

Plant species differ in their ability to reduce allocation to non-beneficial arbuscular mycorrhizal fungi

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Abstract. Theory suggests that cheaters threaten the persistence of mutualisms, but that sanctions to prevent cheating can stabilize mutualisms. In the arbuscular mycorrhizal symbiosis, reports of parasitism suggest that reductions in plant carbon allocation are not universally effective. I asked whether plant species differences in mycorrhizal responsiveness would affect both their susceptibility to parasitism and their reduction in allocation to non-beneficial arbuscular mycorrhizal fungi (AMF) in high-phosphorus soils. In a greenhouse experiment, I found that two C₃ grasses, *Bromus inermis* and *Elymus repens*, effectively suppressed root colonization and AMF hyphal abundance. Increases in soil phosphorus did not reduce the degree to which AMF increased plant biomass. In contrast, two C₄ grasses, *Andropogon gerardii* and *Schizachyrium scoparium*, more weakly reduced root colonization and failed to suppress AMF hyphal abundance. Consequently, they experienced strong declines in their response to AMF, and one species suffered parasitism. Thus, species differ in susceptibility to parasitism and their reduction in allocation to non-beneficial AMF. These differences may affect the distribution and abundance of plants and AMF, as well as the stability of the mutualism.

Key words: arbuscular mycorrhizal fungi (AMF); C₃ grass; C₄ grass; cheaters; mutualism–parasitism continuum; sanctions.

INTRODUCTION

The stability of mutualisms is a long-standing puzzle in ecology and evolutionary biology. In particular, mutualisms that involve the exchange of costly benefits appear vulnerable to “cheaters,” individuals that obtain the benefits of a mutualism but avoid paying the costs by failing to reciprocate. By avoiding the costs, cheaters could outcompete reciprocating members and might drive the mutualism to an antagonism (Bronstein 2001). Cheaters exist in many mutualisms, such as ant–plant protection mutualisms (Edwards et al. 2010), the legume–rhizobia symbiosis (Simms et al. 2006), and the plant–mycorrhizal symbiosis (Bever et al. 2009). Bronstein (2001) noted that some cheaters are conditional, behaving mutualistically in some contexts but parasitically in others. To understand whether conditional cheaters could destabilize mutualisms, it is necessary to understand the conditions under which they act as parasites.

Sanctions are one of the chief mechanisms thought to be important for preventing exploitation and stabilizing mutualisms. To minimize fitness costs imposed by cheaters, partners should reduce their investment (Kiers and van der Heijden 2006). Sanctions occur in some systems, such as the legume–rhizobium symbiosis (Kiers

et al. 2003) and plant–pollinator mutualisms (Pellmyr and Huth 1994). Bronstein (2001) emphasized that different stabilizing mechanisms may operate in different mutualisms, depending partly on whether cheaters are conditional. Whether reductions in allocation can prevent exploitation by conditional cheaters remains an important unanswered question.

The plant–arbuscular mycorrhizal symbiosis is vulnerable to conditional cheaters (Egger and Hibbett 2004). In this symbiosis, plant response ranges along the mutualism–parasitism continuum (Johnson et al. 1997). Plants often benefit from association with arbuscular mycorrhizal fungi (AMF), especially when soil nutrients are scarce (Hoeksema et al. 2010). However, when phosphorus is abundant, plants may receive little or no benefit from the symbiosis, so AMF are not plant mutualists and the interaction functions as a commensalism or a parasitism (Johnson et al. 1997, Johnson 2010). Similarly, when light is scarce, AMF are less likely to benefit plants (Johnson 2010). On the other hand, AMF fitness always depends on plant carbon because they have no independent means of taking up carbon (Johnson 2010). If AMF receive plant carbon when there is no benefit to the plant, they are conditional cheaters.

We expect plants to reduce carbon allocation to AMF in conditions where AMF are not beneficial. There is some evidence that plants sanction AMF; for example, some plants preferentially allocate carbon to more beneficial AMF (Bever et al. 2009, Kiers et al. 2011).

