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Research

Shading decreases plant carbon preferential allocation towards the most beneficial mycorrhizal mutualist

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Summary

• Preferential allocation towards the most beneficial mutualist could maintain mycorrhizal mutualism. Context dependence of preferential allocation could then determine environmental patterns in abundance of mycorrhizal mutualists.

• We assessed the preferential allocation of carbon (C) and differential phosphorus (P) uptake across four light treatments between the host plant *Allium vineale* and two arbuscular mycorrhizal (AM) fungi within a split-root system. The ratios of C allocation and P uptake between the beneficial and nonbeneficial AM fungi were measured using isotopic labelling.

• *Allium vineale* preferentially allocated more C towards roots infected with the most beneficial AM fungus in high light and, in return, received more P from the beneficial fungus. Preferential allocation declined with shading, as *A. vineale* allocated 25% of labelled C to roots infected with beneficial AM fungi in high light, but only 15% with shading, a similar percentage to that allocated to roots infected with nonbeneficial fungi regardless of shading.

• Our findings demonstrate that plant preferential allocation towards the most beneficial mycorrhizal mutualist depends upon above-ground resources, suggesting that the abundance of beneficial mycorrhizal fungi will increase with amount of above-ground resources, with implications for mycorrhizal mediation of plant productivity with anthropogenic change.

Introduction

Mutualisms – interactions that benefit both partners – play an important role in driving the evolution of much of biological diversity (Johnson *et al.*, 1997; Thompson, 2005). However, because the delivery of benefit to another species is costly, there will be partners who do not deliver benefits to, but continue to receive benefits from, their host (Bennett & Bever, 2009; Denison & Kiers, 2011). Theoretically, proliferation of such 'cheaters' could drive the mutualism towards a parasitism (Bronstein, 2001; Verbruggen *et al.*, 2012), and degradation of mutualism has been observed (Bever, 2002). Given this potential instability, the persistence of mutualisms in nature remains a challenging question in ecology and evolution (Denison & Kiers, 2004; Thompson, 2005; Soares *et al.*, 2008; Goto *et al.*, 2010). Currently, several theoretical models (Boucher *et al.*, 1982; Hoeksema & Bruna, 2000; Bronstein, 2001) have been reported to explain the evolutionary persistence of some mutualisms, but they have limited insight into one important mutualism, arbuscular mycorrhizas (Bever *et al.*, 2009).

In the arbuscular mycorrhizal (AM) symbiosis, the bidirectional transfers of organic carbon (C) and nutrients in AM symbioses depend on the identity of AM fungi, which vary from beneficial to nonbeneficial (Bever, 2002; Jones & Smith, 2004; Bennett & Bever, 2009). Based on recent *in vitro* and glasshouse studies (Bever *et al.*, 2009; Kiers *et al.*, 2011), plants can allocate more C to beneficial AM fungi than to nonbeneficial AM fungi. This preferential allocation can overcome the cost of mutualism and stabilize mutually beneficial interactions (Bever *et al.*, 2009; Verbruggen *et al.*, 2012).

The occurrence of beneficial AM fungi has been observed to vary across environments, as AM fungi isolated from high phosphorus (P) soils have been observed to be less beneficial to plant growth than AM fungi isolated from low P soils (Louis & Lim, 1988; Boerner, 1990). Energetic and stoichiometric frameworks have been used to build expectations that mutualistic symbioses between plants and mycorrhizal fungi will be more stable in low-nutrient, high-light environments, because surplus C supplies in the plant host combined with reduced nutrient availability in the abiotic environment should result in increased latitude for beneficial exchanges (Schwartz & Hoeksema, 1998; Kiers & van der Heijden, 2006; Sachs & Simms, 2006). However, these energetic arguments do not identify which factors prevent the spread of cheaters within a population. The environmental dependence of preferential allocation could generate environmental patterns in the abundance of nonbeneficial fungi. One might expect that preferential allocation will be strongest where plant need for resources delivered by fungi is greatest, or that a plant will be less

discriminating as it allocates to any fungi available to fill its resource need. To date, the context dependence of preferential allocation has not been demonstrated.

Plant preferential allocation might depend upon above-ground resources. Plant response to inoculation with mycorrhizal fungi is known to weaken with declining above-ground resources such as light (Son & Smith, 1988; Hunt & Hope-Simpson, 1990). With declining light, plants should allocate more biomass to above-ground structures and invest less in mycorrhizas to adjust resource imbalances (Chapin *et al.*, 1987; Johnson *et al.*, 2008; Johnson, 2010). Consistent with this expectation, several studies have shown reduced investment in mycorrhizal fungi with declining light, as measured by colonization (Tester *et al.*, 1986; Son & Smith, 1988; Gehring, 2003; Hofland-Zijlstra & Berendse, 2009; Clark & St Clair, 2011) and physiological changes of mycorrhizal fungi (Johnson *et al.*, 2006). Similarly, it has been observed that sporulation in fungal cultures is reduced with shading (Morton *et al.*, 1993). However, reductions in infection or investment in mycorrhizal fungi are not always detected (Hurst *et al.*, 2002; Millar & Ballhorn, 2013). Moreover, previous work has not evaluated whether plants differentially reduce allocation to the most beneficial mutualist with shading or to the temporal responsiveness of plant allocation to shifts in light levels.

