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Plant preferential allocation and fungal reward decline with soil phosphorus: implications for mycorrhizal mutualism

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Abstract. Explaining the persistence of mutualism remains a challenge in ecology and evolutionary biology. The evolutionary stability of arbuscular mycorrhiza, a most widespread and ancient mutualistic association, is particularly intriguing because plants lack apparent mechanisms to prevent cheaters from gaining competitive advantages over cooperators. We developed a triple isotopic labeling method (^{14}C, ^{32}P, and ^{33}P) within a split-root design to measure the exchange of carbon (C) and phosphorus (P) between the host plant and two mycorrhizal partners across a soil P gradient. Host plant preferentially allocated more C to the roots associated with the fungus delivering higher P per unit plant C, and the strength of preferential allocation decreased with increasing soil P availability. The host plant received more P per unit of allocated C from the better fungus and this advantageous exchange rate did not depend upon P availability. As a result, the level of preferential allocation was correlated with the differential delivery of P from the two fungi. Our findings suggest that plant preferential allocation to better mutualists can stabilize mutualisms in environments limiting in the traded resource, but as the availability of this resource increases, plant preferential allocation declines. This environmental dependence of preferential allocation generates predictions of declining levels in relative abundance of mutualistic fungi in high-resource environments.

Key words: arbuscular mycorrhiza; carbon; cheater; context dependence; mutualism; phosphorus; preferential allocation.

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Introduction

Mutualistic interactions between individuals of different species are ubiquitous in nature and have played indispensable roles in some key evolutionary transitions through the history of life (Janzen 1985). For example, the widespread mutualism between plants and arbuscular mycorrhizal (AM) fungi is thought to have been instrumental in plant's colonization of land (Redecker et al. 2000). However, the evolutionary stability of mutualisms, such as between plants and AM fungi, remains a major challenge in ecology and evolutionary biology. As the delivery of benefits from one mutualistic partner to the other is costly (Bronstein 2001), individuals that receive benefits from the mutualism but pay less or no costs ("cheaters") can have competitive advantage. This will be particularly problematic for mutualisms such as AM fungi, which are highly promiscuous with individual plants often hosting multiple fungal partners which vary in the benefits they provide (Johnson et al. 1997, Jones and Smith 2004). In fact, less beneficial fungi have been shown to be better competitors in mixture, as is consistent with having reduced

fitness costs relative to the more mutualistic fungi (Bever 2002, Castelli and Casper 2003, Bennett and Bever 2009, Bever et al. 2009). The processes preventing the degradation of mutualism could be important determinants of patterns of mutualism across the environment.

The AM mutualism may be stabilized by plants detecting the variation in the benefits delivered by different fungi, and then preferentially allocating more C to the more beneficial fungus. This possibility is supported by two recent studies. Bever et al. (2009) split roots of individual plants into two adjacent pots that were separately inoculated with two different fungi. In this one plant-to-two fungi system, host plants allocated more C toward the fungus that better promoted plant growth, thereby increasing the fitness of the better mutualists, and allowing the persistence of the mutualism in a spatially structured root system. However, this study did not identify what form of benefits the fungi delivered to the plants. Kiers et al. (2011) fertilized sections of hyphae of a beneficial fungus associated with transformed root cultures and found more C was allocated to the fungal hyphae fertilized with P. C allocation was unresponsive to P fertilization for nonbeneficial fungus. These findings implied that the resource base of plant C allocation could be the P, but the one-to-one system used in this study was not designed for testing preferential allocation of C to the better fungus.

Identifying the relevant resource which triggers plant preferential allocation to the better fungus would be important because it may allow predictions of patterns of preferential allocation across environmental gradients. AM fungi are functionally diverse microbes, but their most prominent function is to increase plant's access to soil P (Jakobsen et al. 1992, Smith et al. 2003). Mycorrhizal P uptake is known to be environment-dependent. For example, when soil P is abundant, the host plant receives little or no benefit from fungi and C allocation to mycorrhizae may decrease. The degree of preferential allocation to the most effective mutualist might be expected to decrease with the increase in soil P content as well. Preferential allocation has been shown to decline with decreasing above ground resource due to restricted light availability (Zheng et al. 2015). Such context dependence of preferential allocation could regulate fungal population dynamics and provide a mechanistic basis for the prediction of the direction in evolution of AM functioning across an environmental gradient (Bever 2015).