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However, evidence is weaker that carbon allocation can keep in check conditional cheaters that are non-beneficial only at high soil nutrients. For example, many studies have found that phosphorus additions decrease the percentage of the plant root system occupied by AMF (Treseder 2004), but root colonization is only an indirect metric of plant allocation. Direct measurement of carbon flux to AMF (Kiers et al. 2011) is the ideal indicator of plant allocation; AMF hyphal abundance in the soil, a surrogate for AMF fitness, may also indicate AMF carbon uptake from plants because AMF have no alternative carbon source. Furthermore, reductions in allocation are insufficient to prevent parasitism; parasitism occurred in at least 15% of studies in a meta-analysis of hundreds of mycorrhizal studies (supplement to Hoeksema et al. 2010). Why do reductions in carbon allocation so frequently fail to prevent parasitism by AMF?

Graham and Eissenstat (1994) hypothesized that variation in plant ability to alter carbon allocation would depend on plant benefit from AMF in low-phosphorus soils, typically expressed as mycorrhizal responsiveness (a measure of plant biomass in the presence of AMF relative to biomass in their absence). Specifically, a plant not benefitting strongly from AMF in low phosphorus (low mycorrhizal responsiveness) would be more likely to reduce allocation to AMF in high phosphorus and therefore less likely to experience parasitism (negative mycorrhizal responsiveness). This reduction in allocation to AMF would drive reductions in AMF hyphal abundance, an indicator of AMF fitness, and might be associated with reductions in root colonization. On the other hand, a plant with high mycorrhizal responsiveness in low phosphorus might only weakly reduce carbon allocation in response to increased phosphorus, driving weak or no reductions in AMF hyphal abundance and root colonization.

I used a well-known model system to investigate differences among plant species in their adjustment of carbon allocation: C₃ and C₄ grasses. Warm-season C₄ grasses are highly responsive to AMF; cool-season C₃ grasses tend to have lower responsiveness (Wilson and Hartnett 1998). These predictable differences allowed me to ask whether mycorrhizal responsiveness at low phosphorus would determine both vulnerability to parasitism and strength of reduction in carbon allocation (indicated by AMF hyphal abundance) at high phosphorus.

METHODS

To address this question, I used plant mycorrhizal responsiveness, calculated from plant biomass, to indicate plant fitness gain from AMF. I used the change in AMF hyphal abundance (a surrogate for AMF fitness) across a phosphorus gradient to indicate reduction in plant carbon allocation. The greenhouse experiment had 480 pots: four plant species, two AMF

treatments, five phosphorus levels, and two light levels, replicated six times.

Plant species and AMF inoculum

I compared two native C₄ prairie bunchgrasses, *Andropogon gerardii* Vitman and *Schizachyrium scoparium* (Michx.) Nash, and two introduced C₃ grasses, *Bromus inermis* Leyss. and *Elymus repens* (L.) Gould. The C₄ species were historically abundant in southwest Michigan, and the C₃ grasses are common dominant species there now. I grew plants and AMF in 0.7-L pots in 90% sand, 10% sieved old-field topsoil, plus 150 mL additional field soil inoculum. I autoclaved the inoculum and sand/soil mix in the nonmycorrhizal treatment but not in the mycorrhizal treatment (see Appendix A for details).

Light and phosphorus treatments

In December 2008, I transplanted one pre-germinated seed into each pot in a heated, lighted greenhouse at the Kellogg Biological Station in southwest Michigan. I placed half the pots under shade cloth that blocked 30% of light (low light treatment). Once weekly, starting three weeks after planting, each pot received a phosphorus fertilizer (0, 0.15, 0.31, 3.1, or 31.0 g/L NaH₂PO₄) that also included nitrogen and micronutrients. The highest phosphorus level was intended to mimic the high availability induced by manure application. At the end of the experiment, I determined water-extractable soil phosphorus using malachite green on a microplate reader (SpectraMax M5, Molecular Devices, Sunnyvale, California, USA; Appendix A).

Harvesting plants and AMF

After nine weeks of fertilization (12 weeks of growth), I clipped seedlings at the soil surface. I dry-sieved the contents of each pot and air-dried the soil for analysis of phosphorus and AMF hyphal abundance, then washed and dried the roots. To determine plant response to AMF, I summed root and shoot biomass and calculated mycorrhizal responsiveness (MR). When plant biomass with AMF (b_{AMF}) was greater than biomass without (b_N), $MR = 100(1 - b_N/b_{AMF})$; when $b_{AMF} < b_N$, $MR = 100(b_{AMF}/b_N - 1)$. MR ranges from -100 to 100; $MR > 0$ indicates that AMF acted as mutualists and $MR < 0$ indicates that AMF acted as parasites to reduce plant biomass. Other indices of MR gave qualitatively similar results. I stained subsamples of dried roots in trypan blue and scored percentage of root colonization. To measure AMF hyphal abundance, I extracted extraradical hyphae from air-dried soils, filtered, and visually identified and counted hyphae at 400 \times (Appendix A).