Our work tests plant preferential allocation towards the most beneficial AM fungal mutualist using the naturally co-occurring plant and AM fungal symbionts used in Bever *et al.* (2009). We quantified C allocation and P uptake across four different shading treatments within split-root chambers that are individually inoculated with beneficial or nonbeneficial AM fungi (Fig. 1). C allocation to each AM fungus and P uptake from each AM fungus were measured within each pot using treble radioactive labelling (^{14}C and $^{32}P/^{33}P$). Our experimental manipulations tested three interrelated hypotheses: plants preferentially allocate towards the AM fungus providing the most P; preferential allocation decreases as plant C is reduced by shading; a longer duration of shading induces larger changes in preferential allocation.

Materials and Methods

Plants, fungi and soil

All the seeds, fungi and soil were obtained from the same field in North Carolina, USA (Bever *et al.*, 1996, 2001). We used *Allium vineale* L. as a host plant associated with two fungi, *Claroideoglomus candidum* (formerly named *Glomus candidum*) and *Gigaspora margarita*. Bever *et al.* (2009) identified that

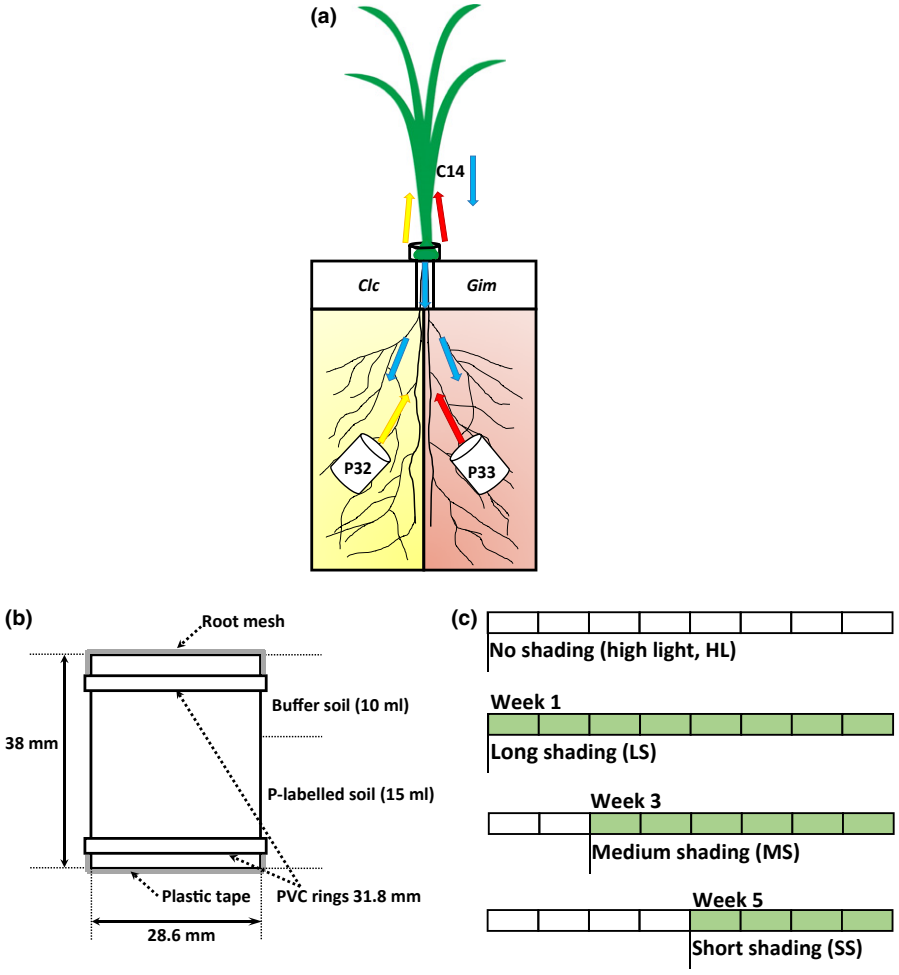

Fig. 1 Schematic of experimental split-root design, radioactive P labelling chambers and shading treatments. (a) The split-root system and radioactive labelling schemes. *Clc*, plant roots inoculated with *Claroideoglomus candidum*; *Gim*, plant roots inoculated with *Gigaspora margarita*. (b) The PVC chamber for ^{32}P and ^{33}P labelling. (c) The shading design.

C. candidum was beneficial to the growth of *A. vineale* while *G. margarita* was not, and that *A. vineale* preferentially allocated C to *C. candidum*. *A. vineale* bulbils were germinated in sterile Metromix (Hummert International, Earth City, MO, USA) and grown for 6 wk before transplanting seedlings to experimental pots in February 2013. AM fungal inoculum consists of spores, mycelium and fine root segments from cultures that were propagated in a sterile mixture of North Carolina soil and sand with host *Sorghum bicolor* in a glasshouse. The inoculum potentials of these two cultures (measured as in Vogelsang & Bever, 2009) were not significantly different ($F_{1,12} = 4.49$). The 1 : 2 (v/v) ratio of soil and sand was thoroughly mixed and then autoclaved for 2 h before potting. The soil and sand mixture contains 4 ppm NO_3^--N, 3 ppm NH_4^+-N and 7 ppm P.