In this study, we evaluate the resource base for and environmental dependence of plant preferential allocation to the most effective mutualist. The major difficulty in evaluating the resource base of preferential allocation of plant C relates to the simultaneously tracking and quantification of the degree of plant C allocation and the variation in the benefits delivered by different fungi on a single plant. Here, we present a novel triple labeling (^{14}C, ^{32}P, and ^{33}P) approach to track and quantify the bi-directional exchange of C and P between host plant and two different fungal partners across a soil P nutrient gradient. We employed this method to evaluate plant preferential allocation and the differential in preferential allocation across a soil P nutrient gradient. We tested if the plant can detect the variation in the benefits delivered by the two fungi, and then preferentially allocate more C to fungus providing more P. Secondly, we tested whether preferential allocation of plant C depends upon environmental P availability. Finally, with our simultaneous measures of C and P, we are able to evaluate whether the relative exchange rate of the two fungi are modified by environment.

Methods

Plant, fungi, and soil

All the seeds, AM fungi and soil used in this study were obtained from the same field as described in previous studies (Bever et al. 1996, 2009, Zheng et al. 2015). The site, located at Duke University (Durham, North Carolina, USA), represents a typical old-field habitat commonly found in temperate regions throughout the Eastern and Midwestern United States. The soil in that area is a sandy loam of the White Store series with low pH and low P contents (Fowler and Antonovics 1981). The host plant (*Allium vineale* L.) we chose is a cool season perennial naturally co-occurring with multiple AM fungal species including *Claroideoglomus candidum* (formerly named as *Glomus candidum*) and *Gigaspora margarita*. Previous results had identified that *C. candidum* promoted the growth of *A. vineale* while *G. margarita* does not (Bever

et al. 2009). Moreover, *A. vineale* was shown to be able to preferentially allocate their fixed C to the beneficial *C. candidum.* Both fungi have been deposited in INVAM (NC172 and NC175, respectively). Single species fungal cultures were maintained on *Sorghum bicolor* in a greenhouse at Indiana University (Bloomington, Indiana, USA).

Plant seeds and field soil were collected in the summer of 2010 and stored in cold condition until use. In early spring of 2011, *Allium* plants were germinated and grown from seeds in sterile Metromix (Hummert International, Earth City, Missouri, USA) for 4 weeks before transplanting to experimental pots. Field collected soil was mixed with sand in a 1:1 (volume/volume) ratio and then autoclaved at 121 °C for 2 h (1 h each time for two consecutive days) for potting. Potting soil prepared in this way contains 73 mg/kg total N and 6 mg/kg soil available P as measured in Mehlich 3 extracts using the colorimetric method. Prior to the experimental setup, single species cultures of *C. candidum* and *G. margarita* consisting of spores, mycelium, and fine root segments were air dried, chopped, and thoroughly mixed with the potting soil in a 1:5 (volume/volume) ratio for use as fungal inocula. Previous data showed that inocula of both fungi prepared in this way had similar inoculum potential (Bever et al. 2009).

Growth assay

To validate our assumption that the primary benefit *A. vineale* receives from the fungi is the enhanced P uptake and this benefit depends on the availability of P in the background soil, we conducted a growth assay to examine the mycorrhizal responses of host plants to each fungus across a gradient of background soil P levels. Growth assay experiment consisted of three fungal treatments (*C. candidum*, *G. margarita*, and non-AM control) and four levels of soil P treatments (1×, 2×, 3×, and 4× of background P level), each treatment combination being replicated six times for a total of 72 pots. In early March of 2011, 4-week-old seedlings of *A. vineale* were transplanted to square plastic pots (13 cm tall by 6 cm wide; Anderson Die Co., Portland, Oregon, USA). Pots were filled from bottom to top with 140 cm^3 potting soil, 120 cm^3 fungal inoculum of *C. candidum*, *G. margarita*, or non-AM control and 80 cm^3 potting soil. In non-AM control pots, the inoculum was the mixture of half volume of sterilized *C. candidum* inoculum and half volume of *G. margarita* inoculum. We added 10 mL of pooled bacterial wash prepared from both fungal inocula to all pot to eliminate possible confounding effects of non-AM microbes. Bacterial wash was prepared by blending soil and water in a 1:2 ratio and passing the slurry through a 20-μm sieve. The gradient of background P levels was achieved by fertilizing the pots with 25 mL calcium phosphate solution containing different amount of P during the fourth week of plant growth. After fertilization, P concentrations in the pots corresponding to 1×, 2×, 3×, and 4× of background P treatments were 6, 12, 18, and 24 mg/kg, respectively. Plants were grown in a greenhouse at Indiana University, Bloomington, Indiana for 9 weeks (early March to late May of 2011). Greenhouse light was maintained above photosynthetically active radiation by either artificial light or ambient sunlight for at least 12 h/d. Plants were watered daily with tap water.