Statistical analysis

I analyzed the data as a split-plot ANCOVA in R (2.10.1; R Foundation for Statistical Computing, Vienna, Austria) package nlme (for plant mycorrhizal responsiveness and AMF hyphal abundance) and package lmer

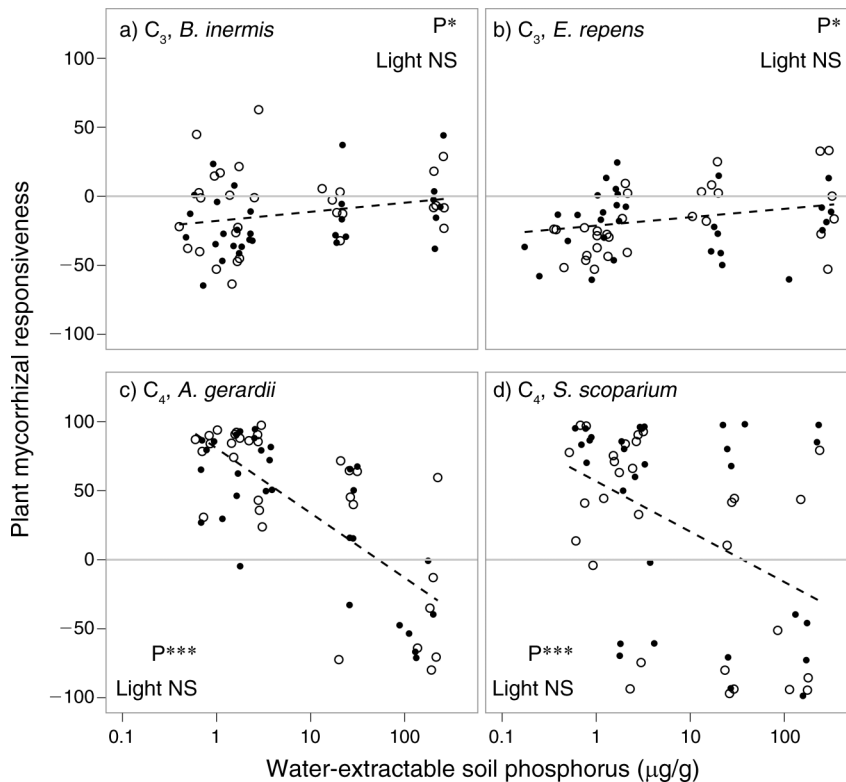


FIG. 1. The effect of soil phosphorus on plant biomass response to AMF (mycorrhizal responsiveness) for (a) *Bromus inermis*, (b) *Elymus repens*, (c) *Andropogon gerardii*, and (d) *Schizachyrium scoparium* at high light (open symbols) or under shade structures (solid symbols). Horizontal lines at zero indicate no effect of AMF, positive values indicate that plants benefited from inoculation with AMF (mutualism), and negative values indicate that plants grew larger when nonmycorrhizal (parasitism). Response to P is plotted as dashed lines. Note the log-scale for the x-axis.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, $P > 0.05$.

(using glmer to accommodate logistic regression for percent root colonization). Plant species (four levels), AMF (two levels), and phosphorus (five levels) were randomized within the light treatment (two levels); each treatment combination was replicated in six blocks (480 pots total). I retained dead individuals in the data set to maintain a balanced design (Appendix A); removing them did not qualitatively change the results. I used log-transformed water-extractable soil phosphorus as a continuous predictor. I simplified models by removing nonsignificant interactions (Crawley 2007). To compare C_3 and C_4 grasses, I combined species into functional groups; if this simplification maintained model fit, species within groups were not significantly different. When there were significant interactions with the phosphorus treatment, I analyzed each species or functional group separately.

