PLANT-MYCORRHIZAL INTERACTIONS AND THE RELATIVE ABUNDANCE OF LIMITING RESOURCES

By

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ABSTRACT

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The relative abundance of limiting resources, particularly light and soil nutrients, may be a key predictor of plant interactions with arbuscular mycorrhizal fungi (AMF). AMF are typically plant mutualists, increasing access to limiting soil nutrients though their extensive network of soil hyphae in exchange for plant carbon. However, when soil nutrients are abundant, relative to light, AMF are less beneficial to plants. In those situations, as plants shift towards light limitation, stoichiometric theory predicts decreases in four metrics of plant-mycorrhizal interactions: plant benefit, fungal benefit, plant root colonization by AMF, and plant carbon allocation to AMF. Indeed, fertilization with soil nutrients does decrease at least some of those metrics of plant-mycorrhizal interactions, but many questions remain.

First, do conceptual models of stoichiometry adequately capture negotiation between plants and AMF? With Chris Klausmeier and Todd Robinson, I developed a mathematical model that points to two key features of trade between mutualists that have previously been ignored: the negotiated exchange ratio of one resource for another, and allocation to self-provisioning of those resources by each partner.

Second, why do AMF sometimes parasitize plants in high nutrient environments? In theory, plants should be able to impose "sanctions" to avoid parasitic carbon drains by "cheating" AMF. In a greenhouse experiment, I show that two C_3 grasses (quackgrass, *Elymus repens*, and smooth brome, *Bromus inermis*) avoided parasitism by effectively reducing carbon

allocation to AMF in high phosphorus environments while one C_4 grass (big bluestem, *Andropogon gerardii*) did not.

Third, why do plant allocation to AMF and AMF abundance not always decrease with increases in nutrient availability? Some field studies have shown no change or even increases in those metrics of plant-mycorrhizal interactions with nitrogen or phosphorus fertilization. In a field fertilization experiment with Todd Robinson, I found that AMF increased in response to nitrogen addition in very nitrogen-poor soils, consistent with AMF nitrogen limitation. In an additional field experiment across a natural productivity gradient, I showed that increases in productivity do not necessarily lead to increases in plant light limitation, calling into question the expectation that increases in fertility should change plant-mycorrhizal interactions.

Finally, do differences among plant species affect how shifts in stoichiometry alter plant-mycorrhizal interactions? In nutrient poor soils, AMF benefit different plant species differentially, but how those species differences affect mycorrhizal response to fertilization is unclear. In a greenhouse experiment, I found that two C_3 grasses did differ from two C_4 grasses in terms of how plant benefit, fungal benefit, and plant root colonization responded to increases in phosphorus availability. In a field fertilization experiment, I again found that a C_3 grass (*B. inermis*) differed consistently from a C_4 grass (*A. gerardii*) in how strongly nitrogen and phosphorus fertilization affected plant-mycorrhizal interactions.

Taken together, these studies show that stoichiometric theory is a powerful tool for understanding plant-mycorrhizal interactions. However, relationships are complex, and differences among species as well as aspects of negotiation and trade also play important roles.

to my mother, the gardener
and my father, the beekeeper/engineer

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CHAPTER ONE

Introduction

The stoichiometry of plant-mycorrhizal interactions

Although only recently termed "ecological stoichiometry" (Sterner and Elser 2002), the study of the dependence of ecological interactions on the ratios of limiting resources has provided insights into ecology for many years (Reiners 1986). Redfield (1958) compared the relative abundances of carbonate, nitrogen, and phosphorus in ocean water and drew important conclusions about the limits of nitrogen and phosphorus cycling that have largely held up to more recent investigations (Sterner and Elser 2002). Resource ratio theory (Tilman 1982 and references therein) can predict the outcome of competition, the coexistence of competitors, and important ecosystem attributes based on the relative abundances of limiting resources (Tilman 1999; Dybzinski and Tilman 2007; Harpole and Tilman 2007). The relative abundance of carbon and nitrogen in decomposing plant litter can determine the rate of decomposition and nutrient cycling (Hobbie 1992). Plant-herbivore and predator-prey interactions, disease dynamics, and the impact of food webs on nutrient cycling can also be determined by the relative abundance of carbon and nutrients in prey tissue (Sterner and Elser 2002; Mayntz et al. 2005; Hall et al. 2009). Ecological stoichiometry may determine biological invasions, toxin production by cyanobacteria, and species traits such as inherent growth rate (Elser et al. 2003; Van de Waal et al. 2009; Gonzalez et al. 2010). Stoichiometry also can mechanistically link organism structure and function across scales, from the chemical constituents of cellular components to ecosystem nutrient cycling (Sterner and Elser 2002). Thus, ecological stoichiometry provides a

unifying framework in which to understand many of the major questions in the field of ecology (Reiners 1986).

The identity and abundance of limiting resources are key factors controlling species interactions. Nutritional mutualisms, such as interactions between plants and arbuscular mycorrhizal fungi (AMF), may be particularly dependent on the relative abundance of limiting resources (Johnson 2010). AMF are ubiquitous fungi that participate in a mutualism with the majority of terrestrial plant species (Wang and Qiu 2006). The benefits of the mutualism for both plants and fungi are based on the exchange of limiting resources. AMF produce extensive networks in soil of very thin hyphae that, per unit length, require less carbon to construct than plant roots (Smith and Read 1997). This growth pattern provides AMF advantages in soil nutrient uptake: for a given mass of tissue carbon, AMF can explore larger soil volumes than roots. As a result, AMF have greater increase access to immobile soil nutrients such as phosphorus, where very slow rates of diffusion limit uptake (Smith and Read 1997). For more mobile nutrients such as nitrate and possibly ammonium, the increased soil volumes explored by AMF may not increase uptake (Hodge et al. 2010; Johnson 2010). After taking up soil nutrients, AMF transport them through their hyphae to points of colonization inside plant roots. At these colonization points, AMF exchange soil nutrients for plant carbon in the form of sugars. Because AMF have no independent source of carbon, they are obligately dependent on plant carbon allocation and benefit strongly from the interaction. Plants also often benefit from the association, especially in environments where soil nutrients are scarce and root uptake is insufficient for plant growth requirements (Smith and Read 1997; Allison and Goldberg 2002; Hoeksema et al. 2010). In these environments, the exchange of carbon for nutrient benefits both partners: plants get more nutrient per unit carbon than they would if they allocated instead to

increased root growth, and fungi get infinitely more carbon than they can take up independently of plants (Tuomi et al. 2001; Geritz et al. 2006).

However, a change in resource availabilities can alter the cost-benefit equation. In environments with abundant soil nutrients, plant roots can take up enough nutrients to satisfy growth requirements. This typically results in a reduction in root:shoot ratio and an increase in plant allocation to acquisition of the new limiting resource: light (Bloom et al. 1985; Tilman 1988; Sterner and Elser 2002; Dybzinski and Tilman 2007). Light limitation, especially in nutrient-rich or fertilized grasslands, constrains the growth and establishment of seedlings and other small-statured plants (Foster and Gross 1998; Hautier et al. 2009; Dickson and Foster 2011). When plants are light limited, as opposed to nutrient limited, carbon allocation to AMF may be less beneficial. Although AMF may protect plants from pathogens (Maherali and Klironomos 2007) or possibly increase access to water or micronutrients (Allen et al. 2003), light limited plants typically benefit less from associating with AMF than nutrient limited plants, at least in controlled greenhouse environments (Graham et al. 1982; Hoeksema et al. 2010). Light-limited plants therefore have two choices: either reduce allocation to AMF and suppress AMF abundance, or suffer parasitic carbon drains. Empirical evidence suggests that both outcomes occur. Many field and greenhouse studies find that AMF abundance declines with fertilization, presumably because of the transition to plant light limitation and reduced plant allocation to AMF (Treseder 2004; Johnson 2010). However, in low light or high nutrient environments, there are also reports of plants experiencing parasitism (defined as greater growth or biomass in the absence than in the presence of AMF) (Graham et al. 1982; Graham and Eissenstat 1994; Johnson et al. 1997; Jifon et al. 2002).

Thus, interactions between plants and AMF are thoroughly stoichiometric. Plant benefit from the association depends on the ratio of soil nutrients to light or photosynthetically fixed carbon (Johnson et al. 1997; Johnson 2010). Plant allocation to AMF is thought to be governed by similar rules: plants should allocate more to AMF when the nutrient:light ratio is low than when it is high (Treseder 2004; Johnson 2010). However, plant-mycorrhizal interactions do not always appear to play by these rules.

The first obvious exception is that some AMF, in some conditions, appear to parasitize plants by obtaining more carbon than is beneficial for plants to allocate (Graham et al. 1997; Jifon et al. 2002). Kiers and van der Heijden (2006) have suggested that plants ought to be able to "sanction" non-beneficial AMF by preferentially allocating carbon to beneficial AMF and reducing carbon allocation to non-beneficial AMF. There is empirical support for this idea (Bever et al. 2009), but the mechanisms and prevalence of these sanctions are not yet understood. One potential mechanism for plant control of carbon allocation to AMF is that plants allocate sugars to root segments where internal phosphorus content is high, either because of direct root uptake in a phosphorus-rich patch or because of trade with mycorrhizal fungi (Fitter 2006). AMF providing phosphate in these root segments could then take up these sugars (Bucking and Shachar-Hill 2005; Javot et al. 2007). This mechanism would allow plants to reduce carbon flows to AMF that do not contribute substantially more phosphorus than uncolonized root segments (Fitter 2006). Other hypotheses to explain how plants may be able to reduce carbon allocation to non-beneficial AMF include acceleration of arbuscule senescence through plant production of H_2O_2, flavonoids, jasmonic acid, or other compounds (Vierheilig 2004; Kiers et al. 2010). Regardless of the mechanism, sanctions are not universally effective: plants experienced parasitism in at least 15% of studies of AMF and ectomycorrhizal fungi in a recent

meta-analysis (supplementary material to Hoeksema et al. 2010), suggesting that plants cannot completely control carbon allocation to AMF. The prevalence of parasitism in the field is unknown.

The second class of exceptions to these rules is that AMF abundance does not always decline with increases in the soil nutrient:light ratio (Johnson et al. 2003a; Treseder 2004). Some studies have found that fertilization has no effect or even a positive effect on AMF abundance (Treseder and Allen 2002; Johnson et al. 2003a; Treseder 2004). Hypotheses have been proposed to explain these counterintuitive patterns. First, plants might increase allocation to fungi, causing increases in fungal abundance, when fertilized with a non-limiting soil nutrient that exacerbates limitation by another soil nutrient (Johnson et al. 2003a). Stoichiometric theory of plant-mycorrhizal interactions typically focuses on above- versus below-ground resource limitation and ignores the fact that many different soil nutrients may limit plant growth. The relative abundance of different soil nutrients may therefore also affect plant-mycorrhizal interactions. Second, fungi might increase in abundance with fertilization because of fungal nutrient limitation in very nutrient-poor soils (Treseder and Allen 2002). However, no consensus on the explanatory power of these hypotheses has yet been reached.

Thirdly, plant species appear to differ in their interactions with AMF. Even when in the same environment, not all plants benefit equally from, and allocate equally to, AMF. The degree to which plants benefit from AMF is thought to be driven by plant traits such as resource uptake efficiencies and life history (Janos 1980; Graham et al. 1991; Hetrick et al. 1992; Johnson 1998; Johnson 2010). However, the degree to which different plant species are able to alter their interactions with AMF depending on resource stoichiometry remains an unanswered question.

The idea that the stoichiometry of limiting resources determines plant-mycorrhizal interactions is a compelling one, but many gaps in our understanding remain (Johnson 2010). First, plant parasitism sometimes occurs. Second, AMF abundance does not always decline with increases in relative abundance of soil nutrients. Third, plant species differences may affect the way in which plant mycorrhizal-interactions respond to changes in resource stoichiometry. In my dissertation work, I explored these ideas in four complementary studies.

The study system

To test the hypothesis that the stoichiometry of limiting resources would affect plant-mycorrhizal interactions differently in different plant species, I studied plants and AMF typical of grasslands and old-fields in southwest Michigan. Grasslands are a model ecosystem for studying the effects of nutrient addition on plant communities, and much attention has been paid to the switch from nutrient to light limitation in grasslands and its effects on diversity, productivity, and species interactions (Tilman 1988; Goldberg and Miller 1990; Suding et al. 2005; Dybzinski and Tilman 2007; Hautier et al. 2009). Previous work done at the W. K. Kellogg Biological Station has shown that nitrogen fertilization increases aboveground biomass and reduces light available beneath the plant canopy, leading to light limitation of seedlings and other small-statured plants (Foster and Gross 1998; Foster 1999). It is therefore an ideal system for investigating how plants and AMF respond to shifts from soil nutrient to light limitation.

In my empirical work, I compared plants of two different functional groups, C_3 grasses and C_4 grasses. These two functional groups of grasses differ in two key attributes: the degree to which they interact with AMF and their characteristic responses to fertilization. Because of

these differences, C_3 and C_4 grasses may differ in terms of how their interactions with AMF depend on the relative abundance of limiting resources. Therefore they comprise a good model system for understanding a range of plant-mycorrhizal responses to changes in resource availability.

From these functional groups, I focused on a few common species. I studied two species from the C_3 grass functional group: smooth brome (*Bromus inermis*) and quackgrass (*Agropyron* or *Elymus repens*). Both species are introduced clonal species that are widespread in abandoned agricultural fields, the dominant grassland type in southwest Michigan (personal observation). I also studied two native C_4 grasses, big bluestem (*Andropogon gerardii*) and little bluestem (*Schizachyrium scoparium*). These two C_4 grasses were once regionally abundant as dominant species in prairies, but are now only a minor component of the Michigan landscape (Foster 1999; personal observation). Along with other C_4 grasses, they are common dominant species in restored prairies.

C_3 and C_4 grasses differ in the degree to which they typically interact with AMF. In general, C_3 grasses associate only weakly with AMF, in some cases benefitting not at all from the interaction even in environments where other plants do (Wilson and Hartnett 1998). In contrast, C_4 grasses usually benefit greatly from the interaction with AMF (Wilson and Hartnett 1998). Their performance without AMF is sometimes so poor that they do not survive to reproduce, so some ecologists consider C_4 prairie grasses to be "obligately" mycorrhizal species (Wilson and Hartnett 1998). Differences in root morphology, specifically root diameter and

specific root length, likely cause the difference between functional groups in response to AMF (Hetrick et al. 1988a; Hetrick et al. 1988b). Because they benefit strongly from the interaction, C_4 grasses often support larger populations of AMF than C_3 grasses (Johnson et al. 1992; Miller et al. 1995).

In addition to differing in their characteristic reliance on AMF, these C_3 and C_4 grasses also differ in their responses to nutrient addition. Long-term fertilization experiments in Minnesota have shown that *E. repens* typically replaces *A. gerardii* and *S. scoparium* in nitrogen-enriched plots (Tilman 1988; Johnson et al. 2008). Furthermore, C_4 grass seedlings rarely colonize abandoned agricultural fields where soil nutrients are abundant and the dominant C_3 grasses are highly productive (Foster 1999). Foster's dissertation work at KBS (Foster and Gross 1998; Foster 1999) showed that light limitation is a likely cause of this reduced C_4 grass establishment in highly productive, fertile old-fields.

I took advantage of these differences between these C_3 and C_4 grasses to ask whether resource stoichiometry would affect plant-mycorrhizal interactions differently in different plant species. These C_3 and C_4 grasses also differ in other ways, such as phenology and their status as native or exotic to southwest Michigan. These additional differences could also affect their interactions with AMF. However, I did not investigate the specific trait differences driving functional group responses to AMF. Instead, I focused on asking whether species that are known to differ in many ways have characteristically different relationships with AMF. I used a combination of theoretical and empirical work to better understand how plant resource limitation

and allocation to AMF would affect plant benefit from the interaction and AMF abundance in response to changes in the abundance of limiting resources.

Outline of the dissertation

To understand to ask whether resource stoichiometry would affect plant-mycorrhizal interactions differently in different plant species, I conducted a series of studies. This dissertation presents the results of a mathematical model (Chapter 2), a greenhouse experiment (Chapter 3), and two field experiments (Chapters 4-5).

In Chapter 2, I describe a model of negotiation and trade between a plant and an arbuscular mycorrhizal fungus. Developed in collaboration with Chris Klausmeier and Todd Robinson, this model allows us to examine how the two partners take up carbon and soil nutrient and how they exchange these resources between them. We varied the relative abundance of light (carbon) and soil nutrient and investigated the effects on plant and fungal gain from trade. We scaled differences in resource availability to empirically observed ranges. We also asked whether plant and fungal gain from trade depended on the traits of the participating species. We looked specifically at trait differences between C_3 and C_4 grasses, exploring ranges of trait values that encompass both functional groups. Given a set of traits and environmental conditions, we allowed the plant and the fungus to adjust their allocation to uptake of both resources and asked whether either partner would specialize on uptake of the resource traded away. We then allowed the partners to negotiate a ratio at which to exchange carbon for nutrient and asked whether this exchange ratio varies predictably with species traits and resource availability. Finally, we asked whether these two key factors of trade, allocation to resource

uptake and the exchange ratio, affect the degree to which the partners benefit from the interaction.

In Chapter 3, I tested the hypothesis in the empirical study system. I manipulated the relative abundance of limiting resources (light and phosphorus) and asked whether different plant species differentially alter their interactions with AMF. In a greenhouse experiment, I measured the response of two C_3 grasses (*B. inermis* and *E. repens*) and two C_4 grasses (*A. gerardii* and *S. scoparium*) to the presence of AMF under different conditions of light and phosphorus availability. I predicted that because of their characteristic weak relationships with AMF, the two C_3 grasses would maintain a positive or neutral response to AMF by reducing carbon allocation to AMF in high phosphorus environments. In contrast, I predicted that the two C_4 grasses would allocate large amounts of carbon to AMF in all phosphorus environments because of their typical reliance on AMF. Consequently, I expected that the two C_4 grasses would be more vulnerable to parasitism by AMF in high phosphorus environments than the two C_3 grasses.

In Chapter 4, I tested an assumption of the hypothesis in the field. I asked whether seedlings experience light limitation in a natural setting. Understanding the prevalence of light limitation is essential for understanding whether seedlings should benefit from association with AMF. To measure light limitation of *A. gerardii* seedlings, I conducted a light addition experiment at each of six sites varying in productivity (Figs. 1.1-1.2). I expected to find that seedlings would respond more positively to light addition at high productivity sites where light availability was lowest. I also asked whether light availability affected seedling response to AMF, using a natural gradient in AMF abundance as a surrogate for mutualistic function. I

predicted that seedlings would be larger in sites with more abundant AMF, and that this positive response to AMF abundance would be even greater when seedling light limitation was alleviated by the light addition treatment.

In Chapter 5, I asked whether the relative abundance of different soil nutrients would affect plant allocation to AMF. Most studies of the ecological stoichiometry of plant-mycorrhizal interactions have ignored the possibility that limitation by multiple belowground resources could affect plant and fungal growth and allocation. In collaboration with Todd Robinson, I conducted a field experiment (Fig. 1.2) where we established plots dominated by either *A. gerardii* or *B. inermis* and applied nineteen different combinations of nitrogen and phosphorus manipulations. We tested two hypotheses to explain potential increases in AMF abundance in response to fertilization. First, we asked whether AMF experienced direct nutrient limitation and would increase in abundance with fertilization in very low nutrient soils. Second, we asked whether nitrogen and phosphorus "imbalance" (N:P ratio) would determine plant allocation to AMF.

Chapter 6 summarizes my major findings. I synthesize my results to test the hypothesis that resource stoichiometry affects plant-mycorrhizal interactions differently in different plant species. I also discuss topics for future research that are motivated by these results.

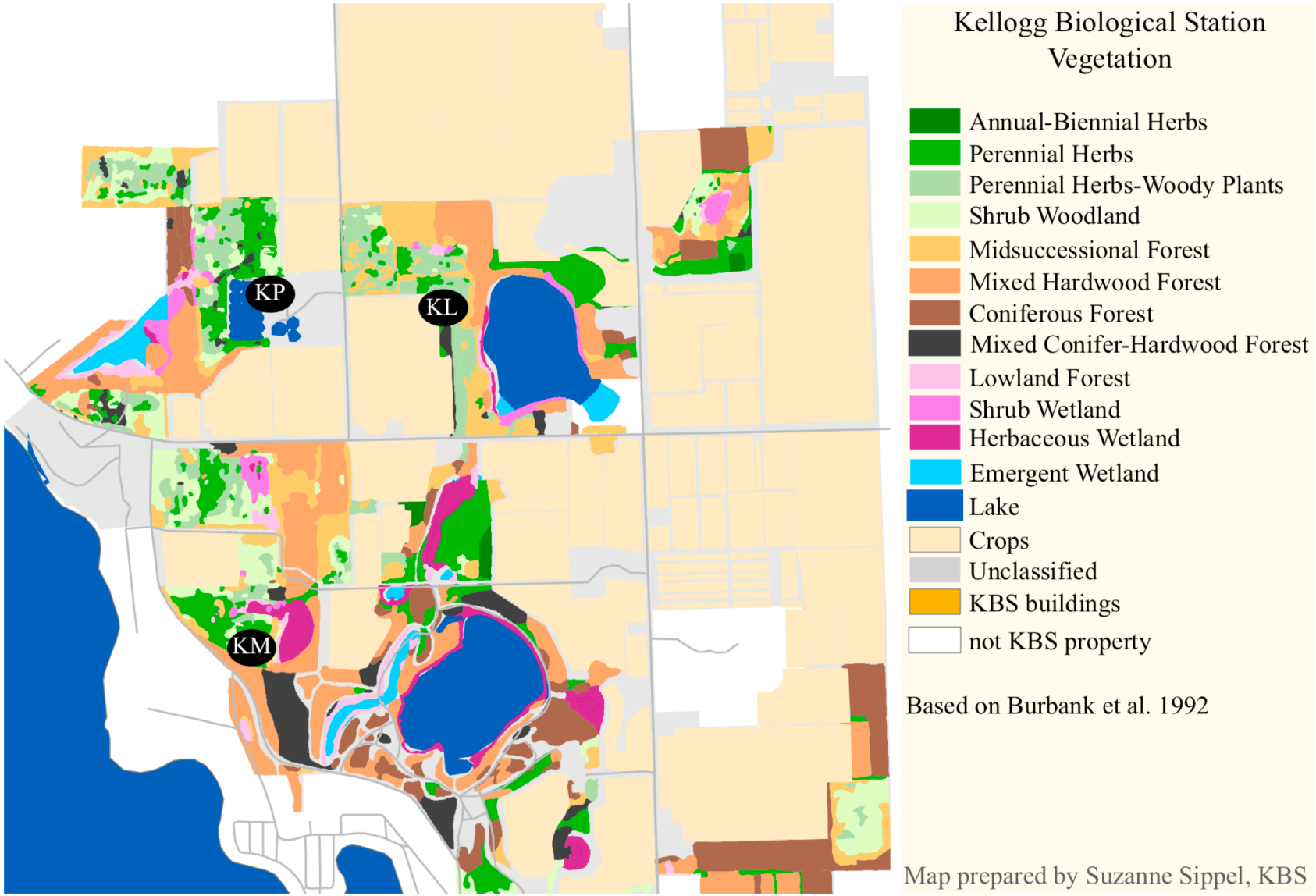
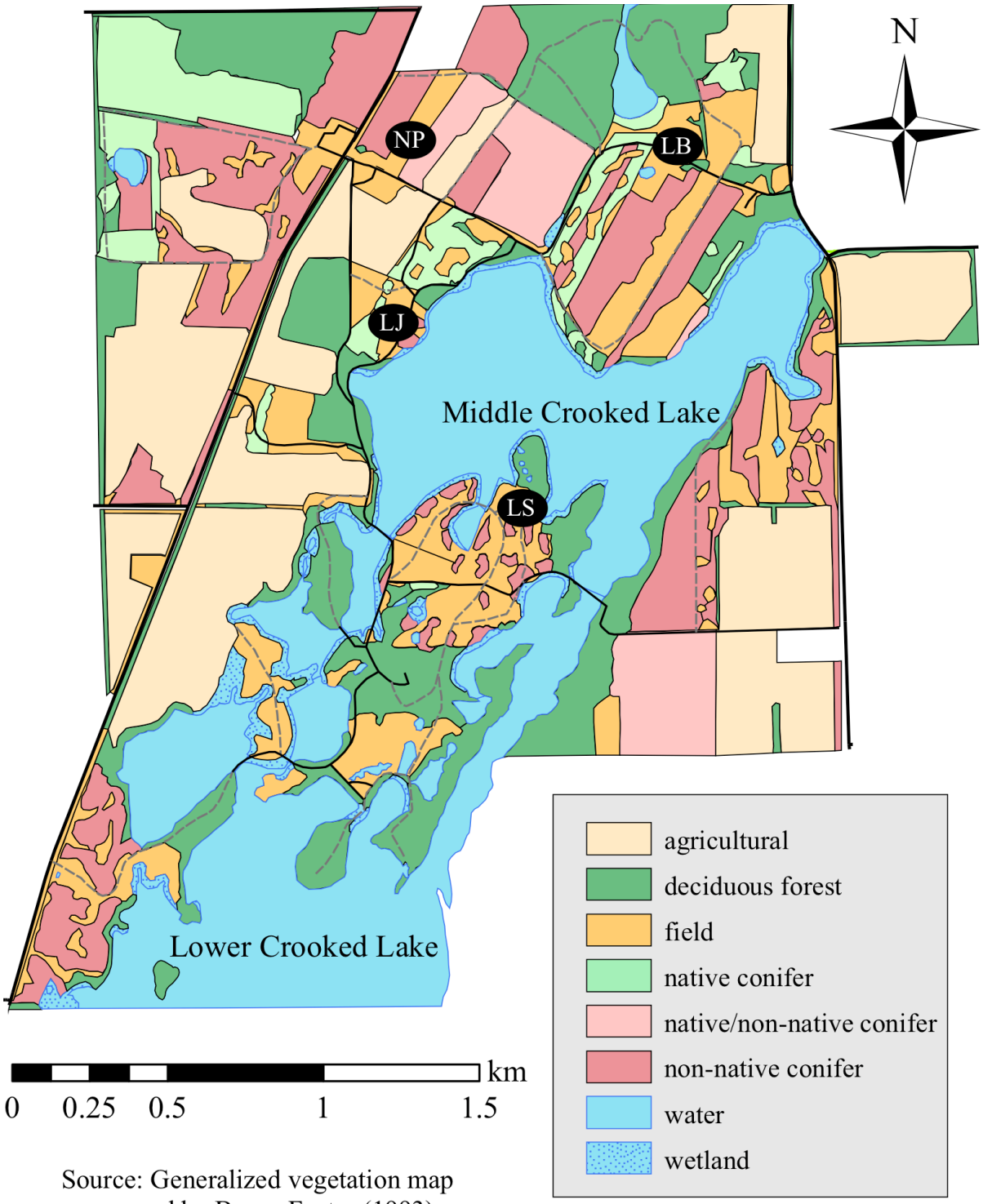


Fig. 1.1: Field sites near the W. K. Kellogg Biological Station's academic center. Sites KM, KL, and KP were all used in the field light addition experiment (Chapter 4). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.