At the end of the experiment, plant shoots and roots were harvested, oven dried at 70 °C for 72 h before being weighed. Shoots of a subset of three replicates for each treatment combination were analyzed for total P content using a modified wet digestion procedure and colorimetric analysis (Sommers and Nelson 1972). Dried shoots were weighted, cut into small fragments, and digested with nitric acid (70%) at 127 °C for 8 h and perchloric acid (70%) at 172 °C for 2 h in a block digestor. P concentrations were colorimetric-determined on the digest against standards.

Quantifying C allocation and P delivery across different soil P levels

To simultaneously measure plant C allocation and fungal P rewards in a one plant-to-two fungi system across different levels of soil P, we first set up a split-root system similar as in Bever et al. (2009). We then employed a novel radioactive labeling approach to track and quantify the differential in plant C allocated to *C. candidum* vs. *G. margarita*, and in the P benefit delivered by these fungi.

Split-root pots were set up in early March of 2011. Two square plastic pots (same size as used

in growth assay) were taped together side by side, and a PVC pipe was clipped over the center (Appendix S1: Fig. S1). Roots of a 4-week-old seedling were divided equally into these two pots, while the bulb of the plant securely sitting inside the PVC pipe. Pots were first filled with 120 cm^3 potting soil at the bottom, then a layer of 120 cm^3 fungal inoculum, and 60 cm^3 potting soil to cover the top. Each pair of pots had different inocula, so that a plant was inoculated with *C. candidum* on one side of the roots and *G. margarita* on the other side. Bacterial wash used in growth assay was added to each pot. Moist sand was added to the PVC pipe in order to prevent the bulb form desiccating.

During the split-root setup, two radioactive forms of P isotopes (^{32}P and ^{33}P) were separately introduced to two different sides of plant roots. To prevent radioactive leakage with watering we concealed ^{32}P and ^{33}P in PVC chambers (Appendix S1: Fig. S1B). One side of the PVC chamber was sealed with plastic, and the other side was covered with root mesh (2 mm openings) which allowed both roots and fungal hyphae to access the radioactively labeled P inside the chamber. The chamber was filled with 15 cm^3 potting soil labeled with 0.925 mBq ^{32}P or ^{33}P in the form of phosphoric acid (supplied in HCl-free water; Perkin Elmer, Waltham, Massachusetts, USA), and then covered with 5 cm^3 buffer soil on both sides. Chambers were buried at about 6 cm depth from the soil surface, and the mesh covered side was inclined upward (Appendix S1: Fig. S1A). Within each split-root unit, one fungal species was paired with ^{32}P in one pot and the other fungal species with ^{33}P in the other pot. The pairing of a fungal species with a radioactive P label was reversed in order to statistically control for any possible differential uptake or differential detection of the two P isotopes. Each P label-fungal species combination was replicated five times (i.e., 10 total replicates). This design was repeated with 1×, 2×, 3×, and 4× of background P on both sides, bringing the total number of split-root units to 40. Background P level manipulation was done by fertilizing the pots with different amounts of calcium phosphate solution during the fourth week of plant growth.

Labeling plants with ^{14}C was done during the last week of plant growth to minimize the confounding effects of respired ^{14}C signals. Previous work demonstrated significant preferential allocation a few days following labeling (Bever et al. 2009), we therefore pulse-labeled plants with ^{14}C-labeled CO_2 three times (2, 4, and 6 d before harvest). The labeling method for ^{14}C was similar as described by Bever et al. (2009). At each labeling time, we sealed the entire shoots of each plant with a plastic bag. We mixed 185 kBq of ^{14}C-labeled sodium bicarbonate (Perkin Elmer) with a few drops of 42% lactic acid into a cuvette inside the bag to release $^{14}CO_2$ for a 30-minute pulse labeling.

As we have previously observed similar results from measurement of C label in roots as in external hyphae (Bever et al. 2009), we only focus on the label in the roots in the present study. Upon harvest after 9 weeks of plant growth, shoots, roots grown with *C. candidum* and roots grown with *G. margarita* from the same split-root plant were kept separate. All these components were weighed after being oven dried at 70 °C for 72 h. To measure the ^{14}C content in roots, each root sample was combusted with an automated biological materials oxidizer (OX-400 model; R. J. Harvey Instrument Co., Hillsdale, New Jersey, USA). The released $^{14}CO_2$ was trapped in 10 mL C-14 Cocktail solution (R. J. Harvey Instrument Co.) and its radioactivity was measured as counts per minute (CPM) with a liquid scintillation counter (Packard Tri-Carb 1600TR; Perkin-Elmer Life Sciences, Inc., Boston, Massachusetts, USA). Scintillation counts were converted to ^{14}C contents based on a formula we constructed with a series of ^{14}C standards.