RESULTS

Effectiveness of the AMF treatment

Three lines of evidence suggest that the pots with autoclaved sand/soil mix and inoculum did not have live AMF. First, root colonization of plants in the nonmycorrhizal pots was $<1\%$ (mean of 80 samples). Second, AMF hyphal abundance in nonmycorrhizal pots did not

respond to phosphorus, light, or plant species ($P > 0.05$). Third, there were more AMF hyphae in mycorrhizal than nonmycorrhizal pots ($F_{1,463} = 4.16$, $P = 0.04$).

Differences among plant species

Plant mycorrhizal responsiveness and AMF hyphal abundance were identical for the two C_3 grasses (*B. inermis* and *E. repens*), so they were combined into a single C_3 functional group. However, *A. gerardii* and *S. scoparium* differed in some respects. Mortality of *S. scoparium* was higher than the other species (35/120, compared to 0/120, 0/120, and 1/120 for *A. gerardii*, *B. inermis*, and *E. repens*) but was unrelated to the treatments. Across resource treatments, the C_3 grasses had lower mycorrhizal responsiveness (Fig. 1; $F_{2,225} = 82.15$, $P < 0.001$) and lower root colonization (Fig. 2; $P < 0.001$) than the C_4 grasses, but equal AMF hyphal abundance (Fig. 3; $F_{2,225} = 0.39$, $P = 0.54$). Species or functional groups responded differently to changes in resource availability.

Light

High light did not affect the mycorrhizal responsiveness of any species (Fig. 1), but it increased root

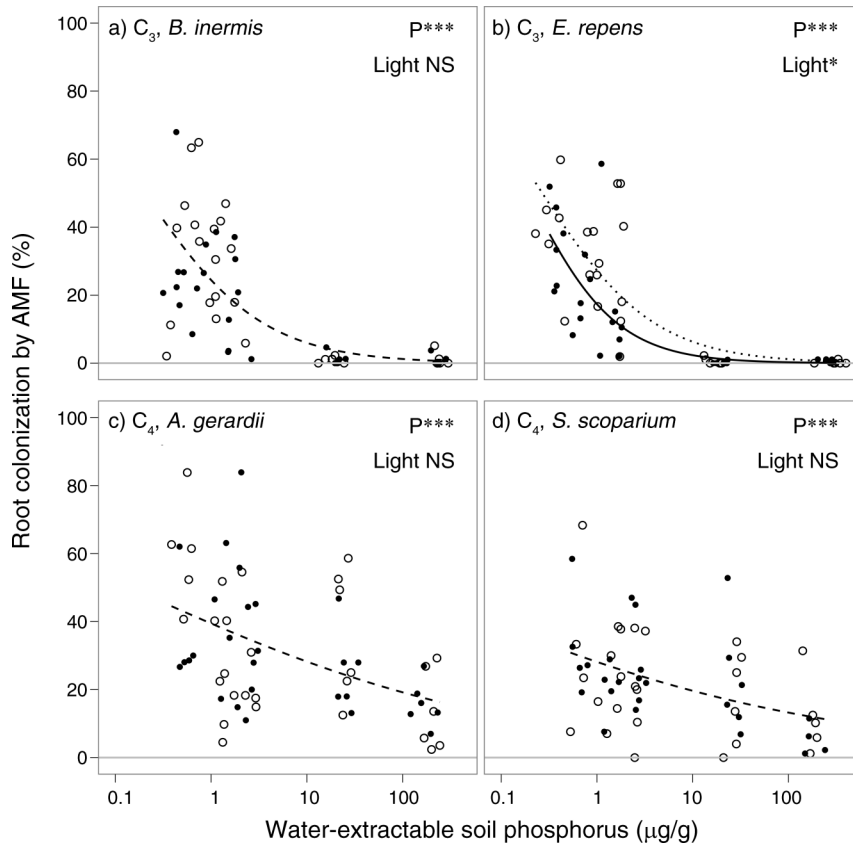


FIG. 2. The effect of phosphorus on the percentage root colonization of (a) *B. inermis*, (b) *E. repens*, (c) *A. gerardii*, and (d) *S. scoparium* at high light (open symbols) or in shade (solid symbols). Response to P is plotted as dashed lines if there was no significant effect of light. If there was a significant effect of light, the response is plotted as dotted lines for high light and solid lines for low light ($P < 0.05$).