Experiment setup

We designed a similar split-root system to that used in Bever *et al.* (2009) to test the plant C allocation and AM fungi P rewards across four light input treatments (Fig. 1). Our system included one host plant (*A. vineale*) and two AM fungal species, *C. candidum* (NC172, INVAM) and *G. margarita* (NC175, INVAM), which were grown in two plastic pots (2 ⅜″ × 5″ short tree band from Anderson Die Co., Portland, OR, USA) connected by duct tape (Fig. 1a). Roots of individual *A. vineale* plants were divided equally into two adjacent pots with *C. candidum* and *G. margarita* inoculated separately on different sides. Each side was filled from the bottom to the top with 120 ml of a sterile soil mix, 120 ml of a 1 : 5 mixture of single inoculum to sterile soil mix, and 60 ml of sterile soil mix. The ^{32}P and ^{33}P chambers (Fig. 1b) were buried on the two sides.

Shading treatments

In order to test whether P uptake and C allocation depend upon different light input in the split-root system, we set up four light input (shading duration) treatments (Fig. 1c). The four light treatments included full light (HL), short shading (SS), medium shading (MS), and long shading (LS).

A sunshade net (deep color nylon mesh) was used to cut out 50% of the full light input. The photosynthetic active radiation (PAR) under full light averaged 1339 ± 71 μmol m^{-2} s^{-1}, and after the 50% shading treatment it was 662 ± 47 μmol m^{-2} s^{-1} ($n = 20$). The HL treatment, as a control, was not shaded. The light availability in this treatment reached a peak of 1806 μmol m^{-2} s^{-1} at midday. As shown in Fig. 1(c), the LS treatment shaded the plants from the beginning of the experiment (week 1) to the harvest (week 8), a period of 8 wk; the MS treatment shaded the plants from week 3 to the harvest, a period of 6 wk; and the SS treatment shaded the plants from week 5 to the harvest, a period of 4 wk.

P and C labelling

We used radioactive labelling to investigate plant C allocation and AM fungi P uptake. The ^{14}C labelling method was as described by Bever *et al.* (2009). The plants were allowed to establish effective symbioses for 4 wk before ^{14}C labelling. We labelled ^{14}C on day 31, day 38 and day 45 and then harvested on day 56. At each labelling time, $^{14}CO_2$ was released into bags containing the *Allium* shoots by mixing 5 μCi (185 Bq, each labelling time) of ^{14}C-sodium bicarbonate (37 mBq per mCi; Perkin Elmer, Waltham, MA, USA) with a few drops of 42% lactic acid into a cuvette within the bag. Plants were pulsed with $^{14}CO_2$ for 30 min before the bags were removed.

The roots of the host plant on both sides were associated with *C. candidum* or *G. margarita* and were labelled with ^{32}P (37 mBq ml^{-1} HCl-free water; Perkin Elmer) or ^{33}P (37 mBq ml^{-1} HCl-free water; Perkin Elmer) in the form phosphoric acid. A PVC chamber (Fig. 1b) containing *c.* 15 ml of ^{32}P or ^{33}P radioactive-labelled soil (0.925 mBq per pot) and 10 ml buffer soil was buried on each side at the experimental setup. A plastic cap was used to seal one side, and root mesh (2 mm) was used to cover the buffer soil side. This mesh allows roots and hyphae access to the label. Previous tests found there was no difference between P uptake in mesh that allowed roots and mesh that excluded roots and only allowed hyphae. The mesh-covered side of the PVC chamber was inclined upward to prevent radioactive leakage with watering.

Eight replicates of each treatment were used, four with ^{32}P on the *C. candidum* side and four with ^{32}P on the *G. margarita* side. The reverse labelling allowed us to statistically control for any possible differential uptake or differential detection of the two P isotopes. All plants were irrigated with tap water daily. All experiments were conducted in a glasshouse at Indiana University (Bloomington, IN, USA).

Harvesting and sample analysis

The bulbs and shoots were harvested, oven-dried (70°C for 72 h), weighed and digested together as total shoots to analyse the uptake of P radioisotopes. The total shoots were digested in 10 ml nitric acid, and 1 ml solution was then transferred into a standard scintillation vial which consisted of 10 ml Bio-safe II cocktail (Research Products International Corp., Mt Prospect, IL, USA) and 4 ml di-H_2O. This mixed solution was used to analyse the contents of ^{32}P and ^{33}P by liquid scintillation counting (Perkin Elmer). The initial contents of ^{32}P and ^{33}P were determined by correcting their scintillation counts for isotopic decay. Formulas for calculating the ^{32}P and ^{33}P contents were obtained from standard curves constructed from test samples with individual radiolabels. These calculations also gave us measurements of ^{14}C in the shoots.

As Bever *et al.* (2009) obtained similar results from measurements of C label in roots as they did from measurements of C label in external AM hyphae, we focused our effort on measurements of C allocation to roots. Each root sample was oven-dried (70°C for 72 h), weighed and oxidized (Harvey Biological Materials Oxidizer, OX-400; Harvey Biological, Hillsdale, NJ, USA). The released $^{14}CO_2$ was trapped in 10 ml C-14 cocktail (Harvey Biological) and analysed by liquid scintillation counting (Bever *et al.*, 2009).

Growth assay

We grew eight replicates of *Allium* with *C. candidum*, *G. margarita* or uninoculated control in the same four shading treatments. Seedlings were transplanted from Metromix to single pots after growing for 6 wk. The same size pot (2 ⅜″ × 5″) for each treatment was filled from the bottom to the top with 140 ml of the sterile soil mix, 120 ml of a 1 : 5 mixture of single inoculum to sterile soil mix (or sterile soil: sand mix for control), and 80 ml of sterile soil mix. At harvest, all plant shoots and roots were oven-dried at 70°C for 72 h before weighing.