To analyze the ^{32}P, ^{33}P, and ^{14}C contents in shoots, the entire shoots were first digested in 10 mL nitric acid (70%) following a modified tissue digestion protocol (Zarcinas et al. 1987). Subsamples (1 mL) of the digests were mixed with 10 mL Bio-safe II cocktail (Research Products International Corp., Mt Prospect, Illinois, USA) and 4 mL di-H_2O in standard scintillation vials and analyzed by liquid scintillation counting (Packard Tri-Carb 1600TR; PerkinElmer Life Sciences, Inc.). Scintillation counts of three separate energy windows (0–156 keV, 156–250 keV, and 250–2000 keV) were recorded for each sample. These counts were used to calculate the contents of ^{32}P, ^{33}P, and ^{14}C based on a set of three formulas we constructed with ^{32}P, ^{33}P, and ^{14}C standards. To best minimize quenching effect, all standards

contained the same amount of plant digest (prepared with unlabeled plants), cocktail, and nitric acid as in samples. The contents of ^{32}P and ^{33}P at time of the experimental initiation were determined by correcting their scintillation counts for isotopic decay.

In the present study, radioactively labeled P was concealed in chambers which were sealed with plastic on one end, and were covered with root mesh on the other end. We referred to chambers covered with root mesh as "root chambers". During the development of our labeling approach a year prior to this study, we also constructed "hyphal chambers", a different type of chambers being covered with hyphal mesh instead of root mesh. The openings of hyphal mesh were 30 μm which allowed fungal hyphae get through but not roots. We tested the differences in plant C allocation and fungal P reward when P labels were introduced with either "root chambers" or "hyphal chambers" with parallel experiments same as described in the present study. We found from these trial experiments across different soil P levels, the differential in labeled P uptake from *C. candidum* roots vs. *G. margarita* roots did not vary between these two chamber types. The same pattern was true for the preferential allocation of labeled C. The absence of difference between "root chambers" and "hyphal chambers" indicated that hyphae were equally involved in accessing the soil P in both cases, which is consistent with *A. vineale* having very limited ability to uptake soil P in the absence of its symbiosis with AM fungi. On the basis of this result, we chose to use chambers that allowed root and hyphae access as this did not exclude different fungal approaches to increasing P availability (Pearson and Jakobsen 1993, Smith et al. 2000).

Data analysis

Plant total biomass data (roots and shoots), P concentration, and total P content obtained from the growth response experiment were analyzed with an ANOVA to assess the effects of fungal species, soil P levels, and their interaction. Plant biomass and total plant P were log-transformed prior to analysis to achieve homogeneity of variance. Mycorrhizal growth response was calculated as the ratio (AM/Ctrl) where AM is the estimated marginal mean of total biomass of mycorrhizal plant and Ctrl is the estimated marginal mean of total biomass of control plant. The standard error of the ratio was calculated following Hinkley (1969).

Differences in C allocation and P uptake within each split-root unit were measured using a metric of the ratio of radioactive C or P from the *C. candidum* over that from *G. margarita*. Differential C allocation and P uptake are best analyzed using the ratio because it controls for different efficiencies of labeling between replicates. We report the absolute values in Appendix S2 (Tables S1–S3).

There were no significant differences in the amount of roots on each side and therefore, for tests of preferential allocation of C, we measured C_C/C_G as an estimate of the preferential C allocation to better fungus, where C_C is the concentration of ^{14}C in roots associated with *C. candidum* and C_G is the concentration of ^{14}C in roots associated with *G. margarita*. The differential in P uptake was measured as P_C/P_G, where P_C and P_G are the amounts of radioactively labeled P delivered from *C. candidum* and *G. margarita* side, respectively. The metrics were log-transformed prior to analysis in ANOVA and then tested for differences from zero. Note that a ratio of >1 indicates preferential allocation (or differential P uptake) and that the log of 1 is zero. For analyses of P uptake, root mass ratios were used as covariates and radioactive label form was also used as a factor to remove effects of detection bias. We also calculated the proportion of ^{14}C in the entire plant allocated to roots infected with *C. candidum* or *G. margarita.* These proportions were Logit transformed for the ANOVA.