* $P < 0.05$; *** $P < 0.001$; NS, $P > 0.05$.

colonization of *E. repens* (Fig. 2; $P = 0.018$) and increased AMF hyphal abundance by 16% in all species (Fig. 3; $F_{1,5} = 7.55$, $P = 0.04$).

Phosphorus effects on plants

The effect of phosphorus differed between the C₃ and C₄ grasses ($F_{1,225} = 63.34$, $P < 0.001$). Phosphorus increased the mycorrhizal responsiveness of *B. inermis* and *E. repens* from negative to neutral (Fig. 1; $F_{1,107} = 6.42$, $P = 0.01$). In contrast, phosphorus decreased the mycorrhizal responsiveness of the C₄ grasses ($F_{1,107} = 52.68$, $P < 0.001$). At low phosphorus, the relationship was mutualistic for *A. gerardii* and *S. scoparium*, increasing biomass by 491% and 656% ($F_{1,11} = 40.27$, $P < 0.001$ and $F_{1,11} = 34.05$, $P < 0.001$). However, at high phosphorus, *A. gerardii* was 49% smaller when mycorrhizal than when nonmycorrhizal, indicating parasitism ($F_{1,11} = 11.57$, $P = 0.006$). *Schizachyrium scoparium* did not respond to AMF at high phosphorus. This difference among species in the effect of phosphorus on mycorrhizal responsiveness suggests that reductions in allocation to AMF should also differ among species.

Phosphorus effects on root colonization

Phosphorus decreased root colonization in *B. inermis* and *E. repens* to nearly zero (Fig. 2; $P < 0.001$ for both species). Both *S. scoparium* and *A. gerardii* also decreased root colonization ($P < 0.001$ for both species), but more weakly than the C₃ species ($P < 0.001$). C₄ grass root colonization ranged from 1% to 31% in high phosphorus.

Phosphorus effects on carbon allocation

As expected, the effect of phosphorus on AMF hyphal abundance also differed between plant functional groups (Fig. 3; $F_{1,225} = 4.33$, $P = 0.04$). AMF hyphal abundance decreased with phosphorus when grown with the two C₃ grasses ($F_{1,107} = 19.98$, $P < 0.001$) but not with the two C₄ grasses. These results indicate that the two C₃ species reduced allocation to AMF in high phosphorus soils, but the two C₄ species did not.

DISCUSSION

There is a growing appreciation that sanctions can be important in preventing cheaters from destabilizing mutualisms (West et al. 2002, Kiers et al. 2003, Jandér

