fertile eastern North American tallgrass prairies. In this system, the native forb, *Allium cernuum*, benefited from *Glomus claroideum* but grew even more with *Scutellospora fulgida* ($F_{1,25} = 4.59$, $P = 0.04$, Fig. 5a). As in the previous study, the outcome of competition depended on spatial structure, with the more beneficial *Scutellospora fulgida* dominating in spore volume when spatially separated, but not when in mixture (Wilkes Lambda $F_{1,37} = 4.6$, $P = 0.04$, Fig. 5b). The shift in AM fungal abundance with spatial structure in this system was more modest than in the previous system.

DISCUSSION

These results provide the first demonstration of preferential allocation of photosynthate by plants to the more mutual-

istic AM fungus (Fig. 3). Previous attempts to look at allocation to individual mycorrhizal fungi did so in comparison to sterile roots (Lerat *et al.* 2003) rather than in comparison with other fungi. Our observation of preferential allocation is analogous to the observations of sanctions (Kiers *et al.* 2003) and preferential rewarding of effective rhizobia (Simms *et al.* 2006) within symbiotic N-fixing bacteria and confirms the potential role of partner choice in a second group of root mutualists. Moreover, while studies with rhizobia do not demonstrate that the partner choice resulted in the increase in the proportion of beneficial symbionts, we confirm that preferential allocation by the plant is associated with a fitness advantage for the beneficial mycorrhizal fungus, provided that the fungi are spatially separated.

Our results indicate that plants preferentially allocated to the better mutualist in both the prairie and old field system, even though there was a reversal in the phylogenetic placement of the better mutualist between the two studies, with the *Glomus* species being the better mutualist in the North Carolina system and the worst mutualist in the Indiana prairie system. The strength of allocation appears to be reduced in the prairie system (Fig. 5), which is consistent with a lower level of preferential allocation due to the smaller difference in growth promotion between *S. fulgida* and *Gl. claroidesum* compared to that between *Gl. 'white'* and *Gi. margarita*. This reduced differential response may also be consistent with reduced sensitivity by the plant to differences in effectiveness of AM fungi in the more fertile soils of the eastern tallgrass prairies (Schultz *et al.* 2001). The evidence of preferential allocation toward the better mutualist across two systems that differ in fertility and in the phylogenetic placement of the better mutualist suggests that these observations may be general, although more work needs to be done across a greater diversity of species and environments.

This work contributes to a synthetic understanding of the dynamics and maintenance of the arbuscular mycorrhizal mutualism. First, we found that the less beneficial AM fungi proliferated in spatially well-mixed environments, a result expected if delivery of greater benefit to the host increases the fitness cost for the fungus that provides it. This result is consistent with previous studies (Bever 2002b) that demonstrated an important role of the cost of mutualism in microbial dynamics. This study suggests that the cost of mutualism is most strongly expressed in competition. Second, we demonstrated that plants can preferentially allocate photosynthate to the better mutualist. Finally, we demonstrated that this preferential allocation can overcome the cost of mutualism and allow beneficial fungi to increase, provided there is sufficient spatial structure within the plant root system.

Spatial structure has been shown to alter the outcomes of antagonistic interactions (Huffaker 1958; Kerr *et al.* 2006)

and has been suggested to be important for the maintenance of mutualism in theory (Doebeli & Knowlton 1998; Bever & Simms 2000; Hoeksema & Kummel 2003), but this is the first experimental demonstration that spatial structure can play a critical role in the maintenance of the mycorrhizal mutualism. Given the dependence of the fungal dynamics on our coarse scale spatial manipulation, we infer that plants do not control preferential allocation at the scale of individual arbuscules. Whether plants control allocation at the scale of a root system or rootlet will require finer scale manipulations. Further work should also test the applicability of our observations to more natural settings, where spatial structure is not as rigidly enforced as in our split root systems. AM fungal systems have been observed to be spatially structured in nature, with variation in fungal composition being observed within root systems of the same plant (Friese & Koske 1991) and within patches of soil on the scale of centimeters (Wolfe *et al.* 2007). While this spatial structure within the soil may be generated by multiple factors (including stochasticity and host specificity), preferential allocation may enhance the success of patches with greater abundance of beneficial fungi.

Our results may have broad implications for our understanding of the evolution of mutualisms in mycorrhizal symbioses and potentially with other root symbionts in natural and managed systems. We hypothesize that most plants have the ability to preferentially allocate to the best provider of soil nutrients, given that allocation to nutrient hotspots has been repeatedly demonstrated in many plant species (Jackson & Caldwell 1991; Caldwell *et al.* 1992; Caldwell 1994). Available data also indicate that patchy distributions of symbionts within a root system is a common feature of the interaction between plants and AM fungi (Friese & Koske 1991; Ritz *et al.* 2004; Wolfe *et al.* 2007) and between plants and other soil microbes (Mummey & Stahl 2003; Nicol *et al.* 2003) in nature. Together these two features of plant–soil microbe interactions may contribute to the evolution of mutualism among the broad taxonomic range of microbial root symbionts.

Moreover, the mechanism that we have described may provide a mechanistic basis for the prediction of mycorrhizal functioning across the landscape. Given variability in spatial structure in plant root systems, the resulting dynamic can explain the persistence of mutualistic fungi as well as the persistence of non-beneficial fungi, both of which are frequently observed in nature. Our results specifically suggest that destruction of soil spatial structure, as might result from tillage in agriculture, will allow for proliferation of less mutualistic microbes. This expectation is consistent with observations of less beneficial varieties of mycorrhizal fungi (Johnson *et al.* 1997) and N-fixing bacteria (Ferreira *et al.* 2000) accumulating in association with conventional agriculture and with tillage.

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