Based on the measurements of P_C/P_G and C_C/C_G within each split-root plant, we tested if the host plant received more P per unit of allocated C from the better fungus across the soil P gradient. The ratio of P delivery per unit of allocated C by *C. candidum* vs. *G. margarita* was expressed as $(P_C/C_C)/(P_G/C_G)$, same as $(P_C/P_G)/(C_C/C_G)$. These ratios were calculated, log-transformed prior to analysis in ANOVA and then tested for differences from zero (> 0 indicates *C. candidum* delivers more P per unit of allocated C) and differences among four P fertilization levels.

All ANOVA analyses were performed using the GLM procedure of SAS 9.2 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Growth of the host plant *A. vineale* varied significantly among the three fungal treatments (inoculation with *C. candidum*, *G. margarita*, and non-AM control) ($F_{2,40}$ = 140, P < 0.0001) and among different soil P levels ($F_{3,40}$ = 34.6, P < 0.001) (Appendix S2, Fig. S1). Across all levels of environmental P (1×, 2×, 3×, and 4× of background P level), *A. vineale* grew significantly larger with *C. candidum* than with *G. margarita* ($F_{1,40}$ = 210, P < 0.001) and with sterile soil ($F_{1,40}$ = 213, P < 0.001). *G. margarita* did not promote plant growth ($F_{1,40}$ = 0.01, P = 0.91). As shown in Fig. 1a, plant growth response ratio to *C. candidum* was significantly >1 at all P levels, but linearly declined as the P level increased ($F_{1,40}$ = 5.78, P = 0.02).

The P concentration in the shoots was strongly affected by AM fungal inoculation ($F_{2,24}$ = 50.6, P < 0.001), with it being highest with *C. candidum* and lowest in the control (Appendix S2, Fig. S2). Interestingly, plant P concentration was greater when inoculated by *G. margarita* than in the uninoculated control ($F_{1,24}$ = 7.67, P = 0.01). The P concentration in plant shoots also increased with P fertilization ($F_{3,24}$ = 3.81, P = 0.02), but the interaction with AM fungal inoculation was not significant ($F_{6,24}$ = 0.53, P = 0.88).

Within the split-root system where half of *A. vineale* roots were colonized by *C. candidum* and the other half by *G. margarita*, *Gl. candidum* provided more P to the host plant than *G. margarita* at all four soil P levels as the ratios of radioactive-labeled P transferred from *C. candidum* roots vs. *G. margarita* roots were significantly >1 ($F_{1,13}$ = 450, 214, 43.4, and 107, for soil P = 1×, 2×, 3×, and 4× of background level, respectively, P < 0.0001 for all cases). These ratios also varied significantly among different soil P levels ($F_{3,13}$ = 11.91, P < 0.001). As shown in Fig. 1b, at the lowest soil P level (background P level, P = 1×), *C. candidum* delivered an average of 16.12 times more P to the host than the nonbeneficial *G. margarita*. As the environmental soil P increased to 2×, 3×, and 4× of the background level, the ratios decreased to 11.54, 5.30, and 5.50 times, respectively.

While there were no significant differences in the amounts of roots on the two sides ($F_{1,28}$ = 0.14, P = 0.71), we observed preferential allocation of C