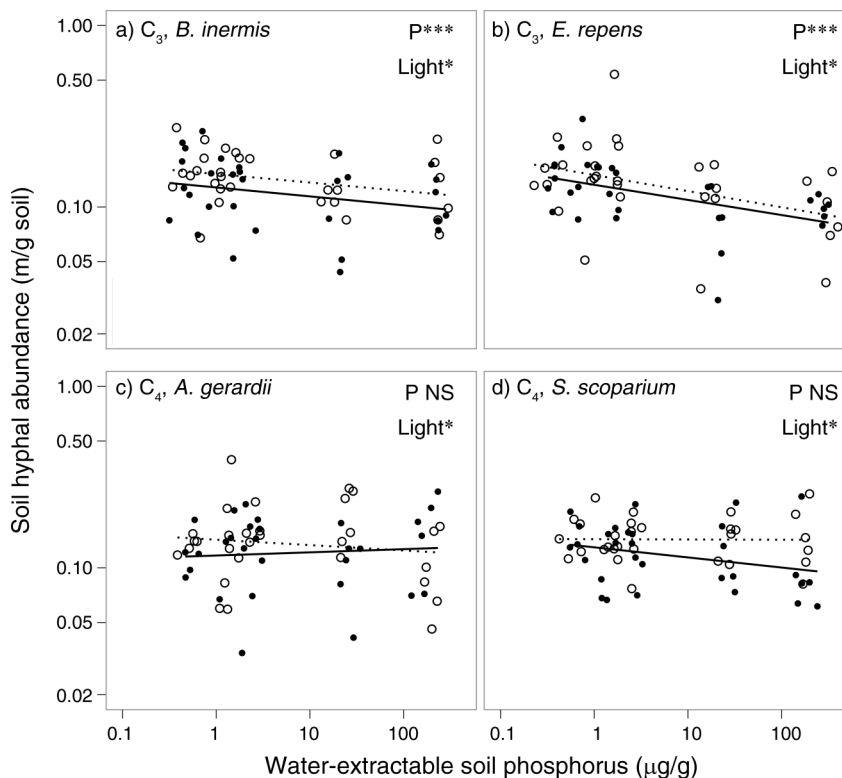


FIG. 3. The effect of phosphorus on the length of AMF hyphae in soil under (a) *B. inermis*, (b) *E. repens*, (c) *A. gerardii*, and (d) *S. scoparium* at high light (open symbols) or in shade (solid symbols). Response to P is plotted as dotted lines for high light and as solid lines for low light.

* $P < 0.05$; *** $P < 0.001$; NS, $P > 0.05$.

and Herre 2010). In the case of arbuscular mycorrhizal fungi (AMF), exciting recent work has demonstrated that plants can adjust carbon allocation to AMF in response to their mutualistic quality (Bever et al. 2009, Kiers et al. 2011). However, not all plants appear to be capable of sufficiently reducing allocation to poor mutualists; AMF can parasitize plants (Hoeksema et al. 2010), especially in phosphorus-rich environments (Johnson 2010). In this study, I show that plant species differ in the degree to which they adjust allocation to non-beneficial AMF in phosphorus-rich conditions and, accordingly, whether they experience parasitism.

The results of this study support Graham and Eissenstat's (1994) hypothesis of a relationship between plant benefit at low phosphorus and plant parasitism at high phosphorus. They also support a relationship between plant benefit at low phosphorus and plant ability to reduce carbon allocation. Neither C_3 grass experienced a benefit from AMF at low phosphorus, but both reduced allocation to AMF in high phosphorus: *Bromus inermis* (smooth brome) and *Elymus repens* (quackgrass) suppressed root colonization and AMF hyphal abundance in the soil, avoiding parasitism. In contrast, the two C_4 grasses, which showed strong positive responses to AMF at low phosphorus, were less effective in reducing allocation to AMF at high

phosphorus: *Andropogon gerardii* and *Schizachyrium scoparium* only weakly suppressed root colonization and failed to reduce soil AMF hyphal abundance. This failure to reduce allocation to AMF led to parasitism in one species (*A. gerardii*). I did not detect parasitism in the other C_4 species, *S. scoparium*, perhaps because high mortality masked effects. Thus, between the functional groups, there were predictable relationships between mycorrhizal responsiveness at low phosphorus and plant vulnerability to parasitism and reduction in allocation to AMF at high phosphorus.

Differences between the functional groups

Many traits differ among the C_3 and C_4 grasses in this study. Some of these may drive differences in mycorrhizal responsiveness at low phosphorus, vulnerability to parasitism, and reduction in allocation to AMF at high phosphorus.

Perennial C_4 prairie grasses are typically strongly mycorrhizal responsive, whereas perennial C_3 grasses tend to have much lower mycorrhizal responsiveness (Wilson and Hartnett 1998). Differences in mycorrhizal responsiveness between the groups are likely determined by root morphology (root diameter and specific root length) and correspondingly nutrient uptake ability

(Hetrick et al. 1988). Root hairs also increase uptake of immobile nutrients and may affect mycorrhizal responsiveness (Schweiger et al. 1995). Anecdotally, I observed that *E. repens* and especially *B. inermis* roots were densely covered in root hairs, but that the C_4 grasses in this study had far fewer. Supporting the idea that these C_4 grasses were less able than the C_3 grasses to take up phosphorus, nonmycorrhizal C_4 grasses showed much stronger growth responses to phosphorus than C_3 grasses (Appendix B). Differences in phosphorus uptake ability may explain why C_4 grasses had much higher mycorrhizal responsiveness than C_3 grasses at low phosphorus. Root morphology and resource uptake ability could also determine a species' susceptibility to parasitism and reduction in allocation to AMF at high phosphorus. Future studies should investigate the mechanisms driving the correlations observed in this study among resource uptake efficiency, parasitism, and reductions in allocation to AMF at high phosphorus.