Analysis

The *Allium* growth responses to inoculation, shading duration and their interactions were analysed using the general linear model (GLM) procedure of SAS 9.2 (SAS Institute Inc., Cary, NC, USA). *Allium* weights were log-transformed before analysis to achieve homogeneity of variance, and initial leaf lengths were used as covariates to remove any variation resulting from initial size. Following analysis, the mycorrhizal growth response (MGR) was calculated as MGR = AM/Ctrl, where AM and Ctrl are the estimated marginal means of the total biomass of mycorrhizal and control plants, respectively. The standard error of the ratio was calculated according to Hinkley (1969).

The preferential C allocation was measured as ^{14}C allocation = $C_{Claroideoglomus}/C_{Gigaspora}$, where $C_{Claroideoglomus}$ is the concentration (mBq g^{-1} DW of root) of ^{14}C in roots associated with *Claroideoglomus*, and $C_{Gigaspora}$ is the concentration of ^{14}C in roots associated with *Gigaspora*. We did not observe any differences in the mass of roots between the two sides of the split-root pots. A ratio of 1 indicates that the allocation is unbiased, whereas a ratio > 1 indicates preferential allocation. The metric was log-transformed before analysis, so that preferential allocation to the most beneficial mutualist was detected as log ratios > 0.

For measurements of ^{14}C in the plant shoot, we calculated the proportion of plant total labelled C allocated to roots as a whole, as well as to roots on the *Claroideoglomus* and *Gigaspora* sides specifically. These measures were arcsine-square-root-transformed before analysis.

Differential P uptake was measured as $P_{Claroideoglomus}/P_{Gigaspora}$, where $P_{Claroideoglomus}$ is the P delivered from roots inoculated with *Claroideoglomus* and $P_{Gigaspora}$ is the P delivered from roots inoculated with *Gigaspora*. Differential C allocation and P uptake are best analysed using the ratio because it controls for different efficiencies of labelling between replicates. We report the absolute values in the Supporting Information, Tables S1–S3.

Metrics of C allocation ratio, the proportion of C allocated, and differential P uptake ratio were analysed using general linear models. The ratio of the root masses on the *Claroideoglomus* and *Gigaspora* sides was used as a covariate in analyses of P uptake, and P labelling orientation (^{32}P vs ^{33}P associated with *Claroideoglomus*) was used as a predictor to remove label bias. Samples in which labelling was not above background levels were excluded from the analyses. For P labelling, samples were excluded when the radioactivity of ^{32}P or ^{33}P in the shoot was below 0.008335 mBq. For C labelling, samples were excluded when root DWs on either side were < 0.001 g and the radioactive count of total C in roots was < 20 004 mBq. As a result, 15 and 28 data points were analysed for P uptake and C allocation, respectively.

Results

Growth assay

Overall, *C. candidum* significantly improved the growth of *A. vineale*, while *G. margarita* did not ($F_{1,41} = 183.25$, $P < 0.0001$, Fig. 2a). Plant growth also varied among different shading treatments ($F_{3,41} = 9.58$, $P < 0.0001$, Fig. 2a). The shoot

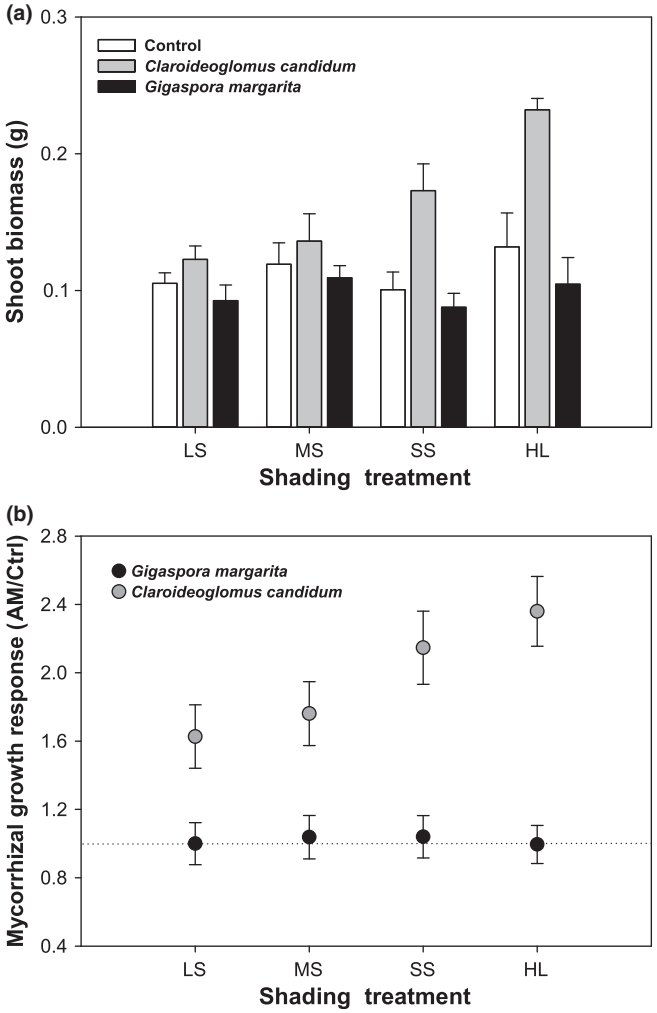

Fig. 2 Growth response of host plant *Allium vineale* to arbuscular mycorrhizal (AM) fungi *Claroideoglomus candidum* and *Gigaspora margarita* across four different shading treatments (HL, high light; LS, long shading; MS, medium shading; SS, short shading). (a) Shoot biomass of all growth response plants. (b) Mycorrhizal growth response was calculated as AM/Ctrl, where AM and Ctrl are the shoot biomasses of mycorrhizal plants and nonmycorrhizal plants, respectively. The dotted line represents a ratio of 1. Estimated marginal means ± SE.