Source: Generalized vegetation map prepared by Bryan Foster (1993) from his 1991 vegetation map.

Map prepared by Suzanne Sippel, KBS, 2005

Fig. 1.2: Field sites in KBS's Lux Arbor Reserve (Prairieville, MI). I conducted the field light addition experiment (Chapter 4) at sites LS, LJ, and LB and the field fertilization experiment (Chapter 5) at site NP. I used soil from site NP in the greenhouse experiment (Chapter 3).

CHAPTER TWO

Ecological specialization and trade affect the outcome of negotiations in mutualism

with Todd M P Robinson and Christopher A Klausmeier

Abstract

By definition, mutualisms involve the exchange of benefits between partners. This trade of one benefit for the other usually increases the growth, survival, or fitness of partners, but sometimes mutualistic interactions have neutral or negative effects. Environmental conditions, species traits, and population sizes can explain some of this variation in the degree to which partners benefit from trade, but much variation remains unexplained. We explored the role of two key features of trade in explaining this variation: allocation to participation and the ratio of benefits exchanged. Using the specific example of the plant-arbuscular mycorrhizal mutualism, we built a model of uptake and trade of two resources, phosphorus and carbon. We found that for most combinations of empirically-derived parameter values, the mycorrhizal fungus allocated to specialization on phosphorus uptake, maximizing its participation in trade, while the plant allocated to uptake of both phosphorus and carbon. In other environmental conditions, for example where phosphorus was abundant and light was scarce, the plant was the specialist partner, taking up only carbon and relying on trade for its phosphorus requirements. Plant and fungal optimal allocation to uptake of one or both resources frequently affected the exchange ratio of carbon for phosphorus. These two key features of trade, allocation and the exchange ratio, varied with environmental conditions and strongly affected the degree to which the plant and the fungus gained from trade. Although we did not model parasitism by mycorrhizal fungi,

we found that environmental conditions, species traits, and population sizes did affect the outcome of trade. Furthermore, the direction and magnitude of the effect was governed by allocation and the exchange ratio. These results suggest potentially important avenues for empirical work linking these key features of trade with aspects of the environment to determine the outcome of mutualism.

Introduction

Mutualisms are ubiquitous, with most species on the planet participating in at least one mutualistic interaction (Bronstein 2001). Furthermore, mutualisms can be an important force structuring communities. Mutualisms may play a role in determining species ranges, perhaps expanding fundamental niches (de Mazancourt and Schwartz 2010). Initially thought to be a destabilizing force (May 1976), it is now appreciated that mutualisms can stabilize communities (Goh 1979; Okuyama and Holland 2008). However, mutualisms are only one conditional outcome of species interactions: interactions that sometimes result in positive outcomes for the participating species may sometimes result in neutral or negative outcomes, depending on the ecological context in which the interaction occurs (Bronstein 1994). Specifically, factors such as the traits of interacting species, population sizes, and environmental conditions can be important in determining how beneficial a mutualism will be for one or both partners. Although many ecologists have recognized this "context dependency" of mutualisms, it has been difficult to develop an understanding of *which* factors are the most important in determining the outcome of a mutualism. This context dependency makes it difficult to understand the role of mutualisms in structuring communities. Explaining and understanding the context-dependent outcome of

species interactions has been a major focus of study, both empirically and theoretically, and continues to be one of the most pressing questions facing ecologists (Agrawal et al. 2007).

Although the outcome of mutualism can vary, all mutualisms share at least one feature: trade. One partner in a mutualism exploits the other for some good or service and, in exchange, allows its partner to obtain some good or service from itself. Behaviors, such as dispersal or defense against herbivory, may be exchanged for other behaviors, or for resources such as sugars. Bronstein (2001) identified 3 major classes of mutualisms (protection, transportation, and nutritional mutualisms) and trade occurs in each class. However, it may be easiest to study or quantify trade in nutritional mutualisms. Nutritional mutualisms involve reciprocal fluxes of resources such as sugars, nitrogen, phosphorus, or other organic or inorganic chemicals. Understanding these fluxes into and out of each organism may help elucidate the variation in the outcome of mutualisms.

Allocation and the exchange ratio are two key features of trade that can cause variation in the outcome of mutualisms. Organisms may influence the amount traded by adjusting their own allocation to direct uptake of the resources exchanged. An organism might "invest" in its partner by increasing uptake of the resource it trades away. For example, plants supporting belowground nutritional mutualists such as rhizobia or mycorrhizal fungi might increase carbon uptake (photosynthesis) in order to increase carbon allocation to their partner (Miller et al. 2002). Clearly, optimal allocation to either trade or independent uptake will depend on how efficiently each partner can provide the resource. When each partner is relatively more efficient at taking up the resource that it trades away than the partner receiving the resource, conditions of relative advantage exist (Ricardo 1817; Schwartz and Hoeksema 1998). Under these conditions of relative advantage, optimal allocation strategies should lead to partners that specialize on uptake

of the resource they trade away (Schwartz and Hoeksema 1998). For plants and belowground mutualists, this would mean that the plant should specialize on uptake of carbon while the belowground mutualist should specialize on nutrient provisioning. However, empirical data indicate that plants do not always allocate carbon to belowground mutualists (Johnson 2010), suggesting that there may be variation in the optimal strategy. To understand the outcome of mutualisms, we must understand the partners' optimal allocation strategies and deviations from those strategies, as well as the costs and benefits of trade.

The second key feature of trade is the exchange ratio. It is clear that the gains obtained from the interaction depend strongly on the magnitude of both the cost (what is exchanged away) and the benefit (what is received in return). In other words, the ratio at which resources are exchanged may affect whether a mutualism is beneficial or not (Schwartz and Hoeksema 1998). Favorable exchange ratios are those for which the price of one resource traded for the other results in "cheaper" acquisition of the obtained resource for both partners. Under conditions of relative advantage, it is possible to identify this range of favorable exchange ratios (Schwartz and Hoeksema 1998). If trade at a favorable exchange ratio is enforced, mutualism based on relative advantage will be evolutionarily stable (McGill 2005).

However, even among exchange ratios that are mutually beneficial, variation could cause differences in the degree to which partners benefit from the mutualism. Each partner would gain the most by minimizing its cost and maximizing its benefit (Schwartz and Hoeksema 1998; Bronstein 2001), creating a conflict of interest between the partners. Although this conflict of interest could lead to selection for one partner to "cheat" and obtain the benefit of trade without paying the cost (West et al. 2002), Bronstein (2001) points out that this need not be the case for all mutualisms. Furthermore, many mutualisms typically result in positive outcomes for both

partners (Karst et al. 2008; Chamberlain and Holland 2009; Hoeksema et al. 2010), suggesting that partners somehow negotiate a mutually beneficial exchange ratio. To determine how the partners would solve this conflict of interest to negotiate a single mutually beneficial exchange ratio, Akçay and Roughgarden (2007a) built a mechanistic model of trade between rhizobia and legumes. The negotiated exchange ratio turned out to be identical to the Nash Bargaining Solution (Nash 1950; Nash 1953), an outcome commonly found in cooperative game theory (Akçay and Roughgarden 2007a). However, they did not explore whether this negotiated exchange ratio would be context dependent, whether it would interact with the partners' allocation strategies, or whether it would explain variation in the outcome of the mutualism.

We asked whether these two key features of trade, allocation and the exchange ratio, could help predict the degree to which two partners would gain from a mutualistic interaction. We then varied species traits and resource availability to explore the context dependency of the relationship. Uniting and building on the key results of Akçay and Roughgarden (2007a) and Schwartz and Hoeksema (1998), we modeled a classic example of a nutritional mutualism, that between plants and mycorrhizal fungi. In this interaction, fungi develop a network of hyphae in the soil and plant roots. Plants transfer photosynthetically-fixed carbon to the fungi in exchange for a range of benefits, primarily increased soil nutrient uptake (Smith and Read 1997). Plants are capable of taking up soil nutrients directly, but mycorrhizal fungi could be more efficient because hyphae require less carbon to build than roots (Smith and Read 1997). Therefore plant allocation to mycorrhizae may be a better investment than allocation towards direct uptake of soil nutrients. Plants and fungi differ in their relative requirements for carbon and nutrients, which could also affect the benefit of trade (Hoeksema and Schwartz 2003). Empirically, plant benefit from fungi is highly variable and the causes of the variation are not yet completely

understood. A recent meta-analysis of hundreds of lab and field studies explained only 23-41% of variation in plant benefit, despite including at least eight important predictor variables such as species identity, species traits, and soil fertility (Hoeksema et al. 2010). This suggests that not all important predictor variables could be included in that analysis because they have not been thoroughly examined. In particular, context-dependent variation in the fluxes of carbon and soil nutrients is insufficiently understood.

We modeled variation in the outcome of trade between a plant and a fungus able to take up soil nutrient and carbon in conditions of relative advantage (Schwartz and Hoeksema 1998) and negotiate an exchange ratio according to the Nash Bargaining Solution (Akçay and Roughgarden 2007a) on a very short (behavioral) timescale. Parasitic outcomes are not possible with use of the Nash Bargaining Solution, but the model does predict variation in the magnitude of benefit experienced by both partners. Although we discuss the model in the context of a specific example (plants and arbuscular mycorrhizal fungi), it is conceptually applicable to all mutualisms because all involve trade. This model allows us to answer three ecological questions: 1) Will both partners specialize on uptake of the resource traded away? 2) What exchange ratio will they negotiate? 3) Can these two features of trade help explain why key predictors fail to fully explain variation in the outcome of mycorrhizal mutualisms? We parameterized the model to explore realistic ranges of variation in critical ecological conditions, specifically the traits of the partners (resource uptake efficiency), community structure (relative population sizes), and environmental conditions (carbon/light and soil nutrient availability).

The model

We model plant growth rate per unit biomass (g_P) as the minimum of the growth allowed by two limiting resources, carbon (C) and a soil nutrient (hereafter "nutrient"; N). We model the plant as a population consisting of a single, clonal individual that is genetically homogenous. Available carbon and nutrient are both assumed to be constant on the timescale of this model. Plant acquisition of available nutrient is the sum of direct uptake and nutrient gained in trade. Nutrient taken up is evenly distributed throughout the population. The rate of direct nutrient uptake per unit biomass is denoted by f_{NP}, which can be thought of as a function of available nutrient in the soil and allocation of effort to direct uptake. The amount of nutrient obtained through trade is determined by X, the total flux of carbon exchange (summed across the entire plant population), and by T, the C:N exchange ratio which determines how much carbon plants give up for a given amount of nutrient gained through trade. The amount of growth allowed by the plant's nutrient income is the product of its total nutrient acquisition and the yield of biomass per unit nutrient (Y_{NP}). Similarly, plant acquisition of carbon is the difference between photosynthetically-driven carbon assimilation (f_{CP}) and carbon lost in trade, which is converted into carbon-limited growth rate by Y_{CP}.

$$g_P = \min\left[Y_{NP}\left(f_{NP} + \frac{X}{PT}\right), Y_{CP}\left(f_{CP} - \frac{X}{P}\right)\right] \quad (1)$$

We model fungal growth (g_F) similarly, except that the fungus gains carbon and loses nutrient as a result of trade. Note that the fungus is capable of direct carbon uptake, so this model is

appropriate for both arbuscular (f_{CF}=0) and ectomycorrhizal fungi (f_{CF}>0), as well as other nutritional mutualisms such as lichens, corals, and legumes and rhizobia.

$$g_F = \min\left[Y_{NF}\left(f_{NF} - \frac{X}{FT}\right), Y_{CF}\left(f_{CF} + \frac{X}{F}\right)\right] \quad (2)$$

Henceforth we measure species biomass in terms of carbon, so we set the yield coefficients Y_{CP}= Y_{CF}=1 without loss of generality; in this case Y_{NP} and Y_{NF} represent the C:N stoichiometry of the plant and fungus respectively.

The plant and the fungus can modify the amount of nutrient and carbon taken up by adjusting allocation to resource uptake. We model allocation as the proportion of effort dedicated to obtaining one resource at the expense of the other. Plant allocation for direct nutrient uptake is A_{NP} and allocation for direct carbon uptake is 1-A_{NP}; fungus allocation for direct carbon uptake is A_{CF} and allocation for direct nutrient uptake is 1-A_{CF}. Uptake is proportional to effort allocated to that resource, so there is a linear trade-off between carbon and nutrient acquisition. Therefore, f_{NP}= $A_{NP}f'_{NP}$, f_{CP}= (1-A_{NP})f'_{CP}, f_{NF}= (1-A_{CF})f'_{NF}, and f_{CF}= $A_{CF}f'_{CF}$. Rates of direct resource uptake efficiency (f'_{NF}, f'_{NP}, f'_{CF}, and f'_{CP}) are functions of fixed traits of the organisms (e.g., species specific morphology) and of resource available in the environment. Because we are interested in situations of relative advantage, we assume the plant is better at taking up carbon than nutrient ($f'_{CP} \geq f'_{NP}$) and that the fungus is better at taking up nutrient than carbon ($f'_{NF} \geq f'_{CF}$). Notation is summarized in Table 2.1.

Table 2.1: List of parameters in the model, their biological interpretations, and estimates of parameter values with references, using phosphorus as the soil nutrient (N) and assuming 50% of biomass was carbon (C). See Appendix for details of calculations. Where possible, values for plant parameters were taken from *Andropogon gerardii*, *Bromus inermis,* and *Elymus repens*. References are as follows: 1 Mahaney et al. 2008, 2 Chapters 3-5, 3 Jonas and Joern 2008, 4 Elser et al. 2000, 5 Allred et al. 2010, 6 Awada et al. 2003, 7 Miller et al. 1987, 8 Cleveland and Liptzin 2007, 9 Miller et al. 1995, 10 Sanders and Tinker 1973, 11 Craine et al. 2002, 12 Pearson and Jakobsen 1993, 13 Lee et al. 2003, 14 Niklas and Cobb 2006, 15 Jakobsen et al. 1992, 16 Schweiger and Jakobsen 1999, 17 Smith et al. 2000, 18 Smith et al. 2004, 19 Smith 1982, 20 McGonigle and Fitter 1988.

Parameter	Interpretation [units]	Range of values	Ref.
A_{NP}	Plant allocation to nutrient uptake [(g C root) (g C plant)$^{-1}$]	0.3–0.8	1, 2, 11
Y_{NP}	Plant carbon yield per unit nutrient [(g plant C m^{-2}) (g plant N^{-1} m^{-2})]	150–500	3, 4, 14
f'_{NP}	Plant nutrient uptake rate [(g N m^{-2})(sec^{-1}) (g root C m^{-2})$^{-1}$]	3.4–600 * 10^{-10}	9-11, 19, 20
V_{CP}	Plant max carbon uptake (photosynthesis) rate [(g C fixed m^{-2})(sec^{-1}) (g shoot C m^{-2})$^{-1}$]	9.7–67.2 * 10^{-7}	5, 6, 13
K_{CP}	Plant carbon half saturation constant [(g C) (μmol m^{-2} sec^{-1})]	200–400	5, 6
A_{CF}	Fungal allocation to carbon uptake [(g C intraradical) (g C fungus)$^{-1}$]	0.33–0.9	7
Y_{NF}	Fungus carbon yield per unit N [(g fungal C m^{-2}) (g fungal N^{-1} m^{-2})]	30–100	8
f'_{NF}	Fungus nutrient uptake rate [(g N m^{-2})(sec^{-1})(g extraradical C m^{-2})$^{-1}$]	1.2–124 * 10^{-6}	9, 15-18
V_{CF}	Fungus max carbon uptake rate [(g C m^{-2})(sec^{-1}) (g intraradical C m^{-2})$^{-1}$]	0	
K_{CF}	Fungus carbon half saturation constant [(g C) (μmol m^{-2} sec^{-1})]	NA	

Table 2.1 (cont'd)

Parameter	Interpretation [units]	Range of values	Ref.
P	Plant biomass [(g plant C) m^{-2}]	686–2128.5	1, 11
F	Fungal biomass [(g fungus C) m^{-2}]	0.006–45	9, 2
N	Nutrient available in the environment [(g N) (g soil)$^{-1}$]	10^{-8}–0.0005	2
C	Carbon (light) available in the environment [µmol m^{-2} sec^{-1}]	100–1600	2
X	Amount of carbon exchanged [(g C) m^{-2} sec^{-1}]		
T	Exchange (trade) ratio of carbon for nutrient [(g C) (g N)$^{-1}$]	0.004–0.151	12

Plant and fungal growth rates are context-dependent in our model. Specifically, they depend on population sizes (P and F), organismal stoichiometry (Y_{NP} and Y_{NF}), and carbon and nutrient uptake rates (f'_{NF}, f'_{NP}, f'_{CF}, and f'_{CP}). Interactions modeled here (allocation decisions and exchange ratio negotiations) take place on much faster timescales than changes in species abundances or nutrient availabilities, so those parameters act as fixed here. Note that although the plant and the fungus take up carbon and nutrient through very different mechanisms (e.g., the plant fixes carbon through photosynthesis in the leaves and takes up nutrient through the roots while the fungus takes up soil carbon and nutrient through extra-radical hyphae), those differences do not matter because we do not model resource depletion. In addition to these species-specific or environmental conditions, plant and fungus growth also depend on the

quantity of nutrients obtained through trade, which could also depend on those species-specific or environmental contexts.

Model analysis

To understand how the two key features of trade, allocation and the exchange ratio, affect the outcome of trade, we analyzed the effect of environmental parameters on three inter-related factors: the amount exchanged (X), allocation to uptake of the resource each species is better at obtaining (A_{NP} and A_{CF}), and the exchange ratio (T). Modeling the outcome of trade between plants and mycorrhizal fungi requires that we solve these three problems simultaneously, but we will describe them sequentially to build up the problem. First, we will fix the allocation (A_{NP} and A_{CF}) and the exchange ratio (T) to examine what determines the volume of trade (X). Second, given X, we then allow plants and fungi to optimize A_{NP} and A_{CF} to maximize their growth rates. Finally, we allow plants and fungi to negotiate T while simultaneously determining X and optimizing A_{NP} and A_{CF}.

1) What determines the amount of resources exchanged, X?

Clearly, the amount of carbon and nutrient traded will determine how beneficial the interaction is for both partners, the plant and the fungus. In general, one partner or the other will limit the amount traded by failing to produce enough resource to "match" its partner's production. Which partner will it be, and how much surplus will the other have left over? We solve this problem by temporarily fixing allocation and the exchange ratio and determining the

amount of surplus plant carbon and surplus fungal nutrient. Surplus plant carbon is simply the amount of carbon taken up, minus the carbon traded away, minus the amount of nutrient acquired (directly or through trade) converted into units of carbon according to plant C:N stoichiometry, Y_{NP}.

$$S_{CP} = f_{CP} - \frac{X}{P} - Y_{NP}\left(f_{NP} + \frac{X}{PT}\right) \geq 0 \tag{3}$$

Surplus fungal nutrient is found similarly:

$$S_{NF} = f_{NF} - \frac{X}{FT} - \frac{1}{Y_{NF}}\left(f_{CF} + \frac{X}{F}\right) \geq 0 \tag{4}$$

If either (3) or (4) fails to hold, trade does not occur (X=0). If (3) and (4) hold, then the plant is nutrient- or co-limited by carbon and nutrient and the fungus is carbon- or co-limited by carbon and nutrient. Since surplus is the resource leftover after trade or growth, at least one partner will have no surplus when matching its partner's contribution. This partner limits trade and specializes on uptake of the resource it trades away. We consider three possible cases: plant-, fungus-, and co-limited trade.

Plant-limited trade: If the plant limits trade, we set $S_{CP}=0$ and solve for X:

$$X_{P\text{-lim}} = \frac{PT\left(f_{CP} - f_{NP}Y_{NP}\right)}{T + Y_{NP}} \tag{5}$$

Substituting (5) into (4) we find surplus fungal nutrient to be

$$S_{NF} = f_{NF} - \frac{f_{CF}}{Y_{NF}} - \frac{P}{F}\cdot\frac{1}{Y_{NF}}\cdot\frac{\left(f_{CP} - f_{NP}Y_{NP}\right)\left(T + Y_{NF}\right)}{T + Y_{NP}} \tag{6}$$

For trade to be plant-limited, we require the fungus to have surplus nutrient, $S_{NF}\geq 0$ or

equivalently,

$$\frac{F\left(f_{NF}Y_{NF} - f_{CF}\right)}{T + Y_{NF}} \geq \frac{P\left(f_{CP} - f_{NP}Y_{NP}\right)}{T + Y_{NP}} \tag{7}$$

Fungus-limited trade: If the fungus limits trade, we set $S_{NF}=0$ and solve for X:

$$X_{F\text{-lim}} = \frac{FT\left(f_{NF}Y_{NF} - f_{CF}\right)}{T + Y_{NF}} \tag{8}$$

Surplus plant carbon is

$$S_{CP} = f_{CP} - f_{NP}Y_{NP} - \frac{F}{P} \cdot \frac{\left(f_{NF}Y_{NF} - f_{CF}\right)\left(T + Y_{NP}\right)}{T + Y_{NF}} \tag{9}$$

For trade to be fungus-limited, we require the plant to have surplus carbon, $S_{CP} \geq 0$ or equivalently,

$$\frac{P\left(f_{CP} - f_{NP}Y_{NP}\right)}{T + Y_{NP}} \geq \frac{F\left(f_{NF}Y_{NF} - f_{CF}\right)}{T + Y_{NF}} \tag{10}$$

Co-limited trade: Clearly the only way for both (7) and (10) to hold is if $S_{CP}=0$ and $S_{NF}=0$, in which case neither partner has surplus and trade is perfectly balanced and co-limited by the plant and the fungus.

2) How do the plant and the fungus adjust their allocation to carbon or nutrient uptake, A_{CF} and A_{NP}?

While finding the amount exchanged (X) helps determine the benefit of trade between the plant and the fungus, it is clear that X depends on how the plant and the fungus allocate to nutrient or carbon uptake on a short behavioral timescale. We model allocation as a linear trade-off scaled to 1: plant allocation to nutrient uptake is A_{NP} and to carbon uptake is $1\text{-}A_{NP}$; fungal allocation to nutrient uptake is $1\text{-}A_{CF}$ and to carbon uptake is A_{CF} (Klausmeier et al. 2007). A plant heavily allocated towards uptake of nutrient (high A_{NP}) will have less surplus carbon to trade than a plant with lower nutrient uptake (low A_{NP}). Should a nutrient-limited plant increase direct nutrient uptake (increase A_{NP}), or should it increase its surplus carbon for trade with a carbon-limited fungus (decrease A_{NP})? Fixing the exchange ratio T and using the process for solving X shown above for plant-limited, fungal-limited, or co-limited trade, we next allow the plant and the fungus to optimize A_{CF} and A_{NP} to maximize their growth rates.