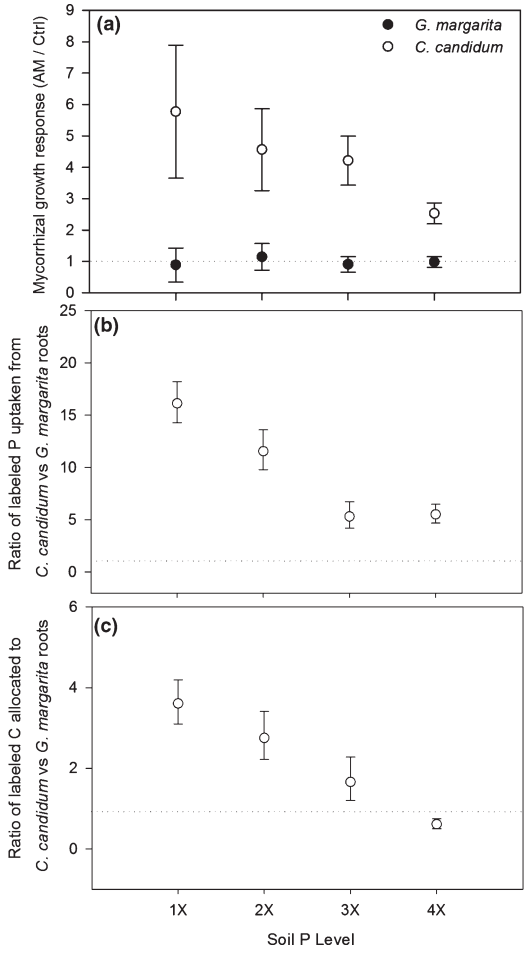

Fig. 1. (a) The mycorrhizal growth response of host plant *Allium vineale* to AM fungi *Claroideoglomus candidum* and *Gigaspora margarita* across different soil P conditions. Mycorrhizal growth response was calculated as (AM/Ctrl), where AM is the total biomass of mycorrhizal plants and Ctrl is the total biomass of the plants receiving no live inoculum and grown in the same background soil as the AM plants. (b) Ratios of radioactive-labeled P delivered to host plant (*A. vineale*) by roots inoculated with *C. candidum* vs. that delivered by roots inoculated with *G. margarita* across four different levels of soil P. (c) Ratios of radioactive-labeled C allocated by host plant (*A. vineale*) to roots inoculated with *C. candidum* vs. that allocated to roots inoculated with *G. margarita* across four different levels of soil P. On all figures, 1×, 2×, 3×, and 4× represent one, two, three, and four times of background P level, respectively. Dotted line represents ratio = 1. Data shown are estimated marginal means and standard errors at each soil P levels.

only when soil P level was limiting. At two lower soil P levels, host plant *A. vineale* allocated an average of 3.60 (P = 1× of the background level) and 2.75 (P = 2× of the background level) times of more labeled C to *C. candidum* than to *G. margarita* ($F_{1,13}$ = 63.2 and 20.4, $P < 0.0001$ and <0.001, for soil P = 1× and 2× of the background level, respectively) (Fig. 1c). However, as soil P increased to 3× and 4× of background level, the differences in the amounts of C allocated to the two fungi became insignificant ($F_{1,13}$ = 1.60 and 2.81, P = 0.23 and 0.12, for soil P = 3× and 4× of the background level, respectively). Overall, there was a significant correlation (R^2 = 0.52, P = 0.006) between the differential in plant C allocation to *C. candidum* vs. *G. margarita* and the differential in P deliveries by these two fungi. As shown in Fig. 2 and Appendix S2, Fig. S3, this correlation appeared to be weaker at higher soil P levels (P = 3×, 4× of the background level).

Although preferential C allocation and the differential in P uptake both decreased with increasing soil P levels, the ratios of P delivery per unit of allocated C by *C. candidum* vs. *G. margarita* did not vary among soil P levels ($F_{3,13}$ = 0.45, P = 0.72) (Appendix S2, Fig. S4). The ratios of $(P_C/C_C)/(P_G/C_G)$ were significantly > 1 across all P fertilization treatments (mean = 4.84, $F_{1,26}$ = 17.14, $P < 0.01$), indicating that the host plant received nearly five times more P per unit of allocated C from the better fungus than the nonbeneficial fungus regardless of the soil P availability.

The proportion of total plant C allocated to roots colonized with *C. candidum* declined from 35% at low P level to 10% at 4× of the background P level ($F_{1,14}$ = 6.22, P = 0.03). The proportion of total plant C allocated to *G. margarita* side of the roots did not vary across the soil P gradient ($F_{1,14}$ = 1.41, P = 0.32) (Fig. 3).

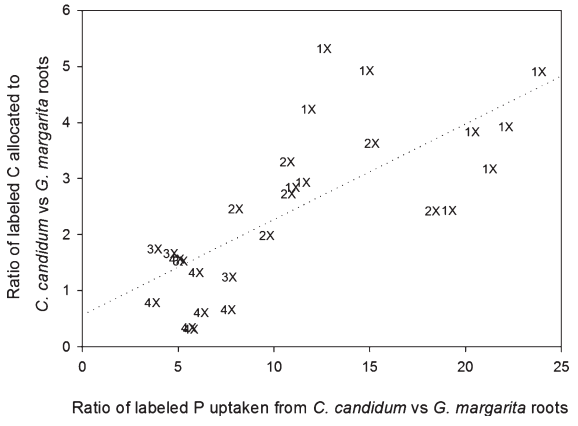

Fig. 2. Correlation between the ratios of C allocation and the ratios of P delivery between shoots of host plant (*Allium vineale*) and roots inoculated with two different AM fungal species (*Claroideoglomus candidum* and *Gigaspora margarita*) across four different levels of soil P (1×, 2×, 3×, and 4× represent one, two, three, and four times of background P level, respectively). Data shown are for each individual samples. Dotted line represents the linear regression line between C ratios and P ratios.