biomass of *A. vineale* was significantly greater when growing with *C. candidum* ($F_{1,41} = 127.28$, $P < 0.0001$, Fig. 2a) than with *G. margarita* ($F_{1,41} = 0.07$, $P = 0.80$, Fig. 2a) as compared with sterile soil across all shading treatments. *Gigaspora* did not promote plant growth ($F_{3,41} = 0.04$, $P = 0.99$, Fig. 2a) across any shading treatments. As shown in Fig. 2(b), the mycorrhizal growth response of *A. vineale* to *C. candidum* was positive across all shading treatments and linearly decreased as the light input reduced ($F_{1,41} = 22.41$, $P < 0.0001$).

P uptake and C allocation

Roots inoculated with *C. candidum* provided more P to the host plant across all shading treatments, because the ratios of P uptake from *Claroideoglomus* vs *Gigaspora* were significantly greater than 1 ($F_{1,7} = 6.50$, $P = 0.04$). Under HL conditions, *C. candidum* delivered an average of 6.25 times more P to the host than did *G. margarita* (Fig. 3). With the light input reduced as a result of shading, the ratios of P uptake between *Claroideoglomus* and *Gigaspora* decreased to 3.51 (SS), 1.22 (MS) and 1.79 (LS). However, these ratios were not significantly different between shading and nonshading treatments ($F_{1,7} = 2.36$, $P = 0.17$).

There were no significant differences in the quantity of roots within pots inoculated with *Claroideoglomus* vs *Gigaspora* ($F_{1,17} = 0.76$, $P = 0.66$). However, the host plant allocated more C to *C. candidum* than to *G. margarita* across all shading treatments ($F_{1,17} = 6.92$, $P = 0.02$, Fig. 4). Although there was no significant difference in the ratios of C allocated to roots inoculated on the *Claroideoglomus* vs *Gigaspora* sides between nonshading and shading treatments ($F_{1,17} = 1.97$, $P = 0.17$), the average ratios were 9.86 when the plant was not shaded, significantly greater than 1($F_{1,17} = 9.00$, $P = 0.01$). The average ratios declined with shading to values that were not significant from 1, with 3.14, 2.51 and 2.04 for 4 (SS), 6 (MS), and 8 (LS) wk, respectively (Fig. 4).

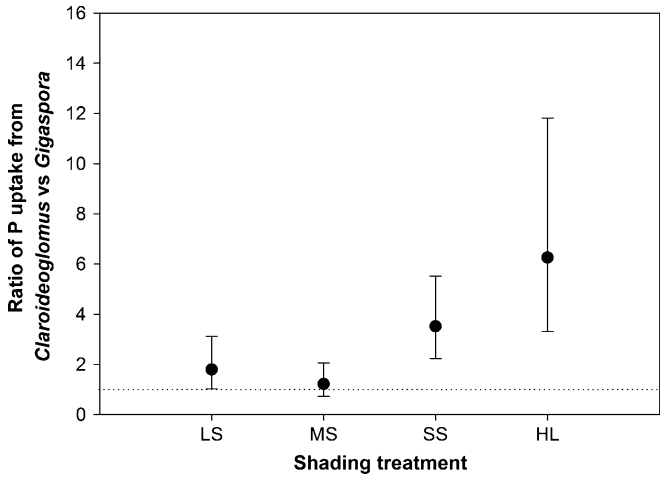

Fig. 3 Ratios of radioactive-labelled phosphorus (P) rewarded to the host plant (*Allium vineale*) from the *Claroideoglomus* side vs that from the *Gigaspora* side across four different shading treatments (HL, high light; LS, long shading; MS, medium shading; SS, short shading). The dotted line represents a ratio of 1. Means ± SE were estimated by back transformation of marginal mean log ratios.

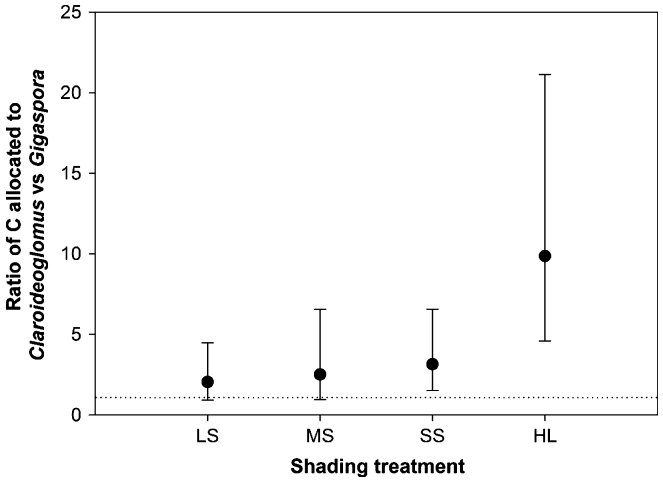

Fig. 4 Ratios of radioactive-labelled carbon allocated by the host plant (*Allium vineale*) to the *Claroideoglomus* side vs that allocated to the *Gigaspora* side across four different shading treatments (HL, high light; LS, long shading; MS, medium shading; SS, short shading). The dotted line represents a ratio of 1. Means ± SE were estimated by back transformation of the marginal mean log ratios.