The species in this study also differed in the degree to which root colonization responded to phosphorus availability. Root colonization varied more widely in the C_3 than in the C_4 grasses, corresponding with stronger reductions in allocation to AMF at high phosphorus in C_3 than C_4 grasses. Root colonization is at best a weak predictor of carbon and nutrient exchange (Noyd et al. 1995, Wilson and Hartnett 1998, Kaeppler et al. 2000, Jifon et al. 2002), so it is surprising that this response was associated with effective reductions in allocation to non-mutualistic AMF.

The grasses in this study also differ in evolutionary origin. The two C_4 grasses are native species, while both C_3 grasses are exotic. This might explain differences in response to AMF; the exotic *B. inermis* and *E. repens* likely lacked their coevolved fungal symbionts, perhaps causing their negative mycorrhizal responsiveness at low phosphorus. However, Wilson and Hartnett (1998) found no difference in mycorrhizal responsiveness between native C_3 and exotic C_3 grasses, suggesting that among C_3 grasses, origin does not affect response to AMF. The inoculum likely also lacked the coevolved fungal symbionts of the native C_4 prairie grasses, as the inoculum came from a former agricultural old field dominated by weeds. Therefore, a species' native or introduced status probably did not affect its response to AMF. However, the difference in origin may affect susceptibility to parasitism or ability to reduce allocation to non-beneficial AMF, but future studies will be required to clarify any links.

Differences in allocation adjustment: other examples from the mycorrhizal symbiosis

Only a few other studies have also compared species' mycorrhizal responsiveness and susceptibility to parasitism and these have produced conflicting results. Citrus genotypes that benefitted more from AMF at low phosphorus were more vulnerable to parasitism at high phosphorus (Graham and Eissenstat 1994) and also lost

more nonstructural carbohydrates to AMF (Jifon et al. 2002). However, there were no differences in reduction of allocation to AMF among two C_4 and one C_3 grasses (Noyd et al. 1995), between *Panicum virgatum* and *Salsola kali* (Johnson 1998), or among 28 maize inbred lines (Kaeppler et al. 2000). Thus, there appears to be no predictable relationship between response to AMF at low and high phosphorus.

Cheating AMF

It is commonly assumed that biomass differences between mycorrhizal and nonmycorrhizal plants in greenhouse experiments can indicate whether the relationship is mutualistic or parasitic, at least in the environmental and temporal context of the study (Hoeksema et al. 2010). Critics of this view correctly point out, however, that the arbuscular mycorrhizal symbiosis is multifunctional, with AMF contributing to nutrient uptake, defense against disease, and perhaps other functions (Maherali and Klironomos 2007). Therefore measuring parasitism in high phosphorus but disease-free environments may not be ecologically informative. An additional complication is that plants might experience parasitism even when obtaining large amounts of phosphorus from AMF (Smith et al. 2009). Furthermore, the AMF species cultured in short-term greenhouse experiments are only a subset of the AMF species encountered in the field, and may represent less beneficial strains. Finally, some plants are very long lived and seedling responses (which tend to be more negative than those of older plants; Johnson et al. 1997) may not reflect true plant fitness responses to AMF in the field. However, it is currently not possible to measure the lifetime fitness benefits of AMF to plants in a field setting. It is also likely true that seedling growth and establishment is an important selective filter. Therefore, while they cannot tell the entire story, greenhouse experiments are still informative.

An important caveat of this study is that the AMF inoculum was composed of a group of field-collected AMF of unknown identity. Shifts in the behavior of the inoculum from mutualistic at low phosphorus to potentially parasitic at high phosphorus could be driven by at least two mechanisms. First, the behavior of individual AMF species could shift from beneficial to non-beneficial with increasing phosphorus. This is the view that considers AMF conditional cheaters. Second, shifts in the relative abundance of AMF species of differential mutualist quality could occur, such that poor quality mutualists dominate communities at high phosphorus. These two mechanisms are not mutually exclusive and, interestingly, might select for different mechanisms driving reduction of plant carbon allocation to AMF (Bronstein 2001). However, these caveats do not diminish the importance of this study's finding that plant species differ in their adjustment of allocation to AMF in high phosphorus.