In the absence of trade (X=0), species maximize fitness when they are colimited by both growth-limiting resources (Bloom et al. 1985; Abrams 1987b; Klausmeier et al. 2007). To find the optimal A_{NP} without trade, we equate the two terms in the minimum (Eq. 1) and solve for A_{NP}, which is

$$A^*_{NP,\text{no trade}} = \frac{f'_{CP}}{f'_{CP} + f'_{NP} Y_{NP}} \tag{11}$$

Similarly, the optimal fungal allocation to carbon uptake without trade is

$$A^*_{CF,\text{no trade}} = \frac{f'_{NF} Y_{NF}}{f'_{CF} + f'_{NF} Y_{NF}} \tag{12}$$

Substituting (11) and (12) into the definitions of plant and fungal growth rates (Eq. 1 and 2 after substituting f' and the appropriate allocation (A) term for f), we get

$$g^*_{P,\text{no trade}} = \frac{f'_{CP} f'_{NP} Y_{NP}}{f'_{CP} + f'_{NP} Y_{NP}} \tag{13}$$

$$g^*_{F,\text{no trade}} = \frac{f'_{CF} f'_{NF} Y_{NF}}{f'_{CF} + f'_{NF} Y_{NF}} \tag{14}$$

When there is trade (X>0), plant and fungal growth rates depend on X, which depends on which partner limits trade. When the plant limits trade, plant fitness (Eq. 1), after substituting the appropriate f' and allocation (A) terms for f, simplifies to:

$$g_{P,\text{P-lim}} = Y_{NP} \frac{A_{NP} f'_{NP} T + \left(1 - A_{NP}\right) f'_{CP}}{T + Y_{NP}} \tag{15}$$

The derivative of plant growth rate with respect to A_{NP} is negative when $T < f'_{CP}/ f'_{NP}$, when the plant limits trade. In this case, the price (T) the plant pays for nutrient by trading carbon is less than the opportunity cost of allocating more effort to taking up nutrient directly. Therefore the plant benefits by decreasing its own uptake of nutrient (decreasing A_{NP}), increasing its uptake of carbon, and allocating more carbon to trade (Fig. 2.1). This is true as long as the plant limits trade and the fungus can "match" plant carbon with fungal nutrient.

When the fungus limits trade, plant fitness is:

$$g_{P,\text{F-lim}} = Y_{NP}\left(A_{NP} f'_{NP} - \frac{F}{P} \cdot \frac{A_{CF} f'_{NF} - \left(1 - A_{CF}\right) f'_{NF} Y_{NF}}{T + Y_{NF}} \right) \tag{16}$$

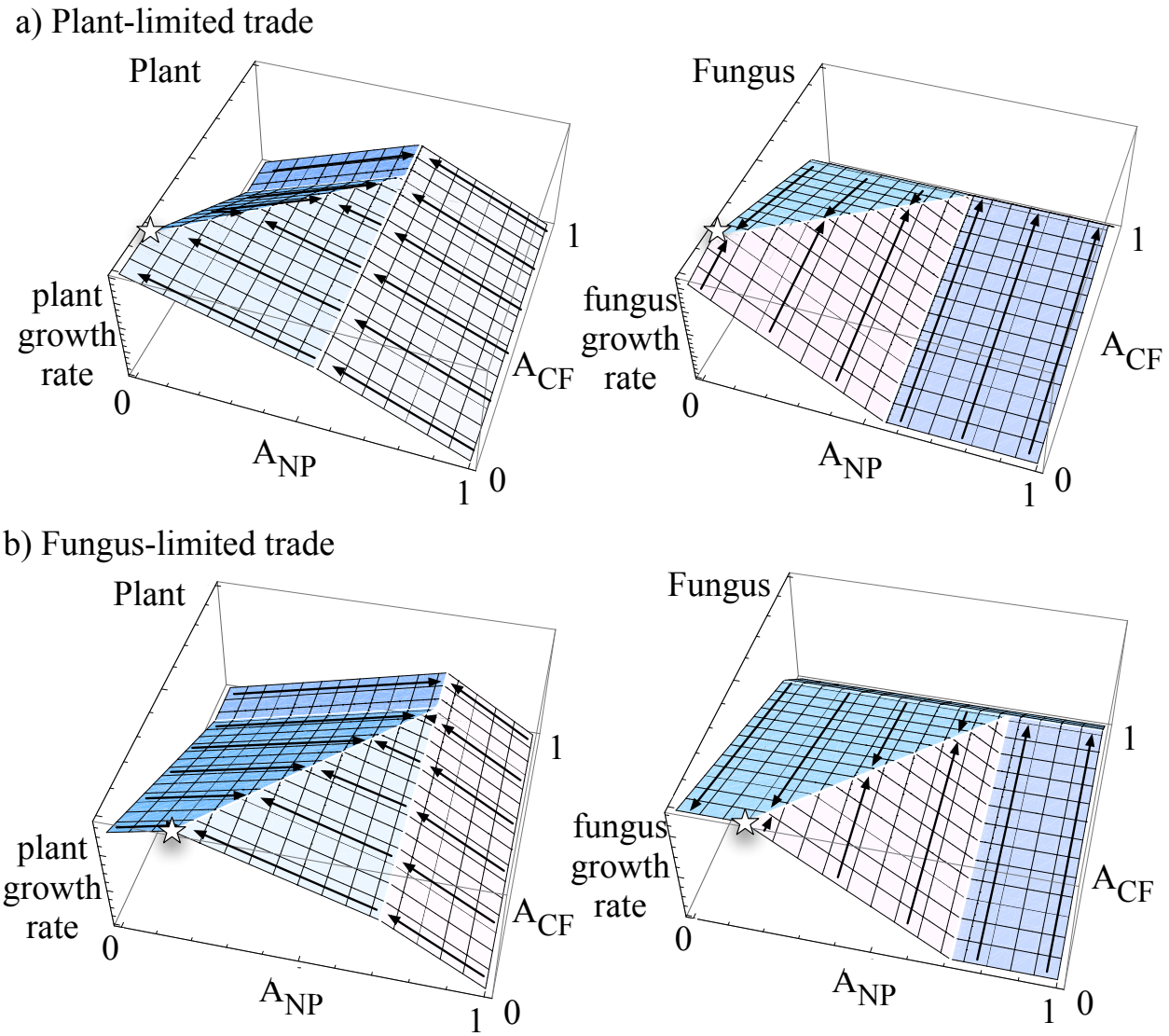


Fig. 2.1: Plant allocation to nutrient uptake (A_{NP}), fungal allocation to carbon uptake (A_{CF}), and their effects on plant and fungal growth rates. Depending on the specific environmental conditions, the plant (a), the fungus (b), or both will limit trade. Arrows indicate the direction of increasing plant or fungal fitness. The star indicates the optimal allocation strategy. Parameters were set at T=1, Y_{CP}=1, Y_{NF}=1, Y_{CF}=1, f'_{CP}=10, f'_{NP}=2, f'_{NF}=10, f'_{CF}=2, F=1, P=2; in a) Y_{NP}=4; in b) Y_{NP}=2.

The derivative of fungus-limited plant growth rate with respect to A_{NP} is always positive, so the plant should increase its allocation to nutrient uptake even though nutrient obtained through trade is cheaper than nutrient taken up directly. This is because when trade is fungus-limited,

increases in surplus plant carbon will not result in greater X because the fungus cannot supply enough nutrient to match trade. Therefore when $T < f'_{CP}/f'_{NP}$, for a given fungus allocation, plant allocation is optimal when trade is matched by the plant and the fungus. If trade is plant-limited for all A_{NP}, then the optimal $A^*_{NP}=0$.

Analysis of the optimal fungal strategy is similar because the fungal growth rate function is symmetrical with the plant growth rate function. We find that when $f'_{CF}/f'_{NF}<T$, for a fixed plant allocation, fungus allocation is optimal when trade is matched if possible, otherwise $A^*_{CF}=0$.

The set of A_{NP} and A_{CF} that eliminates surplus resources is defined by the case of equality in (7) and (10), substituting the appropriate f' and allocation terms for f:

$$F\frac{A_{NF}f'_{NF}Y_{NF}-\left(1-A_{NF}\right)f'_{CF}}{T+Y_{NF}}=P\frac{A_{CP}f'_{CP}-\left(1-A_{CP}\right)f'_{NP}Y_{NP}}{T+Y_{NP}} \qquad (17)$$

This parametrically defines a line where both partners are co-limited by carbon and nutrient and trade is matched by both partners (the ridge in Fig. 2.1). It can be shown that when $f'_{CF}/f'_{NF}<T<f'_{CP}/f'_{NP}$, growth rates of both partners increases as A_{NP} and A_{CF} decrease along the ridge of matched trade. Therefore the optimum allocation strategy for both partners is when one or both of the partners is completely allocated towards uptake of the resource traded away. This optimal allocation strategy will not be affected by the relative rates of plant and fungal allocation adjustments. The optimal allocation strategy is best for both partners, even though each partner acts selfishly to maximize its fitness given the allocation strategy of its partner. Graphically, the optimal allocation strategy is the intersection of the ridge of matched

trade (Eq. 17) with one of two axes: $A_{CF}=0$ or $A_{NP}=0$ (Fig. 2.1). This yields three possible scenarios depending on which partner limits trade.

Plant-limited case: At the optimal allocation strategy, the plant ultimately limits trade when the line of matched trade defined by Eq. 17 intersects the A_{CF} axis (Fig. 2.1a). In this case,

$$A^*_{NP,P-\lim} = 0 \tag{18a}$$

$$A^*_{CF,P-\lim} = \frac{Ff'_{NF}Y_{NF}\left(T+Y_{NP}\right) - Pf'_{CP}\left(T+Y_{NF}\right)}{F\left(f'_{CF}+f'_{NF}Y_{NF}\right)\left(T+Y_{NP}\right)} \tag{18b}$$

Therefore the plant specializes on carbon uptake and the fungus acquires carbon through both trade and uptake. Substituting the optimal strategies $A^*_{NP,P\text{-lim}}$ and $A^*_{CF,P\text{-lim}}$ into plant and fungal growth rates (Eq. 1 and 2), we find

$$g^*_{P,P-\lim} = \frac{f'_{CP}Y_{NP}}{T+Y_{NP}} \tag{19a}$$

$$g^*_{F,P-\lim} = \frac{Y_{NF}\left(f'_{CP}f'_{NF}PT + f'_{CF}\left(Ff'_{NF}\left(T+Y_{NP}\right) - Pf'_{CP}\right)\right)}{F\left(f'_{CF}+f'_{NF}Y_{NF}\right)\left(T+Y_{NP}\right)} \tag{19b}$$

Trade is plant-limited if the optimal fungal allocation strategy includes some direct carbon uptake by the fungus, $A^*_{CF,P\text{-lim}} \geq 0$ or, by setting Eq. 18b ≥ 0 and rearranging, it is equivalently

$$\frac{Ff'_{NF}Y_{NF}}{T+Y_{NF}} \geq \frac{Pf'_{CP}}{T+Y_{NP}} \tag{20}$$

Fungal-limited case: The fungus ultimately limits trade when the ridge of matched trade

defined by Eq. 17 intersects the A_{NP} axis (Fig. 2.1b). The fungus specializes on nutrient uptake and the plant acquires nutrient through both trade and uptake. As above, we can find the optimal strategies and the corresponding growth rates:

$$A^*_{CF,F\text{-lim}} = 0 \tag{21a}$$

$$A^*_{NP,F\text{-lim}} = \frac{f'_{CP}P\left(T+Y_{NF}\right) - Ff'_{NF}Y_{NF}\left(T+Y_{NP}\right)}{P\left(f'_{CP}+f'_{NP}Y_{NP}\right)\left(T+Y_{NF}\right)} \tag{21b}$$

$$g^*_{F,F-\text{lim}} = \frac{f'_{NF}Y_{NF}T}{T+Y_{NF}} \tag{22a}$$

$$g^*_{P,F-\text{lim}} = \frac{Y_{NP}\left(f'_{CP}\left(Ff'_{NF}Y_{NF}+Pf'_{NP}\left(T+Y_{NF}\right)\right) - Ff'_{NF}f'_{NP}TY_{NF}\right)}{P\left(f'_{CP}+f'_{NP}Y_{NP}\right)\left(T+Y_{NF}\right)} \tag{22b}$$

This case holds if $A^*_{NP,F\text{-lim}} \geq 0$ or equivalently,

$$\frac{Pf'_{CP}}{T+Y_{NP}} \geq \frac{Ff'_{NF}Y_{NF}}{T+Y_{NF}} \tag{23}$$

Co-limited case: In this borderline case, both the plant and the fungus expend all their effort taking up resources to maximize trade ($A^*_{NP,co\text{-lim}}=A^*_{CF,co\text{-lim}}=0$) and

$$g^*_{P,co-\text{lim}} = \frac{f'_{CP}Y_{NP}}{T+Y_{NP}} \tag{24a}$$

$$g^*_{F,co-\text{lim}} = \frac{f'_{NF}Y_{NF}T}{T+Y_{NF}} \tag{24b}$$

Notice that trade will always be "matched" at the optimal allocation strategy (always on the ridge

in Fig. 2.1): both partners allocate so that neither has surplus carbon or nutrient leftover after trade. However, trade will be only rarely co-limited. More often, trade will be matched but plant-limited (Fig. 2.1a) or matched but fungus-limited (Fig. 2.1b).

In general, $g_P^* = \min\left(g_{P,P\text{-lim}}^*, g_{P,F\text{-lim}}^*\right)$, $g_F^* = \min\left(g_{F,P\text{-lim}}^*, g_{F,F\text{-lim}}^*\right)$,

$A_{NP}^* = \max\left(A_{NP,P-\text{lim}}^* = 0, A_{NP,F-\text{lim}}^*\right)$, and

$A_{CF}^* = \max\left(A_{CF,P-\text{lim}}^*, A_{CF,F-\text{lim}}^* = 0\right)$.

Notice that there is no conflict of interest between the plant and the fungus when solving the optimal allocation problem: the *same* pair of strategies maximizes growth rates for *both* partners even though each acted selfishly (Fig. 2.1).

These results make intuitive sense: if trade is good, both partners should go "all-in" until one of them is completely specialized and the other is forced to turn to the less profitable avenue of direct uptake to make up its shortfall. Also intuitively, our model predicts that plant and fungal gain from trade ($g^*_P - g^*_{P,\text{no trade}}$ and $g^*_F - A^*_{F,\text{no trade}}$, respectively) depend on T, the C:N exchange ratio (Fig. 2.2).

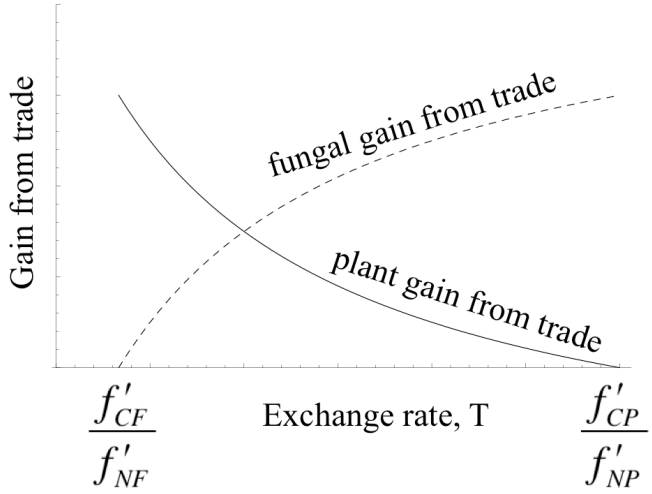


Fig. 2.2: Plant (solid line) and fungal (dashed line) gain from trade (difference between growth with and without trade) as a function of the C:N exchange ratio (T). Trade is beneficial for both partners only when the exchange ratio is between the ratio at which fungi directly take up carbon and nutrient (f'_{CF}/f'_{NF}) and the ratio at which plants directly take up carbon and nutrient (f'_{CP}/f'_{NP}). Parameters were chosen to simulate the case of colimited trade (Y_{NP}=1, Y_{CP}=1, Y_{NF}=1, Y_{CF}=1, f'_{CP}=3, f'_{NP}=1, f'_{NF}=3, f'_{CF}=1, F=1, P=1).

3) How will the plant and the fungus negotiate the exchange ratio, T?

Above, we showed how the plant and the fungus determine the amount of trade based on which partner could offer to trade the smaller amount of surplus resource (plant carbon or fungal nutrient). Next, we showed how the plant and the fungus adjust their allocation to carbon and nutrient uptake to take advantage of trade to maximize their growth rates. It is important to recognize that in both of these processes, there was no conflict between the plant and the fungus. If exchange ratios were favorable to both partners ($f'_{CF}/ f'_{NF} < T < f'_{CP}/ f'_{NP}$), then each partner would seek to maximize the resource it traded away (Fig. 2.1). If exchange ratios were not favorable to both partners, then trade would simply not happen. As long as exchange ratios are favorable, selfish allocation decisions by one partner consistently benefit the other partner.

However, there is conflict in the third problem that the plant and the fungus must simultaneously solve: negotiating the C:N exchange ratio T. Each partner gains more from trade when T is closer to the ratio at which the other partner takes up carbon and nutrient directly (Fig. 2.2). Plant gain from trade is zero when T is equal to the plant C:N uptake ratio (f'_{CP}/ f'_{NP}, the isolation cost ratio in Schwartz and Hoeksema (1998)). Plant gain from trade increases when nutrient becomes cheaper (relative to carbon) through trade than it is through direct uptake. In contrast, the fungus benefits from trade whenever T is greater than the fungal C:N uptake ratio (f'_{CF}/ f'_{NF}), with gain from trade increasing as T increases. This range of mutually beneficial trade is identical to the range identified by Schwartz and Hoeksema (1998). This approach is also similar to an approach comparing the carbon cost for nutrient uptake in mycorrhizal and nonmycorrhizal roots to predict how much plants should invest in the symbiosis (Tuomi et al. 2001; Geritz et al. 2006). However, this intuitive relationship, where the plant gains more from

trade when the exchange ratio is low and the fungus gains more when the exchange ratio is high, generates a conflict of interest. How do the plant and the fungus settle on a single, mutually acceptable T when they have competing interests?

One solution to this bargaining problem is the Nash Bargaining Solution (NBS; Nash 1953), which is the strategy that maximizes the product of the gains to both players (the Nash Product). The NBS was originally derived based on an axiomatic approach (Nash 1953) but has been shown to be the optimal solution of a model of strategic bargaining (Binmore et al. 1986). The NBS has been applied to a wide variety of social and economic situations (van Damme 1986; Border and Segal 1997) but only recently introduced to ecology and evolutionary biology (Roughgarden et al. 2006; Akçay and Roughgarden 2007a). As a central assumption of an alternative theory of social evolution to sexual selection, the NBS has been controversial (McNamara et al. 2006; Roughgarden et al. 2006; Akçay and Roughgarden 2007b). However, Akçay and Roughgarden (2007a) have demonstrated that a negotiation model based on offers and rejections or acceptances of nutrient fluxes between mututalists also leads to the NBS. In that model, each partner acts individualistically to increase its own gain from trade. We assume that such flux-based negotiations take place between a plant and a mycorrhizal fungus, and therefore adopt the NBS to solve the third problem of how the plant and the fungus will negotiate the exchange ratio T. The NBS assumes only that partners can sense the resource they receive from trade, a reasonable assumption in plant-mycorrhizal interactions (Bucking and Shachar-Hill 2005; Javot et al. 2007).

In our model, the Nash Product Π is the product of the gains to the two species. Because fitness depends on whether the plant or the fungus limits trade, we can rewrite it as:

$$\Pi = \left(g_P^* - g_{P,\text{no trade}}^*\right)\left(g_F^* - g_{F,\text{no trade}}^*\right)$$

$$= \left(\min\left(g^*_{P,P-\lim}, g^*_{P,F-\lim}\right) - g^*_{P,\text{no trade}}\right) *$$

$$\left(\min\left(g^*_{F,P-\lim}, g^*_{F,F-\lim}\right) - g^*_{F,\text{no trade}}\right)$$

$$= \min\left(\left(g^*_{P,P-\lim} - g^*_{P,\text{no trade}}\right)\left(g^*_{F,P-\lim} - g^*_{F,\text{no trade}}\right),\right.$$

$$\left.\left(g^*_{P,F-\lim} - g^*_{P,\text{no trade}}\right)\left(g^*_{F,F-\lim} - g^*_{F,\text{no trade}}\right)\right)$$

$$= \min\left(\Pi_{P-\lim}, \Pi_{F-\lim}\right) \tag{25}$$

Therefore, the Nash Product can be composed as the minimum of two unimodal curves, and the NBS is the maximum of the minimum of these two curves (Fig. 2.3). There are three candidate points for this maximum: the maximum of $\prod_{\text{P-lim}}$, the maximum of $\prod_{\text{F-lim}}$, or the intersection of $\prod_{\text{P-lim}}$ and $\prod_{\text{F-lim}}$ (Abrams 1987a). To find the first two candidate NBS's, we set $d\prod_{\text{P-lim}}/d\text{T}=0$ and $d\prod_{\text{F-lim}}/d\text{T}=0$ and solve for $\text{T}_{\text{P-lim}}$ and $\text{T}_{\text{F-lim}}$ respectively. These candidates are

$$T_{P-\lim} = \frac{2f'_{CF}f'_{CP} + f'_{CP}f'_{NF}Y_{NP} + f'_{CF}f'_{NP}Y_{NP}}{f'_{CP}f'_{NF} + f'_{CF}f'_{NP} + 2f'_{NF}f'_{NP}Y_{NP}} \tag{26a}$$

$$T_{F-\lim} = \frac{2f'_{CF}f'_{CP} + f'_{CP}f'_{NF}Y_{NF} + f'_{CF}f'_{NP}Y_{NF}}{f'_{CP}f'_{NF} + f'_{CF}f'_{NP} + 2f'_{NF}f'_{NP}Y_{NF}} \tag{26b}$$

At the NBS, trade is ultimately plant-limited in the first case (so the plant specializes on carbon uptake at the optimal allocation strategy; Fig. 2.3a) and fungus-limited in the second case (so the fungus specializes on nutrient uptake at the optimal allocation strategy; Fig. 2.3c). To find the third candidate NBS we set $\prod_{\text{P-lim}}=\prod_{\text{F-lim}}$ and solve for T. Of the four solutions, two occur at the endpoints of the range of mutually beneficial T's and yield no gain for one partner (Fig. 2.2),

so $\prod$=0 (Fig. 2.3); one solution is also inappropriate because it is always negative; the remaining solution is the third candidate NBS:

$$T_{\text{co-lim}} = \frac{f'_{NF} F Y_{NF} Y_{NP} - f'_{CP} P Y_{NF}}{f'_{CP} P - f'_{NF} F Y_{NF}} \tag{26c}$$

Trade is co-limited in this case, and both partners allocate optimally to take up only the resource they trade away (Fig. 2.3b). The NBS, T^*, is the T of these three candidates that leads to the largest Π, which can easily be found by comparison.

$$T^* = \underset{T \in \{T_{P\text{-lim}}, T_{F\text{-lim}}, T_{\text{co-lim}}\}}{\arg\max} \Pi(T) \tag{27}$$

This comparison also determines whether trade is plant-, fungus- or co-limited and therefore simultaneously solves all three modeling problems.

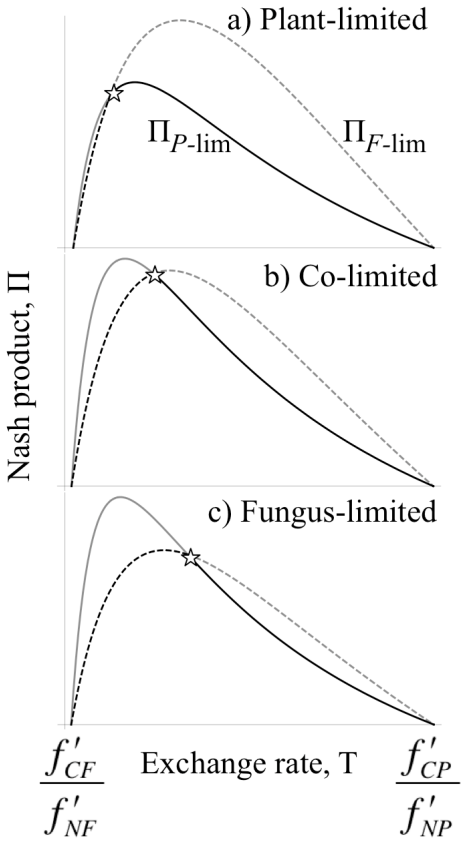


Fig. 2.3: The Nash Bargaining Solution (indicated by the star) is the value of the exchange ratio T which maximizes the minimum of the two Nash Products, $\Pi_{P\text{-lim}}$ (solid curve) and $\Pi_{F\text{-lim}}$ (dashed curve). a) Plant-limited trade: $\Pi_{P\text{-lim}}$ is the smaller Nash Product, so its maximum is the Nash Bargaining Solution. b) Co-limited trade: the minimum of the two Nash Products is maximized at the intersection of $\Pi_{P\text{-lim}}$ and $\Pi_{F\text{-lim}}$. c) Fungus-limited trade: $\Pi_{F\text{-lim}}$ is the smaller Nash Product, so the Nash Bargaining Solution is T_{FLIM}. Parameters used in all three figures were Y_{NP}=1, Y_{CP}=1, Y_{NF}=3, Y_{CF}=1, f'_{NP}=3, f'_{CF}=1, f'_{NF}=10, P=1, F=1; a) f'_{CP}=13; b) f'_{CP}=16; c) f'_{CP}=18.

Having solved the volume of resource exchanged (problem 1), how the plant and the fungus optimally allocate to uptake of carbon and nutrient (problem 2), and at what ratio they exchange carbon for nutrient (problem 3), we explore how these variables depend on organismal traits (organismal stoichiometry and uptake efficiencies), community structure (relative population sizes), and environmental conditions (resource availability). We also explore how the two key features of trade, allocation and the exchange ratio, affect plant and fungal gain from trade. We define gain from trade as the difference between growth rate with trade and growth rate without trade.