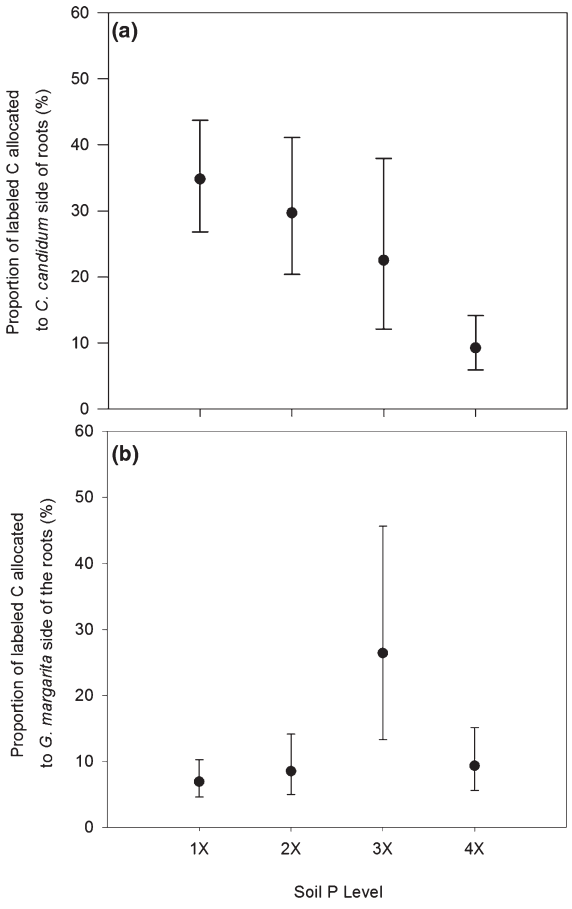

Fig. 3. Proportion of plant total ^{14}C allocated to roots inoculated with *Claroideoglomus candidum* (a) and to roots inoculated with *Gigaspora margarita* (b) across four different levels of soil P (1×, 2×, 3×, and 4× represent one, two, three, and four times of background P level, respectively). Data shown are estimated marginal means and standard errors at each soil P levels.

Discussion

Our findings demonstrate that (1) plants preferentially allocate a greater amount of C to roots that host the fungus delivering more P per unit C, (2) the strength of preferential allocation decreases with increasing availability of soil P, and (3) the host plant received more P per unit of allocated C from the better fungus regardless of the soil P availability. In a resource limiting environment where the cooperating fungus provides more benefits, the host plant rewards its mutualistic partner with more C. This preferential allocation can prevent fungal cheaters from gaining competitive advantages and thereby serve as an effective mechanism for stabilizing mycorrhizal mutualism. The soil P dependence of this allocation can serve as a basis for predicting strength of mutualism across environments.

Based on the results of our growth assay, *C. candidum* is confirmed to be a strong growth promoter for *A. vineale* while *G. margarita* does not benefit plant growth. Moreover, the growth promotion of the beneficial fungus declines when soil P becomes more abundant, indicating the benefits of mycorrhizas are context-dependent, as shown by previous studies (Hoeksema et al. 2010). As expected, P contents in shoots are higher in *C. candidum*-infected *A. vineale* than in *G. margarita*-infected plants or controls, suggesting that enhanced soil P uptake is the primary benefit *C. candidum* provides to its host plant. It is interesting that *G. margarita*, while not promoting plant growth, significantly increases shoot P concentration in *Allium* compared to non-AM controls. Improved P uptake even in the absence of positive growth response has been shown previously (Smith et al. 2003). Our growth assay results are consistent with *G. margarita* being nonbeneficial because of its high C demands per unit P delivery.

By constructing a one host-two symbionts system and using radioactive labeling to simultaneously quantify the ratios of C allocation and P uptake between the plant shoots and the two sides of mycorrhizal roots, we found that *A. vineale* preferentially allocates a greater amount of C to the roots that host the fungus delivering more soil P to the shoots. Although preferential allocation of plant C to more beneficial mutualists has recently been demonstrated (Bever et al. 2009, Kiers et al. 2011) and P delivery as the primary benefit of mycorrhizal fungi has been well documented, this current work is the first empirical study to confirm P as the resource base that triggers plant preferential allocation. Consequently, we observed that preferential allocation to the best mutualist declined with increasing soil P. We note that this pattern in preferential allocation cannot be explained by currently described AM signaling pathways (Harrison 2005, Schmitz and Harrison 2014), but it is consistent with the predictions of some theoretical models developed in the past. For example, Fitter (2006) proposed that the reciprocal exchange of C and P in AM system occurs at the localized region around the arbuscules. When the background P in soil is high, it would become more difficult for the plant to detect the P flux around the arbuscules of a beneficial fungus and then fails to stimulate the allocation of more C to this region. Whether AM signaling pathways can operate in this way remain unknown, which raises interesting opportunities for further work.