Implications of differences in plant species adjustment of allocation to AMF

Variation among plant species in their suppression of allocation to AMF could help explain the distribution of plant species. Both *A. gerardii* and *S. scoparium*, native grasses once widespread in prairies, are now restricted to low-fertility grasslands in Michigan (Foster 1999). Introduced C₃ grasses such as *E. repens* and especially *B. inermis* dominate more productive sites there (Foster 1999; E. Grman, *personal observation*). Long-term experiments in Minnesota also show that *E. repens* typically replaces *S. scoparium* and *A. gerardii* in nitrogen-enriched plots (Tilman 1988, Johnson et al. 2008). One likely mechanism for the extirpation of these C₄ grasses in highly productive soils is reduced seedling establishment driven by low light levels under abundant litter and a C₃ grass canopy (Foster and Gross 1998). These carbon-starved seedlings would be especially vulnerable to the effects of parasitic AMF. In this study, even in the absence of competition and litter, AMF negatively affected *A. gerardii* seedlings. Thus, a second mechanism contributing to C₄ grass loss in fertile soils might be their inability to reduce allocation to non-beneficial AMF. Johnson et al. (2008) found support for this hypothesis: both *E. repens* and *P. virgatum*, which increase in response to long-term nitrogen fertilization, had more plastic root colonization than *A. gerardii*. Johnson et al. (2003) also found that the outcome of competition was better for strongly mycorrhizal species in low nitrogen than in high nitrogen soils. Thus, differences in plant species adjustment of allocation to AMF may contribute to their loss from eutrophic habitats.

Variation among plant species in their adjustment of allocation to AMF could also help explain variation in AMF abundance across fertility gradients. AMF should decline in abundance in fertile soils because of reduced plant allocation, but empirical patterns are variable (Treseder 2004). This study suggests that different dominant plant species may alter the degree of decline in fungal abundance, but field tests of this hypothesis are needed.

Differences in sanction strength: examples from other types of mutualism

In other mutualisms, few studies have compared the effectiveness of sanctions among species or proposed hypotheses to explain the variation. Minchin et al. (1983) and Simms et al. (2006) reported differences in the degree to which legumes sanctioned rhizobia, but I am unaware of any hypotheses explaining the variation. Jandér and Herre (2010) measured variation in sanction strength across six fig species. Among the four species that imposed sanctions on cheating pollinator wasps, sanction strength was negatively correlated with the proportion of wasps not carrying pollen (and potentially cheating). Their study suggests that variation in sanction

strength may impact the ecology and evolution of species interactions.

Other studies have suggested possible reasons for variation in sanction strength. Kiers et al. (2007) showed that newer cultivars of soybeans did not maintain high yields when inoculated with both a good and a bad rhizobial strain, while older cultivars did, possibly indicating that newer cultivars had lost the capacity to sanction. However, this finding is unlikely to explain natural variation in sanction strength. Goto et al. (2010) hypothesized that in obligate pollination–seed-consumption mutualisms, plant ability to impose sanctions may depend on the oviposition behavior of their pollinators. However, they could not test for patterns in sanction strength among species, and it is difficult to generalize this relationship to other mutualisms. In an ant–plant protection mutualism, Edwards et al. (2006) showed that one plant species could effectively sanction cheating ants by reducing the size of domatia (rewards for effective mutualist ants) if ants did not protect leaves from herbivory. In contrast, another species lacked the capacity to sanction cheating ants because it developed domatia before developing leaves (Edwards et al. 2010). While these studies have found differences in species' ability to sanction cheaters, ecologists have only recently begun to understand and predict this variation.

Conclusion

Cheating seems to be a persistent feature of mutualisms (Bronstein 2001). If not held in check, cheaters can have dramatic effects on community structure and evolution, at least in theory. In this study, I found variation in plant species' ability to hold conditional cheaters in check. This variation has important implications for the distribution and abundance of plants and AMF. Both within the mycorrhizal symbiosis and in other mutualisms, there is a growing body of evidence that species differ in sanction strength. However, studies of the causes and consequences of this variation are just beginning. Understanding the frequency of species' ability to sanction cheaters, variation in sanction strength, and the mechanisms of sanction effectiveness may explain aspects of mutualism persistence and community structure, function, and diversity.

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SUPPLEMENTAL MATERIAL

Appendix A

Detailed methods describing greenhouse conditions, sampling procedure, statistical analyses, and references (*Ecological Archives* E093-061-A1).

Appendix B

Figure showing the effects of soil phosphorus and AMF on plant biomass (*Ecological Archives* E093-061-A2).