Effect of environmental and species parameters on the identity of the specialist

A key result of our model is that environmental and species characteristics determine which partner will specialize in uptake of the resource traded away by determining which partner limits trade. In general, the flows of soil nutrient (N) and carbon (C) into negotiations and the demand for those resources determine which partner comes up short. This partner limits trade, failing to provide enough of the resource it trades away to satisfy both its own requirements and its partner's requirements. This occurs despite the limiting partner's adjustment of allocation to specialize on uptake of the resource it trades away. The non-limiting partner, on the other hand, must be a generalist and take up both resources directly from the environment. For example, in a situation where the fungus limits trade, the fungus cannot supply enough nutrient even when it acts as a nutrient specialist, completely eliminating direct carbon uptake and allocating towards maximal uptake of nutrient. Its plant partner must therefore act as a generalist, allocating towards uptake of both carbon (for its own use and to trade away) and nutrient. In borderline

cases, both partners could specialize on uptake of the resource traded away.

What determines which partner will specialize?

To determine when each partner should specialize, we parameterized the model with values from the literature on the symbiosis between temperate grasses and arbuscular mycorrhizal fungi (Table 2.1). We assumed that each partner existed in a genetically homogenous, clonally spreading population where resources taken up were instantaneously evenly distributed across the population. A single plant would represent the entire plant population. Similarly, arbuscular mycorrhizal fungi grow clonally; we modeled a single genetically homogenous fungal individual as the population. We studied soil phosphorus as the soil nutrient, modeling uptake as a type II functional response. Because we assumed that the fungus could not directly take up carbon from the environment ($f'_{CF}=0$), we could model plant carbon uptake as photosynthesis, which is a function of light availability. We ran the model with all 1024 possible combinations of the extremes of empirically-derived parameters listed in Table 2.1 and recorded the identity of the specialist (limiting) partner. Fungal specialization on phosphorus uptake occurred in 89% of combinations of environmental conditions and species traits, 10% of combinations resulted in plant specialization on carbon uptake, and 1% resulted in the borderline case where the plant specialized on carbon uptake and the fungus specialized on phosphorus uptake.

Using a classification tree (library tree in R 2.12.1), we determined which of the parameters were most important in determining the identity of the specialist (limiting) partner (Fig. 2.4). Given the range of empirically measured parameter values, the availability of soil phosphorus was the most important determinant of the identity of the specialist, accounting for

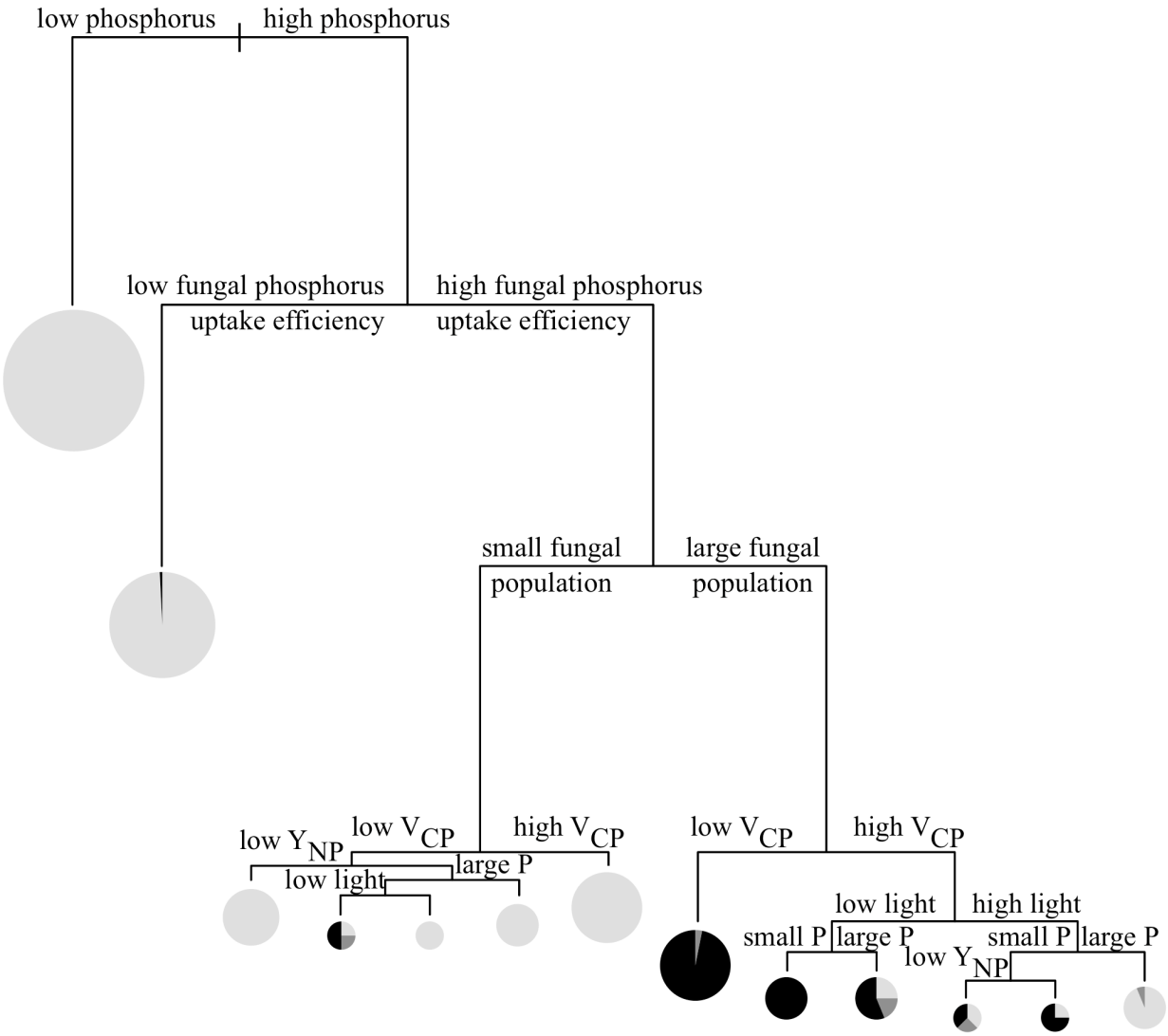


Fig. 2.4: Classification tree indicating the parameters that control the identity of the specialist (limiting) partner. We examined all possible combinations of the extremes of the range of measured parameter values in Table 1 for maximum photosynthetic rate (V_{CP}), photosynthetic rate half saturation constant (K_{CP}), carbon:phosphorus stoichiometries of the fungus (Y_{NF}) and plant (Y_{NP}), population sizes of the plant (P) and fungus (F), light availability, and soil phosphorus availability. We used the extremes of measured phosphorus uptake efficiencies (f'_{NP} and f'_{NF} from Table 1) as maximum uptake rates (V_{NP} and V_{NF}, respectively). There were 2^{10}=1024 combinations of parameter values in total. We estimated a single value for the half saturation constants (K_{NP} and K_{NF}=0.0002). Pie sizes indicate the number of parameter combinations in the branch; the largest pie contains 512 combinations of parameter values and the smallest pie contains 8. The color of the pie indicates the identity of the specialist partner: black indicates that the plant specializes on uptake of carbon (A_{NP}=0), light gray indicates that the fungus specializes on phosphorus uptake (A_{CF}=0), and the intermediate gray indicates parameter combinations where both partners specialize on the resource they trade away.

22% of the total deviance. If phosphorus was low, the fungus always specialized on phosphorus uptake and the plant was a generalist, taking up both carbon and phosphorus directly. If phosphorus was high, the identity of the specialist was determined by fungus maximum phosphorus uptake rate. If the fungus had low capacity for phosphorus uptake, the fungus was the specialist partner in 254 of 256 possible cases.

If phosphorus was high *and* fungus maximum uptake rate was high, fungal abundance became important in determining the identity of the specialist (Fig. 2.4). When the fungus was small, the plant was the specialist in only 4 of 128 cases. All of these cases were associated with very low carbon inputs into the symbiosis (low plant maximum photosynthetic rate, small plant biomass, and low light availability, as well as high plant carbon:phosphorus ratio). On the other hand, when the fungus was large, the plant specialized on carbon uptake in most cases (96/128). This tendency was especially pronounced when carbon inputs into the symbiosis were low.

Over all the empirically-derived ranges of the parameters we investigated, the three most important (phosphorus availability, fungal maximum phosphorus uptake rate, and fungal population size) accounted for 67% of the variation in the identity of the specialist. Less important parameters determined the identity of the specialist partner for some combinations of those three most important parameters. These less important parameters included plant biomass, light availability, and plant species traits such as the maximum rate of photosynthesis and carbon:phosphorus stoichiometry. The remaining parameters, fungal carbon:phosphorus stoichiometry, plant maximum phosphorus uptake rate, and plant photosynthetic half saturation constant, did not appear in the tree. These unimportant parameters occasionally caused variation within the terminal nodes of the tree but explained only 12% of total deviance in the identity of the specialist. Because our model is deterministic, this variation in the terminal nodes was not

error, so we did not perform cross-validation or calculate a misclassification table (Urban 2002; see also examples in Crawley 2007). Instead, we interpret these parameters as unimportant in determining the identity of the specialist partner, perhaps because the ranges of empirically-derived parameters were too small to have much effect.

What are the consequences of changes in the identity of the specialist?

To better understand the causes and consequences of changes in the identity of the specialist partner in the arbuscular mycorrhizal symbiosis, we examined a few combinations of parameters in detail. The availability of resources, especially phosphorus, affected the identity of the specialist partner (Fig. 2.4). When light (carbon) availability was low (Fig. 2.5a,c,e), a habitat with low soil phosphorus would have a specialist fungus and a generalist plant. In these conditions, the fungus specialized because it could not take up enough scarce phosphorus to satisfy fungus and plant requirements. Increases in soil phosphorus within this fungus-specialist region would increase the fungus's ability to trade away phosphorus, increasing the total flux of carbon and increasing both partners' gains from trade but having no effect on the exchange ratio. A habitat with higher soil phosphorus, on the other hand, would allow the fungus to provide enough phosphorus and would challenge the plant to take up enough carbon, leading to both partners specializing on uptake of the resource they trade away (co-limited trade). When both partners specialize, increases in soil phosphorus drive up the exchange ratio (Fig. 2.5a). This occurs because only the fungus can take up the phosphorus that would increase both partners' growth rates when both partners specialize; this power allows the fungus to negotiate very high exchange ratios that favor the fungus (Fig. 2.2). These higher exchange ratios will increase the amount of carbon the plant trades away (Fig. 2.5a), resulting in increased fungal gain from trade

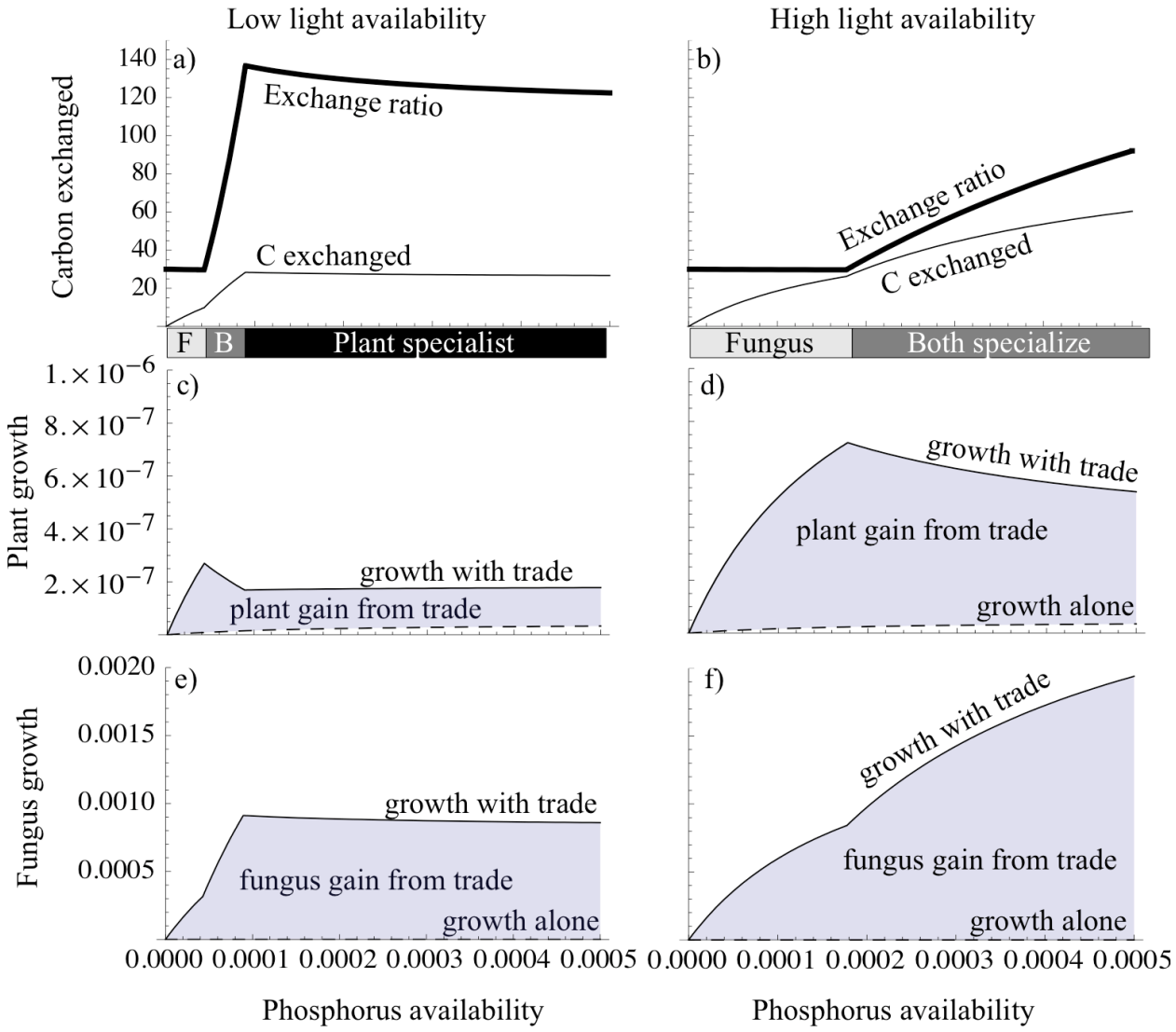


Fig. 2.5: Effect of light and phosphorus availability on the total amount of carbon exchanged per day, the C:N exchange ratio T (a-b), plant gain from trade (c-d), fungal gain from trade (e-f), and the identity of the specializing partner who limits trade (shaded bars in a-b; F indicates fungal specialist, B indicates both partners specialize, and P indicates plant specialization). The parameters were set at Y_{NP}=150, Y_{CP}=1, Y_{NF}=30, Y_{CF}=1, f'_{CF}=0, K_{CP}=200, V_{CP}=9.7*10^{-7}, V_{NP}=3.4*10^{-10}, K_{NP}=0.0002, V_{NF}=1.2*10^{-4}, K_{NF}=0.0002, P=2129, and F=0.36. For low light availability (a, c, e), C=100; for high light availability (b, d, f), C=1600.

but decreased plant gain from trade (Fig. 2.5c,e). Further increases in soil phosphorus and consequent increases in the exchange ratio and the flux of carbon traded away will mean that the plant can no longer supply enough carbon to satisfy both partners. At this level of phosphorus, the plant must continue to specialize on carbon uptake, but the fungus allocates to direct carbon

uptake from the environment (despite the fact that this allocation strategy brings in no carbon in the arbuscular mycorrhizal symbiosis). At high carbon (light) availability, the pattern is similar (Fig. 2.5b,d,f), but both partners specialize at the highest phosphorus availability examined. In situations with phosphorus even higher than the parameter values we examined, the plant would likely remain a specialist while the fungus would attempt to be a generalist. Therefore, changes in the identity of the specialist (limiting) partner have important effects on how carbon flux between partners, the exchange ratio, and plant and fungal gain from trade respond to increases in phosphorus availability.

Fungus phosphorus uptake was also an important determinant of the identity of the specialist (limiting) partner (Fig. 2.4). Examining this parameter more closely, we found that increases in fungus phosphorus uptake efficiency could cause a switch in the identity of the specialist from the fungus, to both partners, to only the plant (Fig. 2.6a). A fungus with very low phosphorus uptake efficiency would be unable to take up enough phosphorus to satisfy plant requirements even though it specialized on phosphorus uptake. The plant associating with this fungus would be a generalist, allocating to both carbon and phosphorus uptake. As long as the fungus was the specialist, a fungus with higher phosphorus uptake would be able to increase the total amount of carbon exchanged between partners; this increased volume of trade would increase both partners' gains from trade. However, a fungus with even higher phosphorus uptake efficiency could satisfy plant requirement for phosphorus, allowing the plant to specialize on carbon uptake (Fig. 2.6a), increasing the carbon exchanged even further because trade would be the plant's only source of phosphorus. In this region, the exchange ratio increases, again because the fungus' monopoly on phosphorus uptake would allow it to negotiate an exchange ratio favorable to the fungus (Fig. 2.2). This shift towards a much higher exchange ratio,

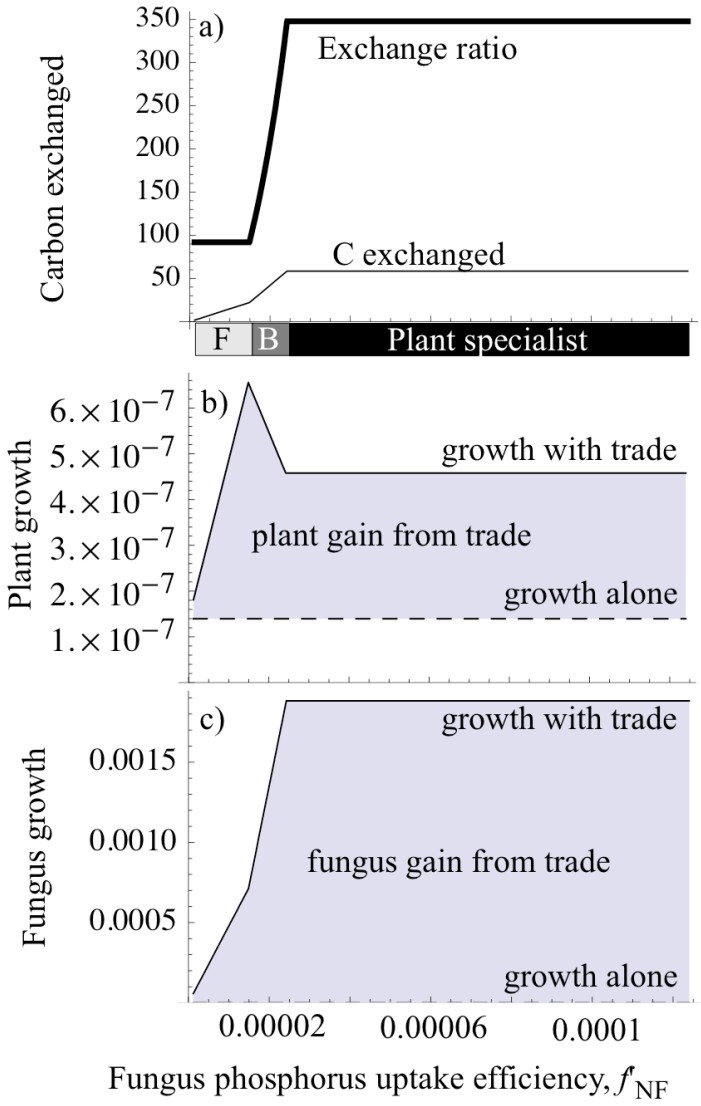


Fig. 2.6: Effects of fungus phosphorus uptake efficiency (a, b, c) on the total amount of carbon exchanged per day, the amount of carbon traded per unit nutrient (C:N exchange ratio, T) (a), plant gain from trade (b), fungal gain from trade (c), and the identity of the specializing partner who limits trade (shaded bars in a; F indicates fungal specialist, B indicates both partners specialize, and P indicates plant specialization). Parameters were set at Y_{NP}=500, Y_{CP}=1, Y_{NF}=100, Y_{CF}=1, f'_{CF}=0, V_{CP}=9.7*10^{-7}, K_{CP}=400, P=2129, F=0.36, C=1600.

accompanied by larger quantities of carbon exchanged, would combine to result in higher fungal gain from trade and lower plant gain from trade (Fig. 2.6b,c). A fungus with still higher phosphorus uptake efficiency would be able to provide more than enough phosphorus for both its own requirements and plant phosphorus requirements, so it would allocate some fraction of effort towards direct carbon uptake, resulting in a specialist plant and generalist fungus. Again, changes in the identity of the specialist partner affected the impacts of shifts in fungal species traits on the outcome of trade.

The biomass of each partner also contributed to determining whether the plant, the fungus, or both would specialize on uptake of the resource traded away (Fig. 2.7). A plant with more biomass was better able to take up enough carbon to satisfy itself and its partner than a smaller plant, leaving a larger plant free to allocate to direct phosphorus uptake as well as direct carbon uptake (Fig. 2.7a,c,e). To understand the dynamics of trade during plant invasion of a habitat occupied only by the fungus, we investigated plant biomasses below the lowest equilibrium biomass listed in Table 1 (686 g plant carbon m^{-2}). Not surprisingly, the plant specialized on carbon uptake during invasion of new habitat. However, even above the lowest equilibrium plant biomass, the plant might still be the specialist partner (Fig. 2.7a). Similarly, a fungus invading a new habitat would specialize. However, in a habitat where the fungus was more abundant, the fungus might be able to take up enough phosphorus to satisfy both plant and fungus requirements. Therefore, with increases in fungus biomass, trade would transition from fungus specialization, to both partners specializing, to plant specialization (Fig. 2.7b,d,f). Again, changes in the identity of the specialist partner affected whether changes in parameters would increase, decrease, or have no effect on the exchange ratio and plant and fungal gain from trade.

These simulations reveal two important points. First, the identity of the specialist partner affects the impacts of changes in parameters on the outcome of the symbiosis. In Figs. 2.5-2.7, sharp breakpoints in the curves occur wherever the identity of the specialist partner shifts. Increases in species uptake efficiencies, biomass, or environmental characteristics can strongly increase the total volume of carbon exchanged, the exchange ratio, or plant and fungal gain from trade while one partner specializes, but strongly decrease them (or have no effect) when the other partner specializes. Second, changes in plant and fungal gain from trade in response to changes in parameter values were frequently counter-intuitive. While the exchange ratio was always

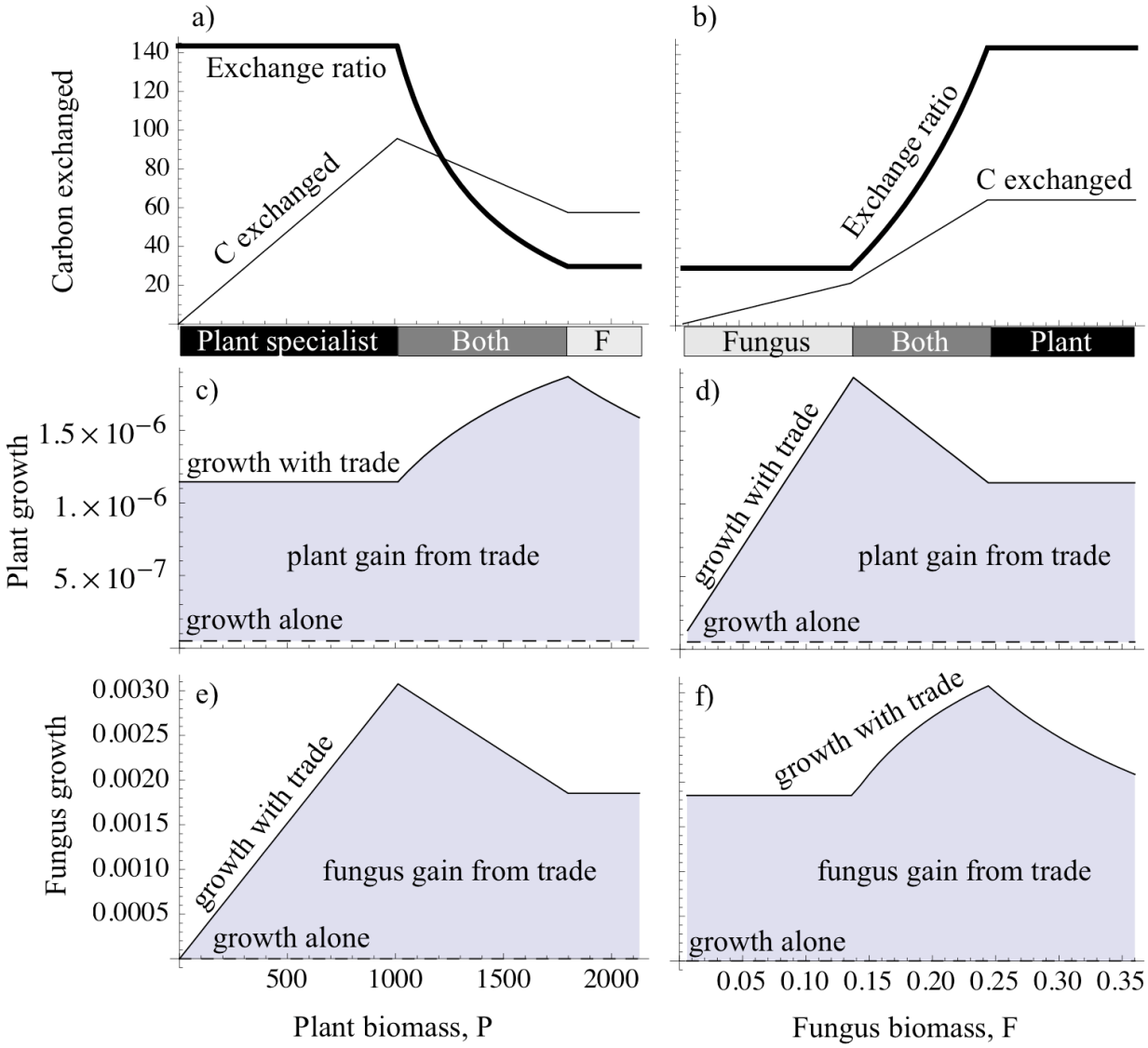


Fig. 2.7: Effect of varying abundances of plants (a, c, e) and fungi (b, d, f) on the total amount of carbon exchanged per day, the amount of carbon exchanged per unit nutrient (C:N exchange ratio, T) (a-b), plant gain from trade (c-d), fungal gain from trade (e-f), and the identity of the specializing partner who limits trade (shaded bars in a-b; F indicates fungal specialist, B indicates both partners specialize, and P indicates plant specialization). The parameters were set at Y_{NP}=150, Y_{CP}=1, Y_{NF}=30, Y_{CF}=1, f'_{CF}=0, K_{CP}=200, V_{CP}=6.72*10^{-6}, f'_{NP}=3.4*10^{-10}, f'_{NF}=1.24*10^{-4}, and C=100. Fungal population size was large when varying plant abundance (F=0.36 in a, c, e); plant population size was small when varying fungal abundance (P=686 in b, d, f).

negotiated according to the Nash Bargaining Solution, some exchange ratios are nearer the lower bound of the beneficial range of trade, an exchange ratio more favorable for the plant (Fig. 2.2).