The proportion of total plant C allocated to roots relative to shoot was not affected by shading ($F_{1,15} = 0.59$, $P = 0.4$). However, the proportion of total plant C allocated to infected roots on the *C. candidum* side significantly declined with shading ($F_{1,15} = 5.68$, $P = 0.03$), whereas the proportion of C allocated to the *G. margarita* side did not change in relation to shading ($F_{1,15} = 0.22$, $P = 0.6$). As shown in Fig. 5, the proportion of total plant C allocated to *C. candidum* declined from 25.0% on average without shading to 15.4, 17.5, and 14.8% for plants shaded for 4 wk (SS), 6 wk (MS), and 8 wk (LS), respectively. The proportion of total plant C allocated to roots infected with *G. margarita* averaged *c.* 11.0% with no apparent trend with shading.

Discussion

We found that plant growth response to a growth-promoting AM fungus declined with shading, confirming previous work (Son & Smith, 1988), but that shading does not alter plant growth response to nonbeneficial mycorrhizal fungus. We found that the plant received greater P from the growth promoter, but that this differential P-uptake declined with shading. While previous work showed that shading reduced investment in AM fungi, here we show for the first time that plant preferential allocation to the most beneficial mycorrhizal fungus also declines with shading. This distinction is critical to the dynamics of AM fungi, as preferential allocation can stabilize the mycorrhizal mutualism against the proliferation of nonbeneficial fungi (Bever *et al.*, 2009; Kiers *et al.*, 2011). The dependence of preferential allocation on light availability has important implications for understanding the environmental dependence of the evolution of mycorrhizal mutualism.

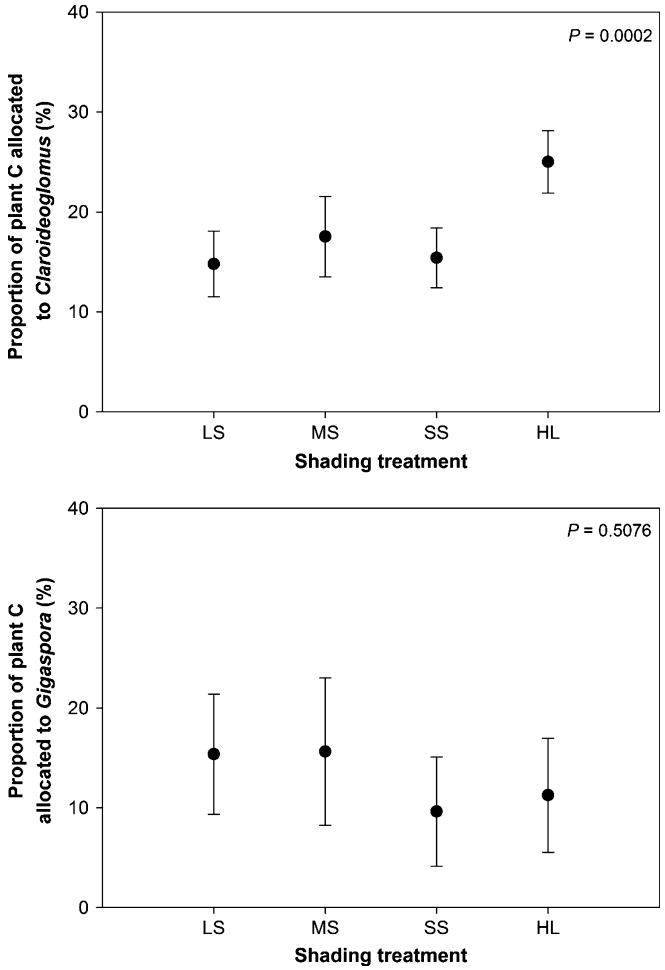

Fig. 5 Proportion of plant total carbon (C) allocated to the *Claroideoglomus* side or the *Gigaspora* side across different shading treatments (HL, high light; LS, long shading; MS, medium shading; SS, short shading). Estimated marginal means ± SE.

As essential resources, C and P can be limiting in nature. The balance of these two resources within a stoichiometric and functional equilibrium model has been suggested as a framework to understand the stability of mycorrhizal mutualism (Chapin *et al.*, 1987; Hoeksema & Schwartz, 2003; Johnson, 2010). When light or carbon dioxide (CO_2) is most limiting, plants should allocate less C below ground to adjust resource limitation imbalances (Johnson *et al.*, 2003, 2008). In this study, shading did not induce shifts by *A. vineale* in allocation to roots as a whole. However, allocation to the beneficial fungus was sensitive to shading, as it declined from 25 to 15% with declining light levels. By contrast, allocation to the nonbeneficial fungus was not sensitive to changes in resource quantities. The decline in preferential allocation to the most beneficial mutualist could allow the nonbeneficial fungi, which do not pay the costs of mutualism, to increase their relative abundance in low light environments. Such a proliferation would generate environmental patterns consistent with stoichiometric models that do not include mechanisms for controlling the spread of cheaters (Hoeksema & Schwartz, 2003). We note that while this study compared beneficial and nonbeneficial fungi, it would be interesting to compare allocation to beneficial AM fungi that vary in their degree of growth promotion.

Our manipulation of the duration of shading allowed evaluation of the immediacy of the effect of shading on plant allocation and uptake. C allocation, which we measured over the final 3 wk of the experiment, appeared to be unaffected by the duration of shading that preceded the labeling (Figs 3, 4). We expected that differential P uptake would mirror C allocation; however, we measured differential P uptake over the entire experiment, which generated a more continuous response to duration of shading, consistent with a temporal averaging of the shading environment. Further work is necessary to evaluate the immediacy of the plant–fungal response to shading.