We found that the beneficial AM fungus provided nearly five times more P per unit C allocated than the nonbeneficial fungus and that this relative exchange rate did not vary across soil P fertility. As our experimental approach focused on relative exchange rates, with P and C exchange being labeled over differing durations, it does not allow estimation of the absolute C and P exchanged. However, we find that the ratio of C to P exchange rates does not change across the soil P gradient. This result is consistent with the exchange rates of the two fungi not being plastic in their response to environmental variation, while the plant is allocating C to the fungus in proportion to its P demand. This host control of allocation combined with constant C to P exchange rate for the two fungi would generate the significant correlation between preferential allocation of C and differential in P uptake (Fig. 2, Appendix S2, Fig. S3).

Our results suggest that a large percentage of total plant fixed C is being preferentially allocated to the beneficial fungus in exchange for soil P. Approximately, 10% of plant-fixed C went to roots associating with *G. margarita* regardless of P fertilization. While some of this plant investment is used to maintain and grow functional roots, we note that *G. margarita* can successfully reproduce in this split-root environment (Bever

et al. 2009), indicating that it is receiving some of this C. However, *Allium* allocates 35% of its fixed C to roots associating with the beneficial fungus at low P and the difference suggests that *Allium* is investing 25% of its fixed C in preferential allocation toward the beneficial fungus. The large investment in AM fungi is consistent with total C measures in other mycotrophic plant systems (Miller et al. 2002). Our results indicate that preferentially allocated C drops rapidly with increasing levels of soil P. Further work is necessary to identify the form of the labeled C in the plant roots as well as to evaluate patterns of preferential allocation in the context of greater ecological complexity including diverse plant and fungal communities.

Our observations of environmental dependence of plant allocation toward the mutualist that provides the most favorable exchange rate for P has important implications for the dynamics of the mycorrhizal mutualism. Previous results in this system show that high levels of preferential allocation observed in low P soils can overcome the competitive advantage of the nonbeneficial fungus *G. margarita* (Bever et al. 2009). Based on the observed decline in plant investment in preferential allocation with increasing soil P, we would suggest that the advantage to beneficial fungi would similarly decline, increasing the likelihood that nonbeneficial fungi proliferate and that the level of mutualism declines (Bever 2015). While this expectation needs to be tested with further work, the context dependence in plant allocation predicts declining efficiency of AM fungal mutualists across fertility gradients. Such patterns have been observed in the field, as AM fungi isolated from infertile soils have been observed to provide greater benefits to plants than AM fungi derived from fertile soils (Louis and Lim 1988, Boerner 1990). This decline in preferential allocation could also explain the proliferation of nonbeneficial fungi in agricultural soils (Johnson 1993, Verbruggen and Kiers 2010) and suggests that decline of mutualism with fertilization will be a predicable long-term cost of high input agriculture.

More generally, the context dependence of preferential allocation provides a mechanism connecting the population dynamic processes that overcome the problem of cheating to predictions of environmental patterns of mutualism in the landscape. For example, context-dependent preferential allocation could generate AM fungi that are adapted to their local soils. AM fungal species are known to vary in the degree of benefit and in the nature of the plant benefit (e.g., drought tolerance vs. P uptake) (Smith and Read 2008). Variation in the strength of preferential allocation and variation in the direction of preferential allocation toward the most efficient fungus for a particular context could result in proliferation of those fungi that were most beneficial in that particular soil type. Such local adaptation in AM fungi had been observed in the context of drought and heavy metal tolerance (Stahl and Smith 1984, Ji et al. 2010) and in the context of soil N:P ratio (Johnson et al. 2010, Ji et al. 2013). While environmental patterns in the efficiency of mutualism and local adaptation of AM fungi have previously been explained from energetic, mutualism market, and plant physiological perspectives (Hoeksema and Schwartz 2003, Johnson 2010), the context dependence of preferential allocation represents a plant physiological response to such environmental gradients that can regulate mutualisms and predict shifts in fungal population and community composition (Bever 2015).

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Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1256/supinfo