Other exchange ratios were nearer the upper bound of the beneficial range of trade, benefitting the fungus more than the plant. Understanding these results relied on knowledge of the identity of the specialist partner and the exchange ratio.

Discussion

Specialization

In contrast to Schwartz and Hoeksema (1998), who found specialization by both partners to be common, our results suggest that only rarely do both partners specialize. Much more commonly, one of the partners specializes, and the other partner acts as a generalist and takes up both resources directly from the environment.

In the majority of cases in our simulations, the fungus was the specialist partner, while the plant took up both carbon and phosphorus. This finding is reassuring, because it is backed by empirical observations that the plant photosynthesizes and takes up soil nutrients and that the fungus only takes up soil nutrients. Because the fungus is the specialist partner in most environments it encounters, it may have experienced selection for the loss of its direct carbon uptake machinery. This hypothesis could explain the apparent evolution of specialization in arbuscular mycorrhizal fungi: in other words, it may explain why arbuscular mycorrhizal fungi apparently have no capacity for direct carbon uptake from the environment.

However, our simulations revealed a few cases where the plant was the specialist partner. This occurred even though we parameterized the model to simulate the arbuscular mycorrhizal case, where the fungus is incapable of direct carbon uptake and is thus incapable of acting as a generalist. In plant specialist situations where it would be advantageous for the fungus to

attempt direct carbon uptake from the environment, negotiations proceeded as if this were possible. Although counter-intuitive, this is realistic because it assumes that the fungus has no knowledge of its capacity for direct carbon uptake and because both partners make allocation and negotiation decisions based on very short time horizons and without regard for their long-term consequences. Interestingly, there may be empirical support for the idea that plants could specialize on carbon uptake and eliminate direct phosphorus uptake when associating with arbuscular mycorrhizas (reviewed in Smith et al. 2009). In fact, suppression of plant direct phosphorus uptake may be proportional to the amount of phosphorus received from the fungal partner (Burleigh et al. 2002).

Combined with empirical evidence suggesting that the identity of the specialist partner in the arbuscular mycorrhizal symbiosis may actually vary, our finding that the identity of the specialist partner affects the outcome of trade is intriguing. Specifically, it suggests that some of the context dependency in plant-mycorrhizal interactions may indeed be driven by variation in the identity of the specialist partner.

Exchange ratio

With a few notable exceptions (Hoeksema and Bruna 2000; McGill 2005; Akçay and Roughgarden 2007a; Golubski and Klausmeier 2010), relatively little theoretical work has explored the implications of variation in the exchange ratio. However, the exchange of costly benefits lies at the heart of any mutualism. Our results support this idea: the exchange ratio affected how much each partner gained from trade, even though exchange ratios were required to be mutually beneficial. These changes sometimes coincided with the expectation that lower carbon:nutrient exchange ratios would benefit the plant and that higher exchange ratios would

benefit the fungus. However, this was not always the case. Changes in the identity of the specialist interacted with changes in the exchange ratio to determine gain from trade.

We modeled negotiation using the Nash Bargaining Solution because it is appropriate for exchanges of resources between single individuals of two guilds (Akçay and Roughgarden 2007a). In nature, however, it is typical for plants to associate with multiple species of mycorrhizal fungus, and similarly for fungi to associate with many plants (van der Heijden and Horton 2009). It is not clear that the Nash Bargaining Solution will be appropriate to predict the outcome of negotiation when either or both partners can chose from among many potential partners. Partner choice is thought to strongly affect the negotiated exchange ratio as well as the benefits each partner gains from the association (Golubski and Klausmeier 2010). Furthermore, the Nash Bargaining Solution requires that partners can enforce this negotiated exchange ratio: in other words, cheating is not allowed. Finally, the Nash Bargaining Solution requires that both partners benefit from the interaction and neither partner be parasitized. Therefore, this bargaining solution is not appropriate for modeling the mutualism-parasitism continuum observed in arbuscular mycorrhizal fungi (Johnson et al. 1997; Hoeksema et al. 2010). Clearly, incorporating cheaters, parasitism, or non-enforceable exchange ratios into a model of negotiation would invalidate the use of the Nash Bargaining Solution and could have profound effects on the outcome of trade, both ecologically and evolutionarily (McGill 2005).

A few empirical studies have measured variation in the exchange ratio in the arbuscular mycorrhizal symbiosis and linked it to fungal species traits (e.g., Pearson and Jakobsen 1993). However, too few studies have reported exchange ratios, and none in conjunction with information on the identity of the specialist, so it is too early to determine whether our model correctly predicts variation in the exchange ratio. The exchange ratio may be even more difficult

to measure in other types of mutualisms where the benefits exchanged are behaviors such as pollination or protection from the environment. It thus remains an understudied component of mutualism, but our results highlight its potential importance for understanding mutualisms.

Gain from trade

The net effect of the arbuscular mycorrhizal symbiosis ranges widely, depending on the specific context (Hoeksema et al. 2010). Several specific aspects of the context are thought to be important, including resource availability, relative population sizes, and the identity of the partners, probably because of differences in traits involving resource uptake efficiency (Hetrick et al. 1992; Allison and Goldberg 2002; Lekberg and Koide 2005; Hoeksema et al. 2010). However, 59-77% of variation in plant growth remained unexplained in a recent meta-analysis (Hoeksema et al. 2010). This surprisingly large amount of residual variation in the outcome of the mutualism suggests that not all important predictor variables have been thoroughly examined. In particular, those investigators were unable to include analyses of the uptake and trade of carbon and soil nutrients. In other words, trade (the reciprocal flux of resources) itself has not been adequately studied.

Our model suggests that two aspects of trade may be important predictor variables. We found that both the identity of the specialist and the exchange ratio help determine the degree to which the plant and the fungus gain from trade. Variation in these two factors is driven by changes in extrinsic forces such as environmental conditions and species traits. However, these two factors also affect the impact of the extrinsic forces. In fact, the partners' gains from trade could increase, decrease, or remain constant with changes in resource availability, the partners' resource uptake efficiencies, or population sizes, depending on the identity of the specialist and

the exchange ratio. Without knowing the identity of the specialist and the exchange ratio, this variation in the response of plant and fungal benefit to environmental drivers would appear to be highly variable and unpredictable, as it does in the recent meta-analysis (Hoeksema et al. 2010). However, radioactive isotopes, genetic techniques, or other methods may make identification of the specialist possible (Smith et al. 2009). Measuring the exchange ratio should also be possible, using some of these same techniques. By identifying the specialist and quantifying variation in the exchange ratio along gradients of key variables such as phosphorus availability, fungal phosphorus uptake efficiency, and fungal biomass, we may be able to explain much of this residual variation in the outcome of the mutualism.

The model in a broader context

Previous models of trade have made important advances in understanding the exchanges of costs and benefits in mutualisms. These models have considered the conditions under which trade could evolve (McGill 2005; de Mazancourt and Schwartz 2010) and could result in a range of positive to negative impacts on populations based on resource availability (Neuhauser and Fargione 2004) or density-dependent functional responses to partners (Holland et al. 2002). Another approach has more explicitly considered costs and benefits by modeling mutualisms as a consumer-resource interaction (Holland and DeAngelis 2010). These and other models (Hoeksema and Bruna 2000; Okuyama and Holland 2008) have also asked whether mutualism can affect community structure. However, none of these have investigated the impacts of plastic allocation to trade as opposed to independent resource acquisition, clearly an important determinant of the magnitude and therefore the impact of trade on individuals and communities.

Although we have analyzed and interpreted our model in the specific example of the

plant-arbuscular mycorrhizal mutualism, many of the central concepts are applicable to other kinds of mutualisms. All mutualisms require the exchange of one benefit for another. Some of these benefits may be costly for partners to provide, opening up the possibility that there may be a conflict of interest in determining the magnitude of resource provisioned (Bronstein 2001). Negotiation of the exchange ratio is therefore likely to be a critical issue in determining the outcome of the interaction. Furthermore, some mutualists may be capable of adjusting allocation to provisioning their partner. Flexible allocation to provisioning raises questions about the optimal allocation strategy, and whether either partner (or both) will specialize on provisioning its partner and becoming an obligate mutualist. Our model is thus conceptually applicable to a wide range of mutualisms.

By uniting two previously disparate fields of theory, we have gained new insight into two factors that should determine the outcome of mutualisms. Specifically, we have incorporated aspects of a biological market (Schwartz and Hoeksema 1998) and an important model of negotiation (Akçay and Roughgarden 2007a). By combining these two modeling approaches, we are able to show that a mutualist's decision to specialize on uptake of a single resource can affect the exchange ratio it negotiates with its partner. We also show that both of these factors, allocation and the exchange ratio, can combine with other important ecological drivers to determine the outcome of trade.

CHAPTER THREE

Plant species differ in their ability to sanction cheating arbuscular mycorrhizal fungi

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 sanctions are not universally effective. I tested the hypothesis that plant species differences in mycorrhizal responsiveness would affect both their susceptibility to parasitism and their ability to sanction cheating (non-mutualistic) arbuscular mycorrhizal fungi (AMF). In a greenhouse experiment, I manipulated resources, plant species, and the presence of AMF and measured plant biomass and fungal abundance in roots and soil. I found that two C_3 grasses, *Bromus inermis* and *Elymus repens*, both effectively sanctioned fungi in high phosphorus soils by suppressing root colonization to near zero and reducing soil fungal abundance. Increases in soil phosphorus did not reduce the degree to which AMF increased plant biomass. In contrast, two C_4 grasses, *Andropogon gerardii* and *Schizachyrium scoparium*, were less effective in reducing root colonization at high phosphorus and failed to suppress soil fungi. Consequently, with increasing phosphorus, they experienced strong declines in the degree to which AMF increased plant biomass. Thus, species differ in susceptibility to parasitism and their ability to sanction cheaters according to their mycorrhizal responsiveness. Two unresponsive species (*B. inermis* and *E. repens*) sanctioned effectively and avoided parasitism, while two more responsive species (*A. gerardii* and *S. scoparium*) did not sanction and one (*A.*

gerardii) suffered parasitism. These differences in ability to sanction cheaters may affect the distribution and abundance of plant and fungal species, as well as the stability of the mutualism.

Introduction

Understanding the apparent stability of mutualisms is a long-standing question in ecology. In particular, mutualisms that involve the exchange of costly benefits appear vulnerable to the rise of "cheaters"—individuals that obtain the benefits of participation in a mutualism but avoid paying the costs by failing to reciprocate. By avoiding the cost of participation, cheaters could outcompete members of their guild who reciprocate. Over evolutionary time, cheaters thus might drive the mutualism to an antagonism (Bronstein 2001). Cheaters are known to exist in a variety of mutualisms, such as plant-pollinator interactions (Tyre and Addicott 1993), 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) pointed out that although cheating is widespread in mutualisms, some cheaters are conditional. These conditional cheaters may behave mutualistically in some environmental contexts but parasitically in other contexts. However, it is not yet clear *what* specific contexts will determine the mutualistic or parasitic outcome of interactions with conditional cheaters. Clearly, to understand whether conditional cheaters could destabilize mutualisms, it is necessary to understand the conditions under which they act as parasites.

One of the chief mechanisms thought to be important for stabilizing mutualisms and preventing exploitation by cheaters is sanctions: to minimize fitness costs imposed by cheaters, partners should be able to reduce their investment in non-mutualistic partners (West et al. 2002;

Kiers and van der Heijden 2006). Sanctions have been shown to occur in some systems, such as the legume-rhizobium symbiosis (Kiers et al. 2003) and plant-pollinator mutualisms (Pellmyr and Huth 1994). However, neither the extent nor the ecological and evolutionary impacts of sanctions are yet fully understood. Bronstein (2001) emphasized that different stabilizing mechanisms may operate in different mutualisms, depending partly on whether cheaters are conditional or not. Whether sanctions can prevent exploitation by conditional cheaters remains an important unanswered question.

The plant-arbuscular mycorrhizal symbiosis is a mutualism vulnerable to conditional cheaters. In this globally widespread symbiosis, in which the majority of terrestrial plant species participate (Wang and Qiu 2006), plant response to the symbiosis ranges widely along the mutualism-parasitism continuum (Johnson et al. 1997; Hoeksema et al. 2010). Plants often strongly benefit from association with arbuscular mycorrhizal fungi (AMF), especially when soil nutrients are scarce (Hoeksema et al. 2010). AMF are thought to be most beneficial in phosphorus-poor environments because fungal hyphae are more efficient than plant roots at scavenging for immobile nutrients such as phosphorus (Smith and Read 1997). However, in environments where phosphorus is abundant relative to other resources such as light, plants may receive little or no benefit from the symbiosis (Allison and Goldberg 2002; Johnson 2010). In high fertility environments where plants receive little or no benefit from the interaction, fungi are not plant mutualists and the interaction functions as a commensalism or a parasitism (Johnson et al. 1997). In contrast, fungal fitness depends heavily on how much carbon AMF obtain from host plants because they have no independent mechanism for taking up carbon (Smith and Read 1997). If the fungi receive plant carbon when there is no benefit to the plant, they are considered conditional cheaters.

We expect plants to sanction these conditional cheaters by reducing carbon allocation in conditions where AMF are not beneficial. There is some evidence that plants have the capacity to sanction AMF when soil resources are abundant. A number of experiments have found a decrease in the percent of the plant root system occupied by AMF in response to phosphorus additions (Smith and Read 1997; Treseder 2004). However, root colonization is only one metric of plant allocation to the symbiosis and may not fully reflect the capacity for plant sanctions. Furthermore, plant sanctions appear to be incomplete; parasitism occurred in at least 15% of studies included in a recent meta-analysis of hundreds of lab and field studies of arbuscular mycorrhizal and ectomycorrhizal mutualisms (supplementary material to Hoeksema et al. 2010). Why does parasitism occur as often as almost one in six interactions? In other words, why does there appear to be such variation in the effectiveness of plant sanctions against cheating AMF?

Graham and Eissenstat (1994) proposed a hypothesis to explain variation in the effectiveness of plant sanctions that focused on the degree of plant benefit from AMF in low phosphorus soils. Plant species are known to vary in the degree to which they benefit from mycorrhizal fungi. This plant benefit is typically measured in low phosphorus soils and expressed as mycorrhizal responsiveness, a measure of plant biomass in the presence of AMF relative to plant biomass in the absence of AMF. Graham and Eissenstat (1994) hypothesized that a plant that benefits greatly from AMF in low phosphorus (high mycorrhizal responsiveness) would be less likely to sanction AMF in high phosphorus conditions and would therefore be more likely to experience parasitism (negative mycorrhizal responsiveness) in high phosphorus conditions (Fig. 3.1a). The strength of plant sanctions would be indicated by a reduction in carbon allocation to AMF in high relative to low phosphorus soils; this might be associated with a reduction in root colonization. These sanctions would result in an ecological indicator of

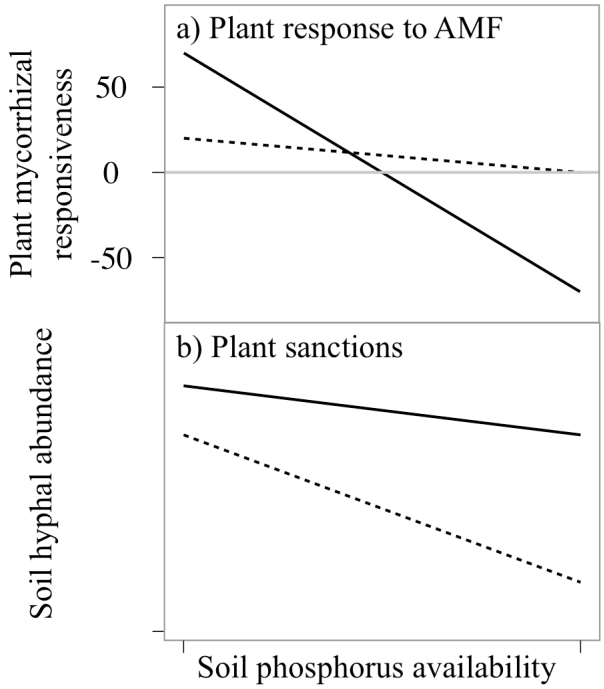


Fig. 3.1: Hypothesized differences between plant species that can (dashed line) or cannot (solid line) effectively sanction non-mutualistic AMF (Graham and Eissenstat 1994) in a) plant response to arbuscular mycorrhizal fungi and b) AMF abundance. Species that show a stronger benefit from AMF at low phosphorus (solid line) might have a stronger decline in mycorrhizal responsiveness with increases in phosphorus (a). If the magnitude of decline is strong enough, then the mutualism could shift to a parasitism in high phosphorus soils (negative mycorrhizal responsiveness). In contrast, a species with low mycorrhizal responsiveness at low phosphorus (dashed line) may experience weaker or no parasitism (near-zero mycorrhizal responsiveness) at high phosphorus. The gray line at zero indicates no response to AMF. These differences in plant species should be reflected in fungal response to soil phosphorus availability (b): a plant species that avoided parasitism (dashed line) should suppress AMF abundance in high phosphorus soils, relative to AMF abundance in low phosphorus soils, indicated by a strong negative slope. The plant species that experienced parasitism (solid line) should not as strongly suppress AMF abundance in high phosphorus soils, indicated by a weak negative or zero slope.

sanction strength: a reduction in AMF abundance in high phosphorus soils, relative to abundance in low phosphorus soils. A plant with weak sanctioning ability would only weakly reduce carbon allocation, root colonization, and AMF abundance in the soil (Fig. 3.1b). In contrast, plants that experience little benefit will sanction effectively and thus experience no parasitism.

To investigate differences among plant species in sanction strength, I tested for a relationship with plant mycorrhizal responsiveness. I asked whether a plant species' mycorrhizal responsiveness at low phosphorus would affect both the likelihood of experiencing parasitism at high phosphorus and the plant's propensity to impose effective sanctions at high phosphorus. To do this, I took advantage of well-known differences between C_3 and C_4 grasses in their mycorrhizal interactions. C_4 grasses are warm-season perennials that are known to be highly mycorrhizal responsive (Wilson and Hartnett 1998). Cool-season C_3 grasses, on the other hand, tend to have lower mycorrhizal responsiveness (Wilson and Hartnett 1998). Both C_3 and C_4 grasses are common in Midwestern old-fields and prairies. In a greenhouse experiment, I grew two species from each functional group and manipulated the presence of AMF and the availability of phosphorus and light. I determined whether species differed predictably in their susceptibility to parasitism and in their ability to sanction AMF.

Methods

To test the hypothesis that a species' mycorrhizal responsiveness would determine its ability to sanction parasitic arbuscular mycorrhizal fungi and thus experience different levels of benefit or parasitism, I conducted a greenhouse experiment. In a factorial design of five phosphorus levels, two light levels, and two AMF treatments (mycorrhizal and nonmycorrhizal), I grew four species of grass: two native C_4 prairie bunchgrasses, big bluestem (*Andropogon*

gerardii) and little bluestem (*Schizachyrium scoparium*), and two introduced C_3 grasses, smooth brome (*Bromus inermis*) and quackgrass (*Agropyron* or *Elymus repens*).

Soil and fungal inoculum. I grew the plants and AMF in 0.7-L pots containing a mixture of 90% sand and 10% field soil collected from an old-field in southwest Michigan (site NP in Fig. 1.2) and sieved through a 2-mm sieve. I autoclaved the sand/soil mix for three hours in the nonmycorrhizal treatment and did not autoclave the mix in the mycorrhizal treatment. The non-autoclaved sand and soil in the mycorrhizal treatment served as a source of AMF. All pots also received 150 mL additional inoculum of field soil, either autoclaved (nonmycorrhizal treatment) or not (mycorrhizal treatment), and an additional 5 cm of autoclaved sand/soil mix on top of the inoculum to reduce cross-contamination among pots. I watered the pots excessively to leach nutrients released during autoclaving. To control for the abundance of other soil microbes, I added to each pot 40 mL of a microbial wash prepared by blending field soil with water in a 1:5 ratio and filtering through a 35 μm sieve. This sieve size excluded mycorrhizae from the microbial wash, but allowed bacteria and other microbes to pass through (Corkidi et al. 2002; Johnson et al. 2008).

Light and phosphorus treatments. In December 2008, I randomly arranged these pots into six replicate blocks on benches in a heated greenhouse with supplemental lighting at the W. K. Kellogg Biological Station in southwest Michigan. I germinated seeds of each species in petri plates and transplanted newly emerged seedlings into the pots to ensure that one individual grew in each pot. To manipulate light availability, I placed half the pots in each block under a shade structure that blocked 30% of incoming light (low light treatment). The other half of the pots received ambient light (high light treatment). To manipulate phosphorus availability, I randomly assigned to each pot one of five fertilizer solutions with 0, 0.15, 0.31, 3.1, or 31.0 g/L NaH_2PO_4.

To balance the pH, I added a different amount of 1M NaOH to each fertilizer solution: 0.05, 0.06, 0.25, 1.5, or 12.5 mL/L, from lowest to highest phosphorus treatment. To ensure that phosphorus was the only limiting nutrient, all of the fertilizer solutions included a mixture of 24 g/L KNO_3, 20 g/L $MgSO_4$ $7H_2O$, 0.5 g/L KCl, 0.11 g/L H_3BO_3, 0.05 g/L $ZnSO_4$ $7H_2O$, 0.03 g/L $MnSO_4$ H_2O, 3.3 mg/L $CuSO_4$ $5H_2O$, and 0.6 mg/L MoO_3. The pots were watered to capacity 5-6 times a week with tap water and fertilizer was added once weekly, immediately after watering the pots. To increase the chance of successful seedling establishment, I began fertilizer applications three weeks after transplanting all seedlings into pots. I added 7.5 mL of the appropriate solution to the pots for the first four weeks of the fertilizer applications and 15 mL during weeks 5-8. The highest level of phosphorus addition was intended to mimic the high phosphorus availability which can result from application of manure to agricultural soils (40-120 µg P/g soil; Andraski and Bundy 2003; Butler and Coale 2005).

Harvesting plants and fungi. I harvested the experiment nine weeks after initiating the fertilizer treatments. To determine plant aboveground biomass, I clipped seedlings at the soil surface and placed the material in coin envelopes or small paper bags. To determine belowground plant biomass, I placed the contents of each pot in a 2-mm sieve and gently agitated the soil and roots to remove as much soil as possible from the roots. I saved and air-dried this soil for later analysis of phosphorus availability and AMF abundance. I then submerged the roots in water and gently agitated them to remove the remaining soil. I placed the wet, cleaned roots in coin envelopes or small paper bags. I dried both root and shoot biomass in an oven at 65˚C to constant mass (at least 48 hours).