Our observation of light levels influencing preferential allocation could be relevant to the direction of change of mycorrhizal mutualism in response to changes in other above-ground resources, such as atmospheric CO_2. Rising concentrations of atmospheric CO_2 have been shown to increase plant investment in mycorrhizas (Garcia *et al.*, 2008; Compant *et al.*, 2010; Drigo *et al.*, 2010). Our work suggests that there should be increased allocation to the most beneficial mycorrhizal mutualist, perhaps increasing the efficiency of mycorrhizal mutualism. Consistent with this expectation, Klironomos *et al.* (2005) observed an increase in benefit of mycorrhizal plants from their soil communities with elevation of atmospheric CO_2. Increased efficiency of mutualism as a result of preferential allocation could contribute to explaining the sustained plant growth stimulation observed in long-term manipulations of atmospheric CO_2 (Drake *et al.*, 2011). Further work is needed to directly evaluate plant preferential allocation across gradients of atmospheric CO_2.

The balance of C costs and P benefits is a key factor in predicting the outcome of AM symbioses (Fitter, 2006; Smith & Read, 2008; Johnson, 2010). Our results provide evidence that C allocation is correlated with P benefit, as both preferential allocation of C to the most beneficial mutualist and differential uptake of P from the most beneficial mutualist declined with shading. Moreover, this shift in preferential allocation with light levels provides a mechanism bridging the predictions of the environmental dependence of mutualisms from stoichiometry models to population models of persistence of mutualism in the face of cheaters. As such, this represents a significant contribution towards a predictive framework of the stability and efficiency of the mycorrhizal mutualism across above-ground and below-ground resource gradients. Our work specifically predicts that the mycorrhizal mutualisms should become more effective precisely under those conditions in which they are most useful to the plant, possibly contributing to observations of local adaptation of the fungi to their local soils (Johnson *et al.*, 2010).

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References

Bennett AE, Bever JD. 2009. Trade-offs between arbuscular mycorrhizal fungal competitive ability and host growth promotion in *Plantago lanceolata*. *Oecologia* **160**: 807–816.

Bever JD. 2002. Negative feedback within a mutualism: host–specific growth of mycorrhizal fungi reduces plant benefit. *Proceedings of the Royal Society B: Biological Sciences* **269**: 2595–2601.

Bever JD, Morton JB, Antonovics J, Schultz PA. 1996. Host-dependent sporulation and species diversity of mycorrhizal fungi in a mown grassland. *Journal of Ecology* **75**: 1965–1977.

Bever JD, Richardson SC, Lawrence BM, Holmes J, Watson M. 2009. Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecology Letters* **12**: 13–21.

Bever JD, Schultz PA, Pringle A, Morton JB. 2001. Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. *BioScience* **51**: 923–932.

Boerner REJ. 1990. Role of mycorrhizal fungus origin in growth and nutrient uptake by *Geranium robertianum*. *American Journal of Botany* **77**: 483–489.

Boucher DH, James S, Keeler KH. 1982. The ecology of mutualism. *Annual Review of Ecology and Systematics* **13**: 315–347.

Bronstein JJ. 2001. The exploitation of mutualisms. *Ecology Letters* **4**: 277–287.

Chapin FS III, Bloom AJ, Field CB, Waring RH. 1987. Plant responses to multiple environmental factors. *BioScience* **37**: 49–57.

Clark AL, St Clair SB. 2011. Mycorrhizas and secondary succession in aspen-conifer forest: light imitation differentially affects a dominant early and late successional species. *Forest Ecology and Management* **262**: 203–207.

Compant S, van der Heijden MGA, Sessitsch A. 2010. Climate change effects on beneficial plant-microorganism interactions. *FEMS Microbiology Ecology* **73**: 197–214.

Denison RF, Kiers ET. 2004. Lifestyle alternatives for rhizobia: mutualism, parasitism, and forgoing symbiosis. *FEMS Microbiology Letters* **237**: 187–193.

Denison RF, Kiers ET. 2011. Life histories of symbiotic rhizobia and mycorrhizal fungi. *Current Biology* **21**: 775–785.

Drake JE, Gallet-Budynek A, Hofmockel KS, Bernhardt ES, Billings SA, Jackson RB, Johnsen KS, Lichter J, McCarthy HR, McCormack ML *et al.* 2011. Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. *Ecology Letters* **14**: 349–357.

Drigo B, Pijl AS, Duyts H, Kielak AM, Gamper HA, Houtekamer MJ, Boschker HTS, Bodelier PLE, Whiteley AS, van Veen JA *et al.* 2010. Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO_2. *Proceedings of the National Academy of Sciences, USA* **107**: 10938–10942.

Fitter AH. 2006. What is the link between carbon and phosphorus fluxes in arbuscular mycorrhizas? A null hypothesis for symbiotic function. *New Phytologist* **172**: 3–6.

Garcia MO, Ovasapyan T, Greas M, Treseder KK. 2008. Mycorrhizal dynamics under elevated CO_2 and nitrogen fertilization in a warm temperate forest. *Plant and Soil* **303**: 301–310.

Gehring CA. 2003. Growth responses to arbuscular mycorrhizae by rain forest seedlings vary with light intensity and tree species. *Plant Ecology* **167**: 127–139.

Goto R, Okamoto T, Kiers ET, Kawakita A, Kato M. 2010. Selective flower abortion maintains moth cooperation in a newly discovered pollination mutualism. *Ecology Letters* **13**: 321–329.