Assessing AMF abundance in plant roots. To determine root colonization by arbuscular mycorrhizal fungi, I cleared haphazardly-chosen subsamples of dried roots in 2.5% KOH for 90-

120 minutes in an oven at 90˚C, soaked roots in 1% HCl overnight, and stained for 90 minutes at 90˚C in trypan blue stain, a solution of 500 mL/L glycerol, 450 mL/L water, 5 mL/L 1% HCl, and 0.5 g/L trypan blue (modified from Koske and Gemma 1989). I then mounted roots on microscope slides with water and assessed percent root colonization using the gridline intercept method (Giovannetti and Mosse 1980) at 100X magnification. I quantified root colonization in all pots in the mycorrhizal treatment and one third of the pots (two of six blocks) in the nonmycorrhizal treatment to test for contamination. Because the mean of root colonization in the nonmycorrhizal treatment was less than 1%, I decided it was not necessary to quantify colonization in the nonmycorrhizal pots in the remaining four blocks.

Assessing AMF abundance in the soil. To determine the abundance of AMF hyphae in the soil (extra-radical hyphae), I thoroughly mixed all the air-dried soil saved from each pot. I extracted the hyphae from a 5-g subsample of each sample by stirring it into 90 mL of 20 g/L sodium hexametaphosphate on a stir plate, sonicating, filtering a subsample through 20 μm mesh, resuspending the hyphae trapped on the mesh in trypan blue stain, staining overnight, filtering through 1.2 μm pore size nitrocellulose filters, mounting the filters with immersion oil on microscope slides, and quantifying AMF hyphal abundance using the gridline intercept method at 400X magnification (modified from Miller et al. 1995). To increase accuracy of quantifying extra-radical hyphae, I counted each slide twice and used the averaged value.

Soil phosphorus analysis. To determine water-extractable phosphorus in the soil at the end of the experiment, I extracted 10 g of air-dried soil in 50 mL water by shaking for 2 minutes, allowing to settle overnight, and filtering through glass fiber filters (modified from Olsen and Sommers 1982). I froze the extract until analysis. To determine the phosphorus content of the extract, I developed color using malachite green and determined intensity of color development

on a microplate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA, USA; modified from D'Angelo et al. 2001).

Mycorrhizal responsiveness (MR). To determine plant response to AMF, I summed root and shoot biomass, paired plants of the same species grown in the same resource environment (phosphorus and light) in each block (and differing only in AMF treatment), and calculated an index of mycorrhizal responsiveness (van der Heijden 2002). When biomass with AMF (b_{AMF}) was greater than biomass without (b_N),

$$MR = 100\left(1 - \frac{b_N}{b_{AMF}}\right);$$

when $b_{AMF} < b_N$, $$MR = 100\left(\frac{b_{AMF}}{b_N} - 1\right).$$

MR ranges from -100 to 100; MR>0 indicates that AMF acted as mutualists to increase plant biomass and MR<0 indicates that AMF acted as parasites to reduce plant biomass. Other indices of mycorrhizal responsiveness (Graham and Eissenstat 1994; Johnson 1998; Kaeppler et al. 2000) gave qualitatively similar results.

Statistical analysis. I conducted all statistical analyses in R (2.10.1, The R Foundation for Statistical Computing), package nlme. I analyzed the data as a split-plot ANCOVA. The plant species (4 levels), AMF (2 levels), and phosphorus treatments (5 levels) were completely randomized within the whole-plot level light treatment (2 levels); all treatment combinations were present in each of six replicate blocks (480 pots in total). I used water-extractable soil P as a continuous predictor instead of the discrete phosphorus treatment variable because the

phosphorus treatment explained 98% of the variation in water-extractable soil P. To better examine responses across the range of tested phosphorus availabilities, which spanned three orders of magnitude, I log-transformed this continuous independent variable. I used ANOVA and ANCOVA models to test for the effects of resources on log-transformed total plant biomass, plant mycorrhizal responsiveness, square-root transformed percent root colonization by AMF, and log-transformed extra-radical hyphal abundance. When there were significant interactions between species and resource availability, I conducted separate ANOVA or ANCOVA on each species to determine how individual species responded to phosphorus or light.

One *E. repens* individual and 35 *S. scoparium* individuals died before the end of the experiment. To maintain a balanced design, I included the biomass collected from those pots in the analyses below. Removing them would not qualitatively change the results. However, root samples of five *S. scoparium* individuals were too small to assess percent root colonization so these data were omitted from analyses of root colonization. I used marginal sums of squares in all analyses including this unbalanced root colonization dataset.

Model simplification. I performed model simplification as suggested in Crawley (2007) to better understand treatment effects and species differences. I removed non-significant interactions. Because I was interested in comparing the responses of C_3 and C_4 grasses, I combined species into the appropriate functional group (C_3 or C_4) and tested whether this simplification (removal of extra terms) affected model fit. A result of no difference ($p>0.05$) between the complex model (containing terms for individual species) and the simple model (containing only terms for functional group) indicated that species within functional groups were not significantly different, either in mean response or in interactions with other treatment variables.

Results

Effectiveness of the AMF treatment

Three lines of evidence suggest that the pots with autoclaved sand/soil mix and autoclaved inoculum did not have live arbuscular mycorrhizal fungi. First, the mean root colonization of plants from the nonmycorrhizal treatments (autoclaved media) was less than 1%. Second, the abundance of soil hyphae in these treatments did not respond to phosphorus ($F_{1,224}$=0.44 p=0.51), light ($F_{1,5}$=0.16 p=0.71), or plant species ($F_{3,224}$=1.75 p=0.16). Third, across all plant species and all resource availabilities, there were more soil hyphae in pots inoculated with live soil than with autoclaved soil ($F_{1,463}$=4.16 p=0.04). Together, these lines of evidence suggest that the AMF in the autoclaved soil were killed by the autoclave treatment and that plants were nonmycorrhizal. Therefore, I only included pots inoculated with live soil in analyses of root colonization and the abundance of soil hyphae. The AMF treatment interacted with other treatments to determine plant biomass responses, so I analyzed mycorrhizal and nonmycorrhizal treatments separately.

Differences among plant species

For all analyses, responses of the two C_3 grasses (*B. inermis* and *E. repens*) were identical, so their responses were combined into a single C_3 functional group category. Combining the two C_4 grasses into a single functional group did not always maintain model fit, indicating that *A. gerardii* and *S. scoparium* differed for some response variables (detailed below).

Mortality of *S. scoparium* seedlings was higher than the other species: 35 of 120 individuals died, whereas mortality of *A. gerardii*, *B. inermis*, and *E. repens* was 0/120, 0/120, and 1/120, respectively. However, *S. scoparium* seedling death did not appear to be related to the treatments (Fig. 3.2; phosphorus, light, and AMF all $p>0.05$) and probably occurred because winter greenhouse conditions were unsuitable for this C_4 species.

Averaged across all resource availabilities, the two C_3 grasses were larger than *A. gerardii* and *S. scoparium*, both when nonmycorrhizal (Fig. 3.3; $F_{2,221}=958.55$ $p<0.001$) and when mycorrhizal ($F_{2,223}=417.32$ $p<0.001$). Across all resource availabilities, the C_3 grasses had lower mycorrhizal responsiveness than the C_4 grasses (Fig. 3.4; $F_{2,225}=82.15$ $p<0.001$) and lower percent of root system colonized by AMF than *A. gerardii* and *S. scoparium* (Figs. 3.5; $F_{2,218}=10.59$ $p<0.001$), but there were no differences in the mean abundance of AMF hyphae in the soil (Fig. 3.6; $F_{2,225}=0.39$ $p=0.54$). However, species or functional groups often responded differently to changes in resource availability (indicated by interaction terms; detailed below).

Response to light availability

Both plants and AMF responded to the manipulation of light availability, but the effect depended on the AMF treatment and differed among species. When nonmycorrhizal (Fig. 3.3a,c,e,g), the two C_4 grasses differed from each other ($p<0.001$): *S. scoparium* doubled in biomass in response to a 30% increase in light ($F_{1,5}=15.67$ $p=0.01$), while *A. gerardii* did not respond ($F_{1,5}=2.40$ $p=0.18$). The two C_3 grasses did not differ from each other ($p=0.86$) and did

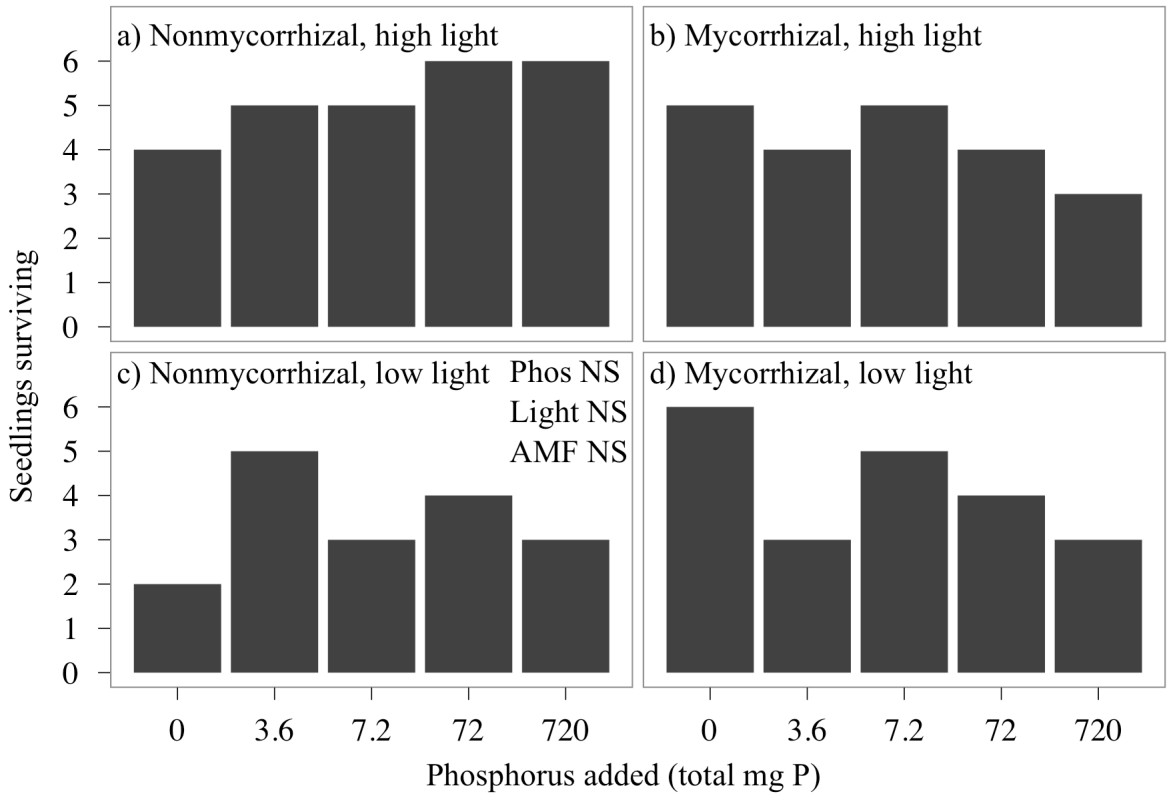


Fig. 3.2: Number of *Schizachyrium scoparium* seedlings surviving in the five phosphorus treatments when grown in a,b) high or c,d) low light conditions, either b,d) with or a,c) without arbuscular mycorrhizal fungi. The maximum number of seedlings possible in each treatment was six.

not show an increase in biomass in response to light ($F_{1,5}$=1.27 p=0.31). The two functional groups responded differently ($F_{2,221}$=8.45 p<0.001).

When mycorrhizal (Fig. 3.3b,d,f,h), plant species again responded differently to increased light ($F_{2,223}$=4.72 p=0.01). The C_4 grasses differed (p<0.001), but neither species significantly responded to light (*A. gerardii* $F_{1,5}$=4.58 p=0.09; *S. scoparium* $F_{1,5}$=0.13 p=0.73). The C_3 grasses again responded identically (p=0.87), increasing biomass in response to light by about 36% ($F_{1,5}$=21.98 p=0.005).

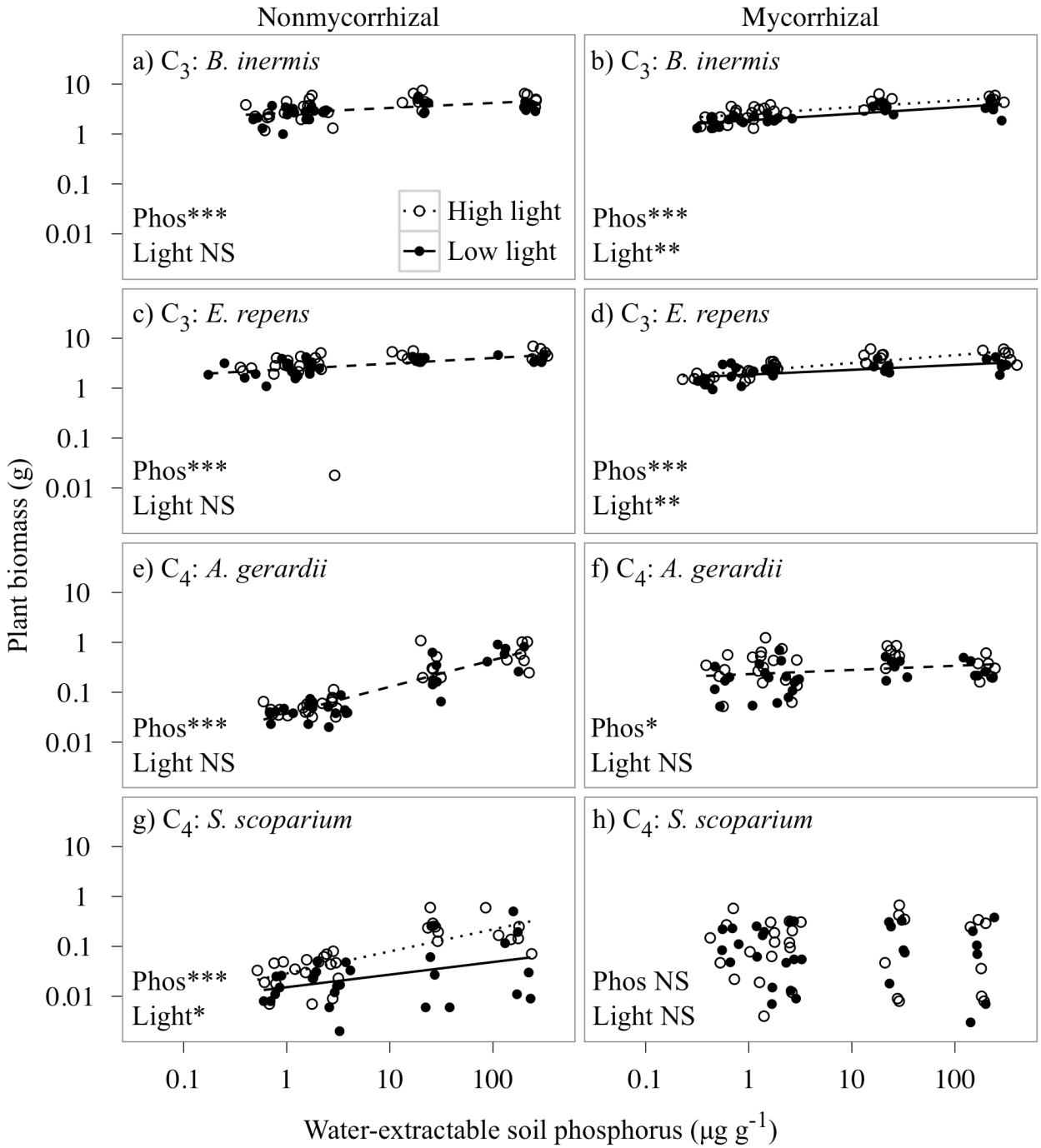


Fig. 3.3: The effect of soil phosphorus on the biomass of a,b) *B. inermis*, c,d) *E. repens*, e,f) *A. gerardii*, and g,h) *S. scoparium*, when grown a,c,e,g) without arbuscular mycorrhizal fungi or b,d,f,h) with AMF. Open symbols and dotted lines are plants grown with high light; closed symbols and solid lines are plants grown under shade structures. Dashed lines are shown when there was no difference between light treatments. Statistical significance of species or functional group responses to light or phosphorus are indicated: $p<0.001$***, $p<0.01$**, $p<0.05$*, $p>0.05$ NS. Note log-scale axes.

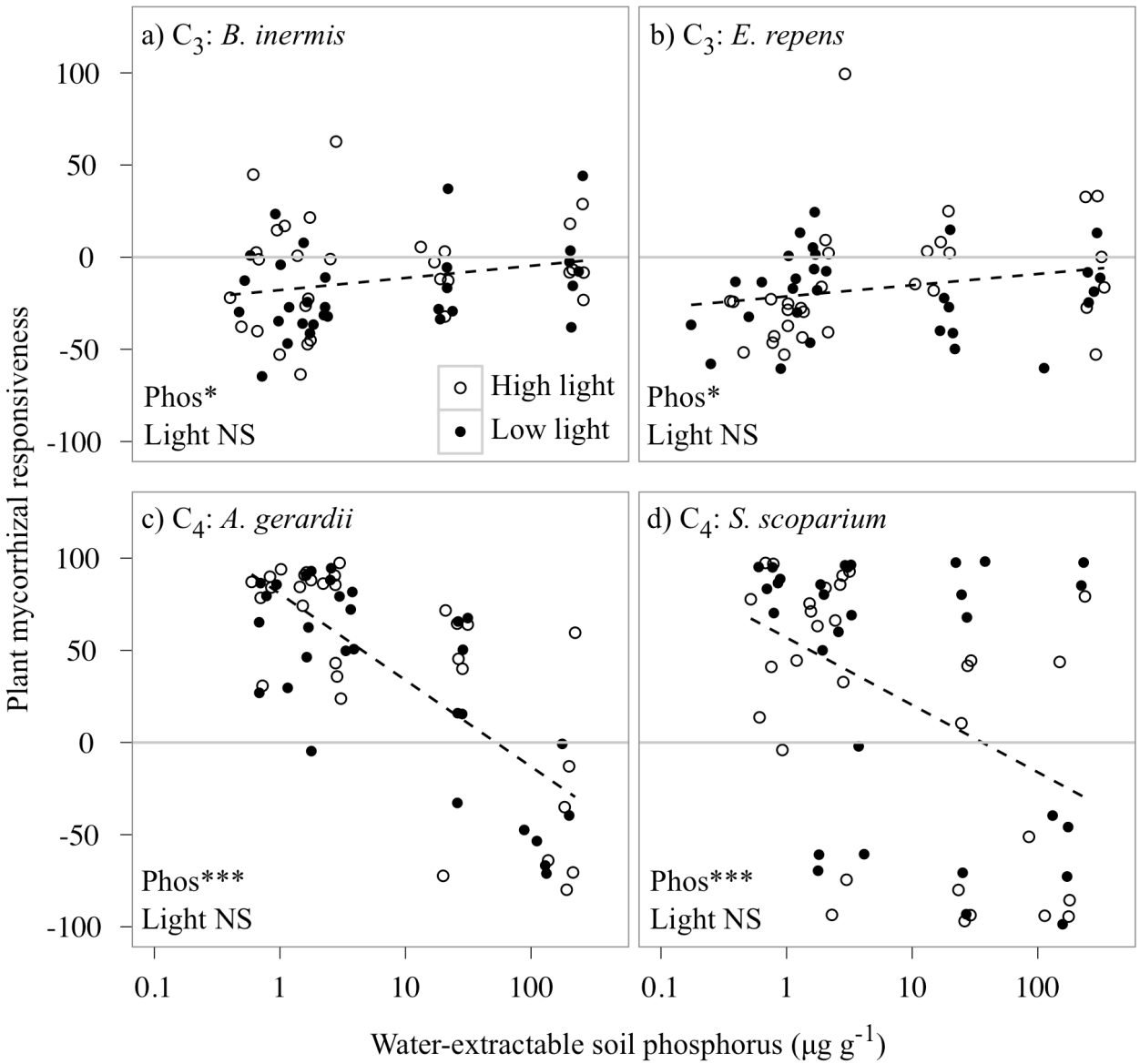


Fig. 3.4: The effect of soil phosphorus on plant biomass response to AMF (calculated as mycorrhizal responsiveness) for a) *B. inermis*, b) *E. repens*, c) *A. gerardii*, and d) *S. scoparium*. Gray horizontal lines at zero indicate no effect of AMF, positive values indicates that plants benefitted from inoculation with AMF (mutualism), and negative values indicate that plants grew larger when nonmycorrhizal (parasitism). Open symbols are plants grown with high light; closed symbols are plants grown under shade structures. Statistical significance of species or functional group responses to light or phosphorus are indicated: $p<0.001$***, $p<0.01$**, $p<0.05$*, $p>0.05$ NS. Note log-scale x-axis.

Because species responses to the treatments depended on whether plants were mycorrhizal or not, I calculated an index of mycorrhizal responsiveness (Fig. 3.4). Light had no

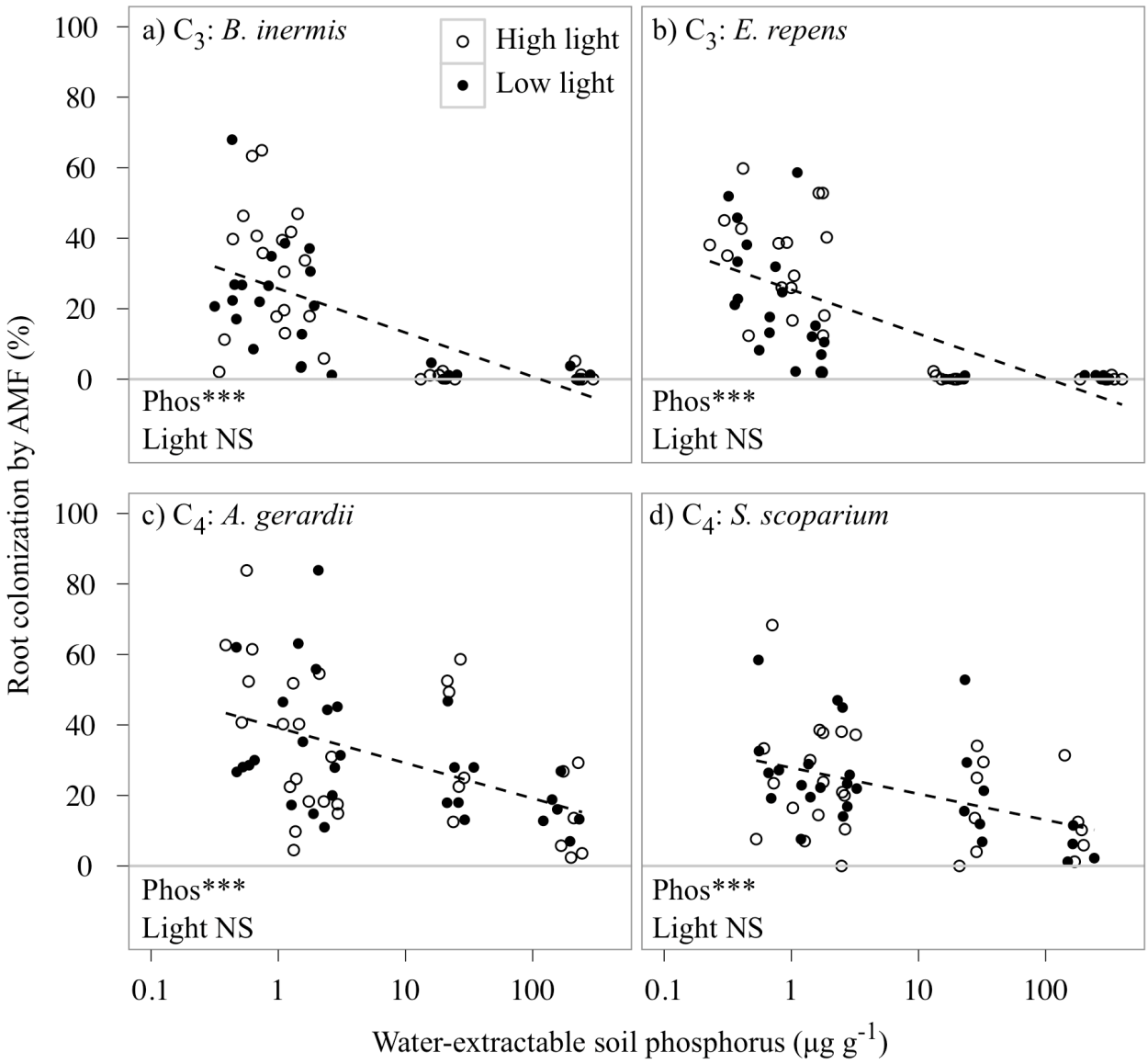


Fig. 3.5: The effect of soil phosphorus on the percent root colonization by AMF of a) *B. inermis*, b) *E. repens*, c) *A. gerardii*, and d) *S. scoparium*. Open symbols are plants grown with high light; closed symbols are plants grown under shade structures. Statistical significance of species or functional group responses to light or phosphorus are indicated: $p<0.001$***, $p<0.01$**, $p<0.05$*, $p>0.05$ NS. Note log-scale x-axis.

effect on the mycorrhizal responsiveness of any plant species ($F_{1,5}=0.03$ $p=0.88$, interaction

$p>0.05$). Light also had no effect on root colonization of any species (Fig. 3.5; $F_{1,5}=1.03$

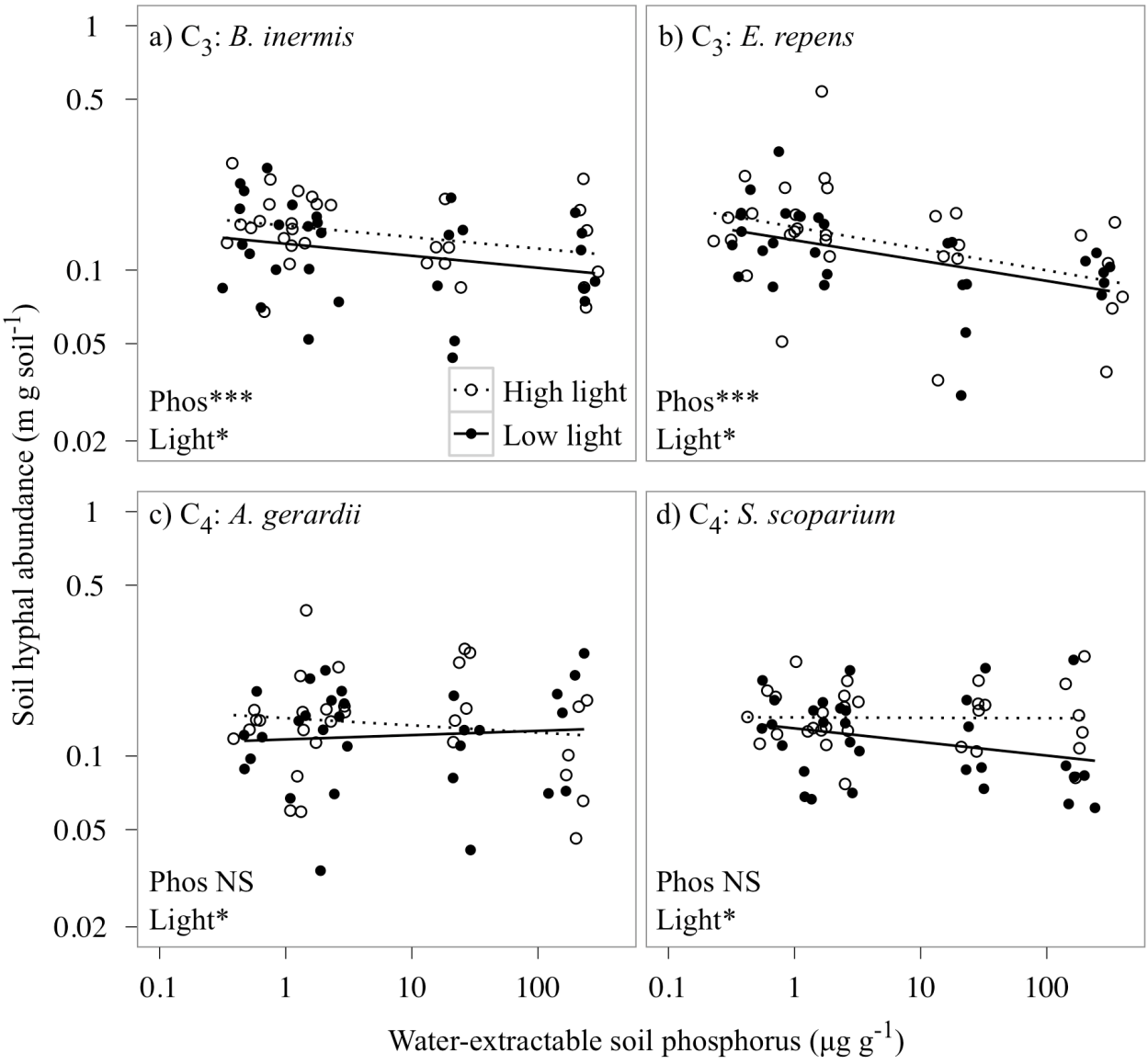


Fig. 3.6: The effect of soil phosphorus on the length of AMF extra-radical hyphae that grew in the soil under single individuals of a) *B. inermis*, b) *E. repens*, c) *A. gerardii*, and d) *S. scoparium*. Open symbols and dotted lines are plants grown with high light; closed symbols and solid lines are plants grown under shade structures. Statistical significance of species or functional group responses to light or phosphorus are indicated: $p<0.001$***, $p<0.01$**, $p<0.05$*, $p>0.05$ NS. Note log-scale axes.

$p=0.35$, interaction $p>0.05$). However, increasing light increased the abundance of AMF hyphae in the soil by about 16% (Fig. 3.6; $F_{1,5}=7.55$ $p=0.04$).

Response to phosphorus availability

Plants and AMF also responded to the phosphorus availability treatments, but again responses differed among species and depended on the AMF treatment. When nonmycorrhizal, all four species responded positively to increased phosphorus (Fig. 3.3a,c,e,g; $F_{1,221}$=89.31 p<0.0001), but the strength of the relationship differed among *A. gerardii*, *S. scoparium*, and the C_3 grasses (phosphorus x species $F_{2,221}$=34.08 p<0.0001). The two C_3 grasses did not differ in their response (p=0.86) and showed the weakest response to phosphorus availability, increasing 93% from the lowest to highest phosphorus treatment ($F_{1,107}$=22.01 p<0.001). The two C_4 species differed from each other (p<0.001): *S. scoparium* showed strong phosphorus limitation, increasing 741% ($F_{1,47}$=29.03 p<0.001), and *A. gerardii* showed the strongest phosphorus limitation, increasing 1434% ($F_{1,47}$=245.35 p<0.001) across the gradient of phosphorus availability.

When mycorrhizal, these species again responded differently to phosphorus (Fig. 3.3b,d,f,h; phosphorus x species interaction $F_{2,223}$=4.72 p=0.01). The two C_3 grasses responded similarly (p=0.87), increasing in biomass by 151% with increased phosphorus ($F_{1,107}$=147.85 p<0.001). *Andropogon gerardii* differed from *S. scoparium* (p<0.001), increasing in response to phosphorus by 33% ($F_{1,47}$=5.08 p=0.03) whereas *S. scoparium* did not ($F_{1,47}$=0.36 p=0.55).

Analysis of the index of mycorrhizal responsiveness again clarified these differences between mycorrhizal and nonmycorrhizal plant responses to phosphorus (Fig. 3.4). *Bromus inermis* and *E. repens* had similar mycorrhizal responsiveness (p=0.88). Their response to AMF increased with increases in phosphorus ($F_{1,107}$=6.42 p=0.01). With no added phosphorus, C_3

grasses were significantly smaller when mycorrhizal than when nonmycorrhizal ($F_{1,35}$=12.33 p=0.001) but this negative response to AMF disappeared at high phosphorus ($F_{1,35}$=1.48 p=0.23). However, the effect of phosphorus differed between the C_3 and C_4 grasses ($F_{1,225}$=63.34 p<0.001). Mycorrhizal responsiveness was similar between *A. gerardii* and *S. scoparium* (p=0.07), decreasing sharply with phosphorus ($F_{1,107}$=52.68 p<0.001). Separate ANOVA for each species revealed that when grown with no added phosphorus, the relationship was mutualistic for both *A. gerardii* and *S. scoparium*, increasing biomass by 491% and 656% ($F_{1,11}$=40.27 p=0.0001 and $F_{1,11}$=34.05 p=0.0001, respectively). However, in the highest level of added 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 the highest level of phosphorus ($F_{1,11}$=0.55 p=0.47). These differences in the effect of phosphorus on the mycorrhizal responsiveness of C_3 and C_4 grasses suggests that these species should also differ in the degree to which they sanction the growth of AMF at high phosphorus.

Species differences in sanction strength

To test the hypothesis that the two C_3 and the two C_4 grasses would differ in the degree to which they sanction AMF in high phosphorus environments, I measured the abundance of AMF both inside roots (percent of root system colonized by hyphae, vesicles, or arbuscules) and in the soil (extra-radical hyphae) along the phosphorus gradient. Root colonization declined in

response to increased phosphorus (Fig. 3.5; $F_{1,218}$=18.28 p<0.001) at different rates among the species ($F_{2,218}$=12.58 p<0.001). Phosphorus had similar effects on root colonization in the two C_3 grasses (p=0.86): colonization declined sharply with increasing phosphorus in both species (Fig. 3.5a,b; $F_{1,107}$=218.03 p<0.001). In the two highest phosphorus addition treatments, most C_3 individuals had 0% root colonization and the maximum level detected was 5%. In the two C_4 species, the strength of root colonization declined with phosphorus, but differed between *A. gerardii* and *S. scoparium* (p=0.005), declining in *S. scoparium* (Fig. 3.5d; $F_{1,42}$=14.05 p<0.001) and declining more weakly in *A. gerardii* (Fig. 3.5c; $F_{1,47}$=20.05 p<0.001). Levels of root colonization ranged from 1-31% in the highest phosphorus treatment in these species (Fig. 3.5c,d). Patterns in percent of root systems colonized by arbuscules or vesicles were qualitatively similar to those in total percent colonization (Figs. 3.7-3.8).

The abundance of soil AMF hyphae (extra-radical hyphae) also declined with increasing phosphorus (Fig. 3.6; $F_{1,225}$=14.96 p<0.001) but differed between the two functional groups ($F_{1,225}$=4.33 p=0.04). Specifically, AMF grown with the two C_3 grasses were less abundant in the soil with high phosphorus than with low phosphorus (Fig. 3.6a,b; $F_{1,107}$=19.98 p<0.001) but did not differ between *B. inermis* and *E. repens* (p=0.37). In contrast, the fungi grown with the two C_4 grasses showed no response to phosphorus availability in terms of soil hyphal abundance (Fig. 3.6c,d; $F_{1,107}$=1.00 p=0.32) and did not differ between *A. gerardii* and *S. scoparium*

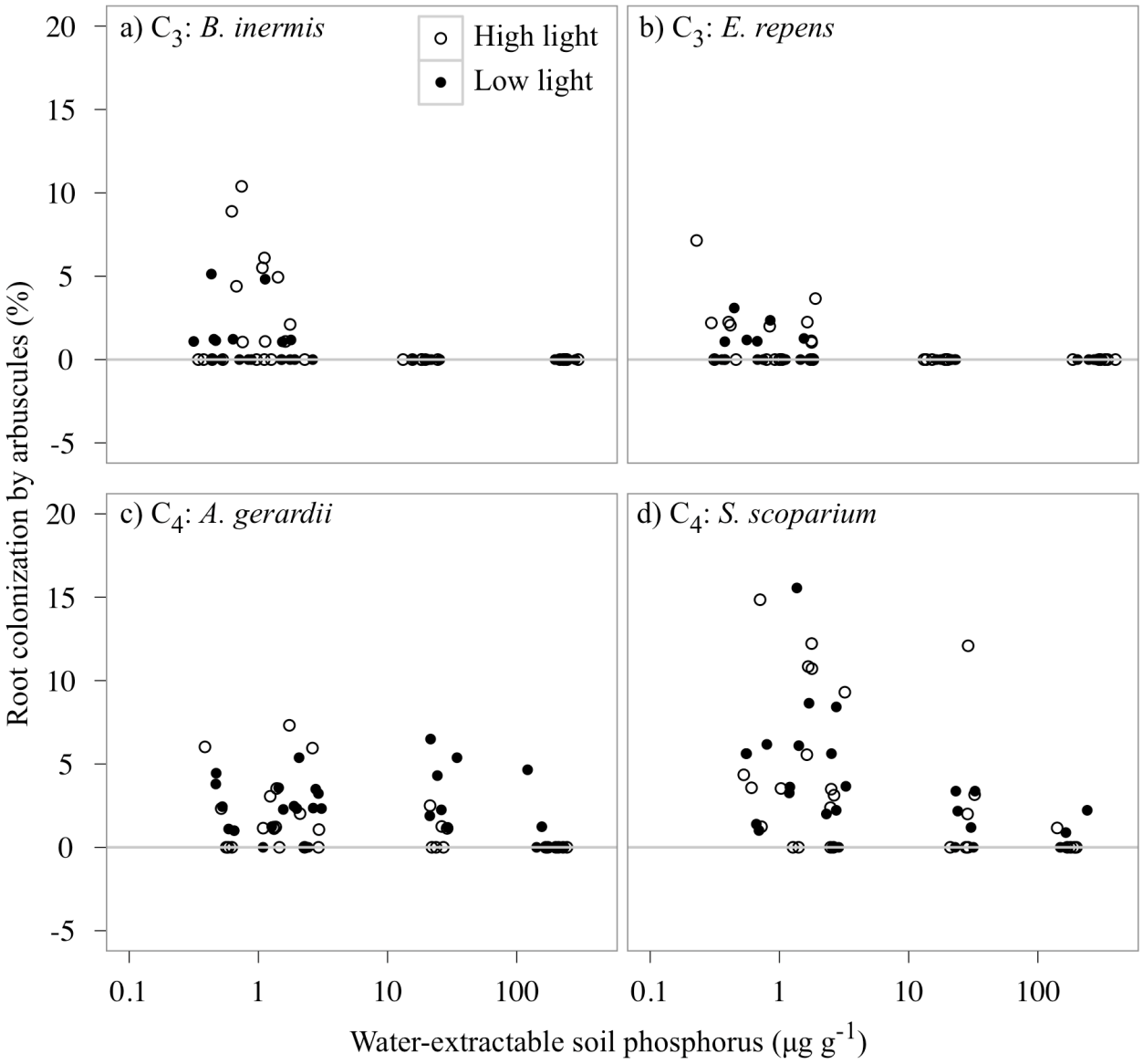


Fig. 3.7: The effect of soil phosphorus on the percent root colonization of arbuscules by AMF of a) *B. inermis*, b) *E. repens*, c) *A. gerardii*, and d) *S. scoparium*. Open symbols are plants grown with high light; closed symbols are plants grown under shade structures. Note log-scale x-axis.

(p=0.83). These results indicate that the two C_3 species sanctioned AMF in high phosphorus soils, while the two C_4 species did not.

To better understand what was driving these differences among plant species, I conducted two additional tests. First, because the C_3 and C_4 species differed in biomass, I investigated

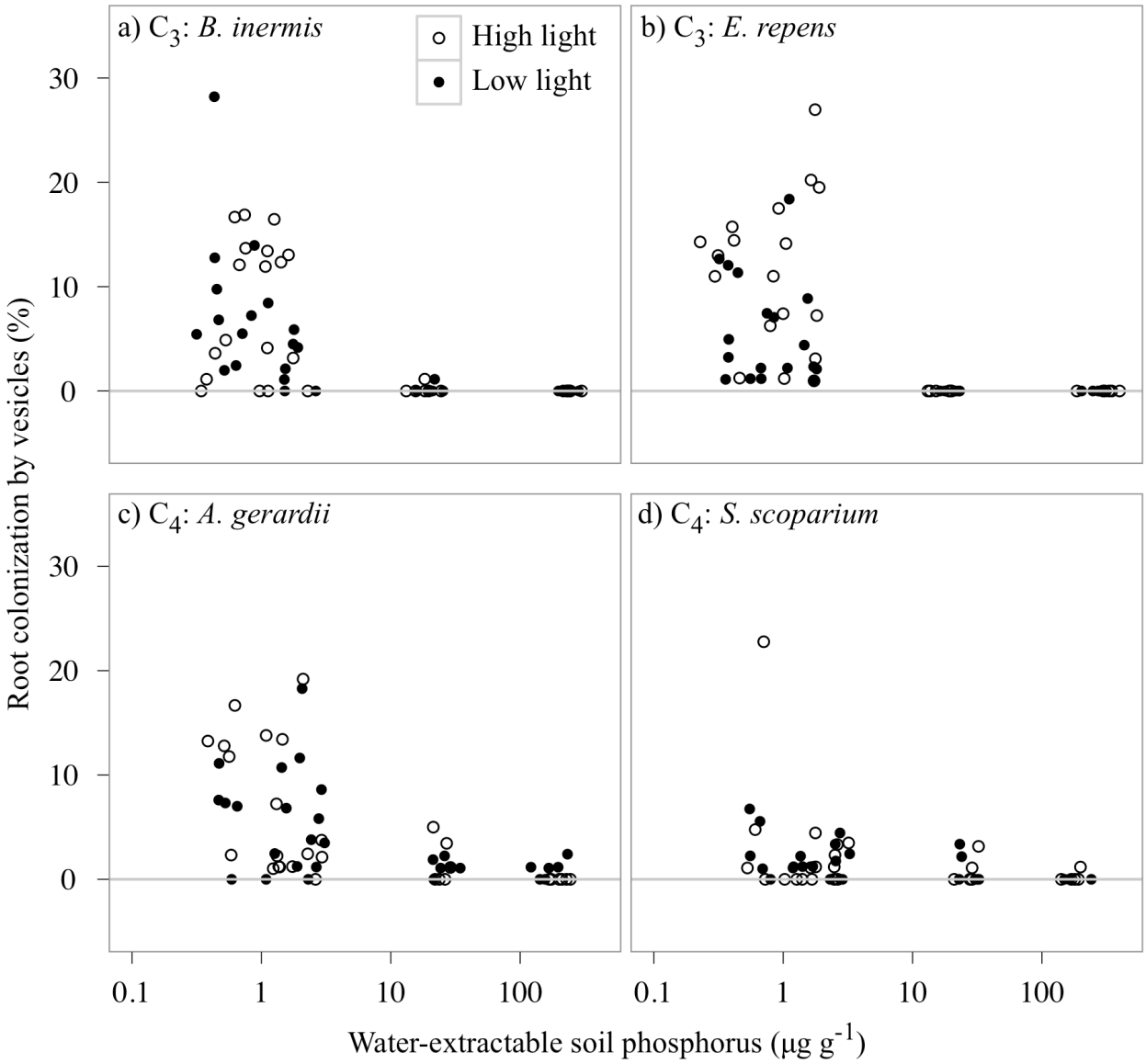


Fig. 3.8: The effect of soil phosphorus on the percent root colonization of AMF vesicles of a) *B. inermis*, b) *E. repens*, c) *A. gerardii*, and d) *S. scoparium*. Open symbols are plants grown with high light; closed symbols are plants grown under shade structures. Note log-scale x-axis.

differences in the size of plant species. I asked whether larger plants (with presumably greater photosynthetic capacity) might allocate larger amounts of carbon to AMF and support larger populations of soil hyphae. To do this, I repeated the analysis of soil hyphae but removed the effect of plant biomass by including a covariate of log(plant biomass). Interestingly, this analysis indicated that larger plants did not necessarily support larger fungal populations

($F_{1,224}$<0.01 p=0.98). It also indicated that statistically controlling for differences in biomass removed the difference in AMF abundance between the functional groups ($F_{1,225}$=1.23 p=0.27). Although phosphorus still significantly reduced AMF abundance ($F_{1,224}$=14.89 p<0.001), the relationship was the same for AMF grown with all four plant species (interaction p>0.05). However, plant biomass interacted significantly with phosphorus ($F_{1,224}$=3.91 p<0.05): at high phosphorus, larger plants had fewer soil hyphae than smaller plants. This analysis confirmed that AMF abundance responded negatively to phosphorus when grown with the larger plants (the two C_3 grasses *B. inermis* and *E. repens*) but not with the smaller plants (the two C_4 grasses *A. gerardii* and *S. scoparium*) and suggests that plant size may partially be responsible for the differences between the functional groups in their ability to sanction.

Second, I investigated whether a species' root colonization affected soil hyphal abundance. Because points of root colonization are the putative sites of carbon transfer to AMF, reduced root colonization might reflect reduced carbon allocation to fungi. I used ANCOVA to account for variation among plant species in root colonization while testing for the effect of phosphorus, light, and species on soil hyphal abundance. Soil hyphal abundance was positively associated with root colonization ($F_{1,219}$=6.03 p=0.01) and negatively associated with increases in phosphorus availability ($F_{1,219}$=4.37 p=0.04), but these factors interacted ($F_{1,219}$=3.85 p=0.05): the negative effect of phosphorus was weaker when root colonization was high. In this analysis, there were no differences between the plant functional groups ($F_{1,219}$=1.61 p=0.21). This result suggests that differential impacts of the two C_3 and the two C_4 grasses on soil hyphae

were mediated through impacts on root colonization. Specifically, plants with high root colonization had more hyphae, especially when root colonization remained high in high phosphorus—as it did in the two C_4 grasses.

Discussion

An important unanswered question in our understanding of mutualisms is whether sanctions can control the outcome of interactions with conditional cheaters such as arbuscular mycorrhizal fungi (AMF). Ecologists have long appreciated that plants "shed" their mycorrhizal associations in high nutrient environments (Smith and Read 1997), reducing their allocation to AMF when the symbiosis is not beneficial and sanctioning the conditional cheaters. However, not all plant species appear to be able to sanction sufficiently: AMF are known to parasitize plants (Hoeksema et al. 2010), especially in phosphorus-rich environments (Johnson 2010). It has been difficult to reconcile these two contrasting views on how plants should respond to AMF in high fertility environments. In this study, I offer a resolution. I found that plant species differ in their ability to sanction AMF and that these differences affect plant vulnerability to 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 (Fig. 3.1a). They also support a relationship between plant benefit at low phosphorus and plant sanction strength at high phosphorus (Fig. 3.1b). Neither of the two C_3 grasses experienced a benefit from AMF at low phosphorus, but both sanctioned AMF in high phosphorus environments: *Bromus inermis* (smooth brome) and *Elymus repens* (quackgrass) suppressed AMF abundance in roots and in the soil. As a result, these two species avoided parasitism in

high phosphorus soils. In contrast, the two C_4 grasses, which showed strong positive responses to AMF at low phosphorus, were less effective in sanctioning AMF at high phosphorus: *Andropogon gerardii* and *Schizachyrium scoparium* only weakly suppressed root colonization and failed to reduce soil hyphal abundance. This failure to sanction AMF led to parasitism in high phosphorus soils in one species (*A. gerardii*). I did not detect parasitism in the other C_4 species, *S. scoparium*, perhaps because high mortality increased measurement error. Thus, between the plant functional groups, there was a predictable relationship between mycorrhizal responsiveness at low phosphorus and plant sanction strength. There was consequently a relationship between mycorrhizal responsiveness at low phosphorus and vulnerability to parasitism.

Differences between the functional groups

Many characteristics differ between the C_3 and C_4 grasses included in this study. Some of these may be responsible for differences in mycorrhizal responsiveness at low phosphorus, vulnerability to parasitism, and sanction strength.

Other studies have noted differences between C_3 and C_4 grasses in mycorrhizal responsiveness at low phosphorus (Wilson and Hartnett 1998; Hoeksema et al. 2010). Wilson and Hartnett (1998) found that 15 of 16 perennial C_4 prairie grasses were strongly mycorrhizal responsive, whereas all 14 C_3 perennial grasses tested tended to have much lower mycorrhizal responsiveness. Root morphology, and therefore plant ability to take up soil resources, is the leading hypothesis explaining differences in mycorrhizal responsiveness between the two

groups. The root systems of C_3 and C_4 prairie grasses differ in terms of root diameter and specific root length; these differences in root morphology are correlated with differences in mycorrhizal responsiveness (Hetrick et al. 1988a; Hetrick et al. 1988b). Root hairs are also important for taking up relatively immobile mineral nutrients such as phosphorus (Bates and Lynch 2001) and may predict differences in mycorrhizal responsiveness (Schweiger et al. 1995). Observation of root morphology in this study supports this idea. The roots of these C_3 grasses, *B. inermis* in particular, were densely covered in root hairs, many exceeding 1 mm in length. The C_4 grasses, on the other hand, had far fewer root hairs. Supporting the idea that the C_4 grasses in this study were less able than the C_3 grasses to take up scarce soil phosphorus, I found that nonmycorrhizal C_4 grasses showed much stronger growth responses to phosphorus addition than C_3 grasses. These root morphology traits may also determine a species' susceptibility to parasitism, but I am unaware of systematic tests of this idea.

The species in this study also differed in their plasticity of root colonization. Greater plasticity in root colonization of the two C_3 grasses, compared to the two C_4 grasses, could explain differences between the plant functional groups in how effectively they sanction AMF in high resource environments. Indeed, root colonization did predict soil hyphal abundance, but it interacted with soil phosphorus availability such that less plastic species suppressed soil AMF abundance more weakly. However, because differences in plasticity of root colonization were confounded with functional group and I could not test for the effect of root colonization alone, differences among the functional groups might be driven by other unique traits. 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 plant response was associated with effective sanctions against non-mutualistic AMF.

Another difference among the species in this study was that the two C_3 grasses tended to be larger than the two C_4 grasses at all levels of resource availability. Plant size is known to affect plant allocation to above and belowground biomass (Weiner 1994) and may also affect allocation to AMF. In this study, large plants at high phosphorus had stronger negative impacts on AMF abundance than small plants, but including plant biomass as a predictor removed the difference between the C_3 and C_4 grasses. This finding suggests that an important factor associated with differences in fungal response to phosphorus between these C_3 and C_4 grasses was the larger size of *B. inermis* and *E. repens* compared to *A. gerardii* and *S. scoparium*. However, plants in the two functional groups were consistently different in size, and there was no direct relationship between plant biomass and AMF abundance within each group. Therefore, it was not possible to determine whether *B. inermis* and *E. repens* suppressed soil hyphae at high phosphorus more effectively than *A. gerardii* and *S. scoparium* because of their larger size, or because of some other unique trait. It is also likely that this relationship with plant size would differ if plants were allowed to reach their adult size. These C_4 grasses, when mature, produce much more aboveground biomass than the C_3 grasses (Mahaney et al. 2008). Therefore, it is unlikely that size differences are the primary driver of differences in sanction strength among the species in this study.