Hinkley DV. 1969. On the ratio of two correlated normal random variables. *Biometrika* **56**: 635–639.

Hoeksema JD, Bruna EM. 2000. Pursuing the big questions about interspecific mutualism: a review of theoretical approaches. *Oecologia* **125**: 321–330.

Hoeksema JD, Schwartz MW. 2003. Expanding comparative–advantage biological market models: contingency of mutualism on partner's resource requirements and acquisition trade-offs. *Proceedings of the Royal Society B: Biological Sciences* **270**: 913–919.

Hofland-Zijlstra JD, Berendse F. 2009. The effect of nutrient supply and light intensity on tannins and mycorrhizal colonisation in Dutch heathland ecosystems. *Plant Ecology* **201**: 661–675.

Hunt R, Hope-Simpson JF. 1990. Growth of *Pyrola rotundifolia* ssp. *maritima* in relation to shade. *New Phytologist* **114**: 129–137.

Hurst SE, Turnbull MH, Norton DA. 2002. The effect of plant light environment on mycorrhizal colonization in field-grown seedlings of podocarp-angiosperm forest tree species. *New Zealand Journal of Botany* **40**: 65–72.

Johnson NC. 2010. Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytologist* **185**: 631–647.

Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist* **135**: 575–585.

Johnson NC, Hoeksema JD, Bever JD, Chaudhary VB, Gehring CA, Klironomos JN, Koide R, Miller RM, Moore J, Moutoglis P *et al.* 2006. From Lilliput to Brobdingnag: extending models of mycorrhizal function across scales. *BioScience* **56**: 889–900.

Johnson NC, Rowland DL, Corkidi L, Allen EB. 2008. Characteristics of plant winners and losers in grassland eutrophication-importance of allocation plasticity and mycorrhiza function. *Ecology* **89**: 2868–2878.

Johnson NC, Rowland DL, Corkidi L, Egerton-Warburton L, Allen EB. 2003. Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. *Ecology* **84**: 1895–1908.

Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proceedings of the National Academy of Sciences, USA* **107**: 2093–2098.

Jones MD, Smith SE. 2004. Exploring functional definitions of mycorrhizas: are mycorrhizas always mutualisms? *Canadian Journal of Botany* **82**: 1089–1109.

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A *et al.* 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* **333**: 880–882.

Kiers ET, van der Heijden MGA. 2006. Mutualistic stability in the arbuscular mycorrhizal symbiosis: exploring hypotheses of evolutionary cooperation. *Ecology* **87**: 1627–1636.

Klironomos JN, Allen MF, Rillig MC, Piotrowski J, Makvandi-Nejad S, Wolfe BE, Powell JR. 2005. Abrupt rise in atmospheric CO_2 overestimates community response in a model plant-soil system. *Nature* **433**: 621–624.

Louis I, Lim G. 1988. Effect of storage of inoculum on spore germination of a tropical isolate of *Clomus clarum*. *Mycologia* **80**: 157–161.

Millar JA, Ballhorn DJ. 2013. Effect of mycorrhizal colonization and light limitation on growth and reproduction of lima bean (*Phaseolus lunatus* L.). *Journal of Applied Botany and Food Quality* **86**: 172–179.

Morton JB, Bentivenga SP, Wheeler WW. 1993. Germ plasm in the international collection of arbuscular and vesicular-arbuscular mycorrhizal fungi (INVAM) and procedures for culture development, documentation and storage. *Mycotaxon* **48**: 491–528.

Sachs JL, Simms EL. 2006. Pathways to mutualism breakdown. *Trends in Ecology and Evolution* **21**: 585–592.

Schwartz MW, Hoeksema JD. 1998. Specialization and resource trade: biological markets as a model of mutualisms. *Ecology* **79**: 1029–1038.

Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis*. New York, NY, USA: Academic Press.

Soares MC, Côté IM, Cardoso SC, Bshary R. 2008. The cleaning goby mutualism: a system without punishment, partner switching or tactile stimulation. *Journal of Zoology* **276**: 306–312.

Son CL, Smith SE. 1988. Mycorrhizal growth responses: interactions between photon irradiance and phosphorus nutrition. *New Phytologist* **108**: 305–314.

Tester M, Smith SE, Smith FA, Walker NA. 1986. Effects of photon irradiance on the growth of shoots and roots, on the rate of initiation of mycorrhizal infection and on the growth of infection units in *Trifolium subterraneum* L. *New Phytologist* **103**: 375–390.

Thompson JN. 2005. *The geographic mosaic of coevolution.* Chicago, IL, USA: University of Chicago Press.

Verbruggen E, Mouden CE, Jansa J, Akkermans G, Bücking H, West SA, Kiers ET. 2012. Spatial structure and interspecific cooperation: theory and an empirical test using the mycorrhizal mutualism. *The American Naturalist* **179**: 133–146.

Vogelsang KM, Bever JD. 2009. Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. *Ecology* **90**: 399–407.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Shoot and root DW of *Allium vineale* on the *Claroideoglomus candidum* side and the *Gigaspora margarita* side across all shading treatments

Table S2 P uptake (mBq) from *Allium vineale* roots inoculated with *Claroideoglomus candidum* and *Gigaspora margarita* across all shading treatments

Table S3 Carbon counts in *Allium vineale* roots ($\times 10^7$) inoculated with *Claroideoglomus candidum* and *Gigaspora margarita* across all shading treatments

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