The grasses in this study also differ in their evolutionary origin. The two C_4 grasses are native prairie species, while both C_3 grasses are introduced species. This difference might

explain differences in how the grasses responded to AMF in low phosphorus: perhaps *B. inermis* and *E. repens* lacked their coevolved fungal symbionts and this caused their negative mycorrhizal responsiveness. However, Wilson and Hartnett (1998) found no difference in mycorrhizal responsiveness between native and exotic C_3 grasses, suggesting that the evolutionary origin was not an important factor determining plant interactions with AMF. Furthermore, the soil inoculum used in this experiment likely also lacked the coevolved fungal symbionts of the native C_4 prairie grasses as the inoculum was taken from a 12-15 year old field abandoned from agriculture (a corn-soybean rotation) and dominated by weedy native and exotic species. Therefore, the native or introduced status of the species was probably not the most important factor determining response to AMF at low phosphorus. The difference in evolutionary origin may also have affected species' susceptibility to parasitism at high phosphorus or ability to sanction cheaters, but it is unclear how a species' native or introduced status could explain the observed results.

Differences in sanction strength: other examples from the mycorrhizal symbiosis

Despite the widespread distribution of mycorrhizal symbioses (Wang and Qiu 2006) and large differences in plant benefit (Hoeksema et al. 2010), only a few other studies have also compared species' mycorrhizal responsiveness and susceptibility to parasitism and these have produced conflicting results. Graham and Eissenstat (1994) found that citrus genotypes that benefitted more from AMF at low phosphorus were more susceptible to parasitism at high phosphorus. Between two of those genotypes, the gain-more-lose-more genotype (sour orange) lost more nonstructural carbohydrates to AMF at high phosphorus than the other genotype (sweet orange), resulting in parasitism in sour orange (Jifon et al. 2002). However, other studies have

shown no differences in species ability to sanction fungi at high phosphorus. Noyd et al. (1995) compared two C_4 grasses and one C_3 grass and found that all three species prevented parasitism, reduced root colonization, and suppressed soil hyphal abundance. Johnson (1998) also found evidence against this relationship: *Panicum virgatum* (a highly mycorrhizal C_4 grass) benefitted strongly from AMF at low phosphorus but was weakly parasitized at high phosphorus, while *Salsola kali* (a forb belonging to a family thought to be nonmycorrhizal) gained little from AMF at low phosphorus but was strongly parasitized at high phosphorus. Among 28 genotypes of maize inbred lines, Kaeppler et al. (2000) found no relationship between mycorrhizal responsiveness at low and high phosphorus. Thus, among these studies, there appears to be no predictable relationship between a species' or genotype's response to AMF at low and high phosphorus.

Implications of differences in plant species ability to sanction AMF

Variation among plant species in their ability to sanction non-mutualistic AMF could help explain the distribution of plant species across fertility gradients and their responses to eutrophication. Both *A. gerardii* and *S. scoparium*, native prairie grasses once widespread in the Midwestern USA, are now restricted to low fertility grasslands in Michigan (Foster 1999). Introduced C_3 grasses such as *E. repens* and especially *B. inermis* now dominate more productive sites in this region (Foster 1999, Grman pers. obs.). Long-term fertilization experiments in Minnesota have also shown 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 C_4 grasses in highly productive soils is reduced seedling

establishment driven by low light availability under abundant litter and a canopy of C_3 grasses (Foster and Gross 1998; Foster 1999). These carbon-starved seedlings would be especially vulnerable to the effects of parasitic AMF. This study has shown that AMF can negatively affect *A. gerardii* seedlings, even in the absence of competition and litter. Thus, a second mechanism contributing to C_4 grass loss in fertile soils might be their inability to sanction cheating AMF. The dominant C_3 grasses, on the other hand, would avoid this added carbon cost of AMF in nutrient-rich soils by effectively sanctioning AMF and avoiding parasitism. Johnson et al. (2008) found support for the hypothesis that a species' loss from eutrophied habitats might be associated with an inability to reduce allocation to AMF: both *E. repens* and *Panicum virgatum*, which increase in response to long-term fertilization, were more plastic in allocation to AMF than *A. gerardii*. Also supporting this idea, Johnson et al. (2003b) found that the outcome of competition was better for several strongly mycorrhizal species in low nitrogen than in high nitrogen soils. Thus, differences in plant species ability to sanction AMF may contribute to the loss of some species from eutrophied communities.

Identifying patterns in variation among plant species in their ability to sanction non-mutualistic AMF could also help explain variation in AMF abundance across fertility gradients. AMF are thought to decline in abundance in fertile soils because of reduced plant allocation when AMF would not be beneficial (Treseder 2004). In general, this study supports this idea, with an important caveat. Differences in the mycorrhizal responsiveness of dominant plant species across the fertility gradient may alter the degree of decline in fungal abundance. Under strongly mycorrhizal responsive species, AMF abundance may not change dramatically with increases in fertility, but under more weakly mycorrhizal responsive species, AMF may decline

more sharply. Changes in the abundance of AMF could have important impacts both on plant community structure (Klironomos et al. 2011) and on ecosystem functions such as soil stability, carbon sequestration, and nutrient cycling and retention (Wilson et al. 2009; van der Heijden 2010). If we are to understand controls on the abundance of AMF, this hypothesis needs further testing.

Differences in sanction strength: examples from other types of mutualism

Among other types of mutualisms, few studies have attempted to compare the relative effectiveness of sanctions among species or propose hypotheses to explain the variation. Nearly 30 years ago, Minchin et al. (1983) reported differences in the degree to which legume species sanctioned rhizobia experimentally prevented from fixing nitrogen, but I am unaware of any hypotheses proposed to explain this variation. Similarly, Simms et al. (2006) reported variation in the ability of different legume genotypes to sanction worse rhizobial mutualists, but they did not investigate this variation. Jandér and Herre (2010) measured variation in sanction strength across six species of fig tree. Among the four fig species that imposed sanctions on cheating pollinator wasps, sanction strength was negatively correlated with the proportion of wasps not carrying pollen (and thus potentially acting as cheaters). 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 effectively against the poor quality mutualist. While this hypothesis of artificial selection makes sense in the particular human-dominated

system in that study, it is unlikely to explain natural variation among genotypes or species. 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 specific pollinators. However, they could not test for patterns in sanction strength among species, and it is difficult to generalize this relationship to other types of 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 plant species lacked the capacity to sanction cheating ants because it developed domatia before developing the leaf (Edwards et al. 2010). This developmental constraint prevented the second species from sanctioning cheating ants. 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 (Ferriere et al. 2007; Jones et al. 2009). In this study, I found variation in the ability of plant species to hold conditional cheaters in check. This variation has important implications for the distribution and abundance of plant and AMF species. Both within the mycorrhizal symbiosis and across other types of 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.

CHAPTER FOUR

Light limitation and plant-mycorrhizal interactions across a natural productivity gradient

Abstract

A key idea in plant community ecology is that the identity of the limiting resource shifts from soil nutrients in low productivity sites to light in high productivity sites. This shift in the limiting resource has critical implications for interactions between seedlings and arbuscular mycorrhizal fungi (AMF). To test the idea that seedling light limitation would increase in strength across a natural productivity gradient, and that light limitation would affect seedling response to natural variation in AMF abundance, I conducted a cross-site field experiment. I found that transplanted seedlings of big bluestem (*Andropogon gerardii*) responded positively to a light addition (tie-back) treatment that increased light availability. However, seedling response to the treatment did not increase across the natural productivity gradient. Furthermore, light availability did not affect seedling response to variation in AMF abundance across the gradient. Complex interactions between seedlings and their competitors and mutualists that differed across sites combined to determine seedling growth. It is not possible to extrapolate to the field results from greenhouse experiments showing that plant-AMF interactions depend on the identity of the limiting resource. The importance of shifts in the limiting resource for seedlings establishment in the field and interactions with AMF must be explicitly tested in manipulative field experiments.

Introduction

The outcome of species interactions can be strongly dependent on the identity of the limiting resource, but the identity of the limiting resource varies with the specific environmental context. For plants, one important shift in the identity of the limiting resource is thought to occur along gradients of soil fertility (Tilman 1988). In low fertility soils, plants are typically limited by soil nutrients such as nitrogen or phosphorus. In higher nutrient soils, soil nutrient limitation is alleviated, allowing plants to grow larger, decrease root:shoot ratios, and produce greater aboveground biomass (Dybzinski and Tilman 2007). Greater aboveground productivity leads to more shading and lower light availability under the canopy (Dybzinski and Tilman 2007; Hautier et al. 2009). Thus, we expect plants to experience a shift towards light limitation with increases in aboveground productivity. The effects of the transition from soil nutrient to light limitation on species interactions underpins much of plant community ecology. For example, shifts in the identity of the limiting resource from soil nutrients to light strongly impact plant interactions with other organisms and thus can determine the distribution of plant species (Dybzinski and Tilman 2007), plant successional trajectories (Tilman 1988), and plant community responses to elevated carbon dioxide (Reich 2009) and atmospheric nitrogen deposition (Hautier et al. 2009).

Shifts in limiting resources may be particularly important for determining plant interactions with one type of mutualist, arbuscular mycorrhizal fungi (AMF) (Johnson 2010). AMF are ubiquitous fungi that participate in a mutualism with the majority of terrestrial plant species (Wang and Qiu 2006), transferring soil nutrients to plants in exchange for photosynthetically fixed carbon. AMF are most beneficial to plants in low soil nutrient environments (Smith and Read 1997; Hoeksema et al. 2010), where they provide the plant with

limiting soil resources and obtain from the plant non-limiting photosynthetically fixed carbon (Johnson 2010). In high nutrient soils, on the other hand, AMF provide a non-limiting resource (soil nutrient) but obtain a plant-limiting resource (fixed carbon) from the plant. In this environment, the nature of the relationship can change: plants can reduce or eliminate allocation to AMF such that they do not associate with AMF and the mutualism disintegrates (Smith and Read 1997). Supporting this prediction, many studies find decreased root colonization in high nutrient (Smith and Read 1997; Treseder 2004) or low light environments (Johnson 2010). Alternatively, in high nutrient soils the mutualism may switch to a parasitism, where plants experience negative growth responses to AMF (Johnson et al. 1997; Johnson 2010). This contingent outcome of plant-mycorrhizal interactions, where plants benefit from AMF in poor soils but do not benefit in fertile soils, is likely driven by changes in the identity of the limiting resource (Johnson 2010).

Shifts from nutrient to light limitation may impact seedlings more than adult plants for two reasons. First, seedlings are smaller than adult plants and fall victim to the unidirectional supply of light and asymmetric light competition. Second, seeds contain nutritional resources for initial seedling growth and seedlings are most limited by carbon (Johnson et al. 1997). These two factors contribute to make seedlings particularly prone to light limitation. For example, seedlings are frequently lost from fertilized communities, likely because of increased light limitation (e.g., Foster and Gross 1998; Hautier et al. 2009; but see Dickson and Foster 2011). These two factors also cause seedling interactions with AMF to be particularly context dependent (Johnson et al. 1997). For example, in a greenhouse experiment (Chapter 3), I showed that AMF increased big bluestem (*Andropogon gerardii*) seedling biomass in low phosphorus environments where the seedlings were phosphorus limited. In high phosphorus

environments, AMF decreased *A. gerardii* growth and acted as plant parasites, likely because the seedlings were light limited (although I did not detect light limitation, perhaps because light manipulations were too subtle). Many other greenhouse experiments have also found that transitions from nutrient to light limitation can affect the outcome of seedling interactions with AMF (Johnson 2010).

However, it is difficult to extrapolate these results to seedling establishment in the field for several reasons. First, several studies have investigated the effects of AMF in a community context, but they have not been able to separate direct effects of AMF on seedlings from indirect effects mediated through altered competitive environments. They have generally found, however, that resource availability does affect the impact of AMF on communities and that plant species responses are idiosyncratic (Johnson et al. 2005; van der Heijden et al. 2008; Collins and Foster 2009). Second, pot studies show that seedlings tend to benefit from pre-established networks of mycorrhizal fungal hyphae ("common mycorrhizal networks"; van der Heijden 2004; van der Heijden and Horton 2009). However, too few of these studies have been conducted to ask whether seedling benefit from established fungal networks depends on the identity of the limiting resource. Third, in field sites where disturbance has reduced AMF abundance, inoculation with AMF frequently increases the establishment and growth of crop plants (Lekberg and Koide 2005) and late-successional species that are dependent on AMF (Johnson 1998; Smith et al. 1998). However, it is unclear whether the benefits of inoculation also depend on resource availability (Lekberg and Koide 2005). Finally, while studies have manipulated light available to seedlings or small statured plants in the field (e.g., Dickson and Foster 2011 and refs therein), they have not specifically investigated the role of AMF in seedling

establishment. Whether seedling responses to AMF depend on the identity of the limiting resource in the field is therefore an important unanswered question.

What is the role of light limitation in determining seedling response to established networks of arbuscular mycorrhizal fungi across natural gradients in the field? To address this question, I conducted a light addition experiment with *A. gerardii* seedlings in six old-fields in southwest Michigan. I asked three specific questions. First, are seedlings light limited in the field? Second, does seedling light limitation vary across sites that vary in aboveground productivity? Third, does light limitation affect *A. gerardii* seedling response to the abundance of AMF in the field?

Methods

Site selection: To measure seedling light limitation across a natural productivity gradient, I chose six sites in old-fields in southwest Michigan. Three sites were at the W. K. Kellogg Biological Station in Kalamazoo County (Fig. 1.1) on Kalamazoo loam (sites KP and KL) or Oshtemo sandy loam (site KM) soils. Three sites were in the Kellogg Biological Station's Lux Arbor Reserve in Barry County (Fig. 1.2) on Oshtemo sandy loam soils (sites LB, LJ, and LS). All six sites were dominated by smooth brome (*Bromus inermis*), a non-native clonal C_3 perennial grass. None of them contained natural populations of big bluestem (*Andropogon gerardii*), but one (site KM) was approximately 200 m from *A. gerardii* experimentally planted in 1995 (Foster 1999) and 2005 (Mahaney 2007). The other species in the community were mostly C_3 grasses such as *Elymus repens*, *Poa* spp., *Dactylis glomerata*, and *Phleum pratense*, although a few forbs such as *Centaurea maculosa* and *Hieracium* sp. were occasionally present.

At each site in 2007, I set up fifteen 0.5 x 0.5 m plots. Each plot was randomly assigned to one of three plot types (site characterization, light addition treatment, and control) within five replicate blocks at each site.

Site characterization: In late June 2007, when the brome was setting seed, I collected site characterization data from 3 or 4 plots at each site; these plots were not used later in the experiment. Specifically, I clipped aboveground biomass and sorted it to live biomass or litter. I dried and weighed the biomass to estimate site productivity. I collected soil samples from the top 10 cm of soil, sieved the samples, and analyzed the samples for gravimetric soil moisture (Robertson et al. 1999). After air-drying the samples, I measured water-extractable soil phosphorus by extracting with water, using the ascorbic acid method to develop color, and determining phosphate content on a spectrophotometer (modified from Kuo 1996). I also conducted laboratory nitrogen mineralization on the soils to estimate net nitrification and net mineralization (Robertson et al. 1999). I incubated the soils at about 60% water-filled pore space and 25 degrees C for 34 days, comparing the difference in 1M KCl-extractions on subsamples at the beginning of the incubation (T_0) and at the end of the incubation (T_{34}). Extracts were frozen and analyzed for NO_3 and NH_4 on an O. I. Analytical Flow Solution IV Analyzer (O. I. Analytical, College Station, TX). I also analyzed the air-dried soils for the abundance of extra-radical hyphae of arbuscular mycorrhizal fungi. To quantify AMF, I extracted 2 g air-dried soil in 200 mL of 20 g/L sodium hexametaphosphate by stirring, sonicating, and filtering through 20 µm mesh. I then stained the mesh and the hyphae trapped on it in trypan blue stain. I resuspended the hyphae and re-filtered them onto a nitrocellulose filter, then quantified the hyphae of AMF by mounting the nitrocellulose filter on a microscope slide and using the gridline intercept method, examining 100 haphazardly chosen fields at 200x (modified from Miller et al.

1995). All six sites were maintained through 2008, but the two sites of highest aboveground biomass (KL and LJ) were excluded in 2009 because of site disturbances beyond my control.

Establishing A. gerardii seedlings: In July 2007, I germinated seeds of a northern Indiana genotype of *A. gerardii* (purchased from Native Connections, Three Rivers, MI) on moistened filter paper in Petri dishes in a greenhouse at the Kellogg Biological Station. After germination, I transplanted the seedlings into trays of peat moss. I transplanted the seedlings into the field plots at the end of September to avoid mid-summer droughts. I planted nine seedlings into each plot, replacing seedlings that died until the end of October. Most seedling mortality was probably from slug herbivory. Seedling mortality was higher than expected, so I started a second set of seedlings from the same seed source in the greenhouse in April 2008. After germination, I transplanted each seedling into a 3 oz plastic drinking cup filled with potting soil and a few g of sieved field soil or 4 oz peat pots filled with sieved field soil. I transplanted these seedlings into the plots in the field in early June 2008 to achieve four live seedlings per plot. Spring seedling mortality was very high, so I continued transplanting to replace mortality until the end of June. I covered the seedlings with 5cm x 5cm x 5cm cages of hardware cloth from early June 2008 until spring 2009.

Light addition treatment: I established light addition treatments at five randomly selected replicate plots in each site in early June 2008. I increased light available to the target seedlings by tying back the vegetation around the seedlings with string anchored to the ground with nails. I also gently pushed litter to the side of seedlings, reducing the effect of shading by litter. I removed the nails and string in early October 2008. I then re-established the treatment when the *A. gerardii* seedlings emerged in late May 2009 and removed them in early October 2009. These manipulations were timed to be in place during the part of the growing season when *A. gerardii*,

a warm-season C_4 grass, is most active (June-September). Five additional plots per site were unmanipulated controls.

Effect of the treatment on the abiotic environment: I measured the effect of the light addition treatment on several abiotic variables: light available to seedlings, soil temperature, soil moisture, nitrogen availability, and phosphorus availability. I measured light availability three times over the experimental period, in mid-July 2008, early September 2008, and early September 2009. I used a Li-Cor LI-185B Quantum/Radiometer/Photometer point sensor (Li-Cor, Inc., Lincoln, NE) to measure photosynthetically active radiation (PAR) at the top of the seedling cages, about 5cm from the soil surface, and compared this measurement to full sun readings taken above the brome canopy. I used a soil thermometer to measure effects of the treatment on soil temperature in the top 12 cm of soil in early July 2008, early August 2008, and mid-August 2009. I measured the effects on soil moisture by collecting 15-cm soil cores and analyzing them for gravimetric soil moisture in early July 2008, early August 2008, and mid-August 2009.

To determine whether the treatment affected soil nitrogen availability, I installed one anion and one cation resin strip into each plot in mid-June 2009 and covered them with the hardware cloth cages to reduce rodent damage. I prepared and analyzed the resin strips following the KBS LTER's protocol (http://lter.msu.edu/protocols/105). In mid-July 2009, I collected the strips (27 day incubation) and installed a second set of strips, which I collected in mid-August 2009. During the second incubation, rodents dug up and destroyed five of ten cation resin strips at site KP (four in control plots) and one of ten (a control plot) at site KM. Remaining values were highly correlated with values from the first incubation ($r=0.87$), so I threw out the data from the second incubation. To measure the effect of the treatment on

phosphorus availability, I analyzed air-dried soil samples collected in August 2009 for water-extractable soil phosphorus as described above. To determine the phosphorus content of the extract, I developed color using malachite green and determined intensity of color development on a microplate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA, USA; modified from D'Angelo et al. 2001).

Treatment effects on the plants and AMF: I also measured the effect of the light addition treatments on several biotic response variables likely to affect the target seedlings. These variables included the abundance of live and dead neighboring plants and litter and the abundance of soil (extra-radical) hyphae of AMF. To measure the effect of two consecutive years of the treatment on the neighboring plants, I clipped aboveground biomass in early October 2009. I sorted this biomass into live *B. inermis* (the dominant species), other live biomass, standing dead biomass (which represents spring growth of the plant community), and litter. I dried the biomass to constant mass and weighed it.

To measure the effect of the treatments on the growth of soil hyphae of AMF, I installed bags of polyester window curtain fabric (mesh size approximately 250 μm, bags about 2 cm in diameter and 5 cm long) filled with play sand into each plot. In October 2008, I buried two bags per plot into holes pre-drilled with a soil corer so the top of the bag was flush with the soil surface. I removed the bags in early October 2009, after approximately one year of fungal growth into the bags, and composited the contents of the two bags. I extracted extra-radical fungi from the sand by first removing plant roots with forceps, then stirring and sonicating about 5 g of the sand in 20 g/L sodium hexametaphosphate solution and processing the extracts as described above for bulk soil hyphal abundance in 2007.

To measure the effects of the treatment on *A. gerardii* seedling growth and survival, I counted the number of surviving individuals in each plot in late September 2008 and mid-August 2009. In late September 2008 and early October 2009, I clipped seedlings at ground level to estimate their aboveground biomass, pooling all seedlings from a plot into a single biomass sample and drying and weighing as above.

Statistical analysis: To determine if there were differences among the sites that might affect seedling response to the light addition treatment, I conducted one-way ANOVA followed by Tukey's HSD test on the site characterization data collected in 2007. To assess the effect of the light addition treatment on abiotic and biotic response variables, I conducted ANOVA with two fixed factors, treatment (control vs light addition) and site (6 and 4 sites in 2008 and 2009, respectively), and a random blocking factor (5 blocks per site). If the site by treatment term was significant ($p<0.05$), I conducted paired-sample *t*-tests and report sites at which the treatment had a significant ($p<0.05$) effect. If not reported, main effects and interaction terms were not significant. To improve normality and homoskedasticity, I square-root-transformed the resin strip nitrogen data and log-transformed *A. gerardii* seedling biomass (2008 and 2009), standing dead biomass, and hyphal length. To analyze site and treatment effects on number of seedlings surviving, I used a chi-square test. I conducted all ANOVA and ANCOVA analyses in library nlme in R (2.10.1, The R Foundation for Statistical Computing).

Because the light addition treatment affected several biotic and abiotic variables, and sites differed in many characteristics, I used partial least squares regression (PLSR; Wold et al. 2001; Fraterrigo and Downing 2008; Carrascal et al. 2009) to determine which of these factors were most important in determining seedling biomass. This technique has advantages over multiple regression: it allows many predictor variables despite low replication and correlations

among the predictor variables. It constructs "latent" components that are linear combinations of the predictor variables, chosen such that the components maximize the explained variance in the dependent variable (Fraterrigo and Downing 2008; Carrascal et al. 2009). To choose the number of these components to use in interpretation, I performed cross-validation by dividing the dataset into ten segments and re-running the analysis ten times, each time removing one of the segments (Wold et al. 2001). I used the components to predict seedling biomass; differences between predicted and observed seedling biomass, summed across all ten runs, indicated the predictive residual sum of squares (PRESS). I used the PRESS to determine whether the components were statistically significant (Wold et al. 2001; Fraterrigo and Downing 2008). All the components presented here are statistically significant; I chose to keep only the first two components for interpretability. To interpret the relationships between the original predictor variables and seedling biomass, I examined the loading weights of each component. If the squared loading weight of a predictor variable was greater than 0.1, I considered that variable to be a significant contributor to the explanatory power of that component (a compromise between loading weights of 0.05 and 0.2 in Carrascal et al. 2009). I compared site-level data collected in 2007 (averaged across the 3-4 replicate samples per site) and plot-level data collected in 2008 and 2009. I analyzed log-transformed seedling biomass in 2008 and 2009 separately. I centered all predictor variables by standardizing to a mean of zero and a standard deviation of one (Fraterrigo and Downing 2008). This standardization prevents highly variable predictors (such as productivity, which ranged from about 500-1200 g/m^2) from masking the effects of less variable predictors (such as soil moisture, which ranged from about 0.01-0.2 g water/g soil). I conducted PLSR using R (2.10.1, The R Foundation for Statistical Computing) library pls (Mevik and Wehrens 2007).

Results

Site differences

The six sites used for this experiment differed in a number of biotic and abiotic characteristics measured in 2007 (Fig. 4.1). The sites differed in live aboveground biomass (Fig. 4.1a; $F_{5,13}$=6.14 p=0.004) and litter ($F_{5,13}$=7.30 p=0.002). The sites also differed in soil resources, including soil moisture (Fig. 4.1b; $F_{5,13}$=10.16 p<0.001), net nitrification (Fig. 4.1c; $F_{5,13}$=19.14 p<0.001), net nitrogen mineralization (Fig. 4.1d; $F_{5,13}$=9.15 p<0.001), and water-extractable soil phosphorus (Fig. 4.1e; $F_{5,13}$=14.53 p<0.001). I used the sum of live aboveground biomass and litter as an index of productivity because they were positively correlated (Spearman's rho=0.44). Site productivity was positively correlated with most measurements of soil resource availability, including soil moisture (rho=0.38), net nitrification (rho=0.37), and net nitrogen mineralization (rho=0.43), but was negatively correlated with water-extractable soil phosphorus (rho=-0.28), likely because of a single site (site KM). The abundance of soil hyphae of arbuscular mycorrhizal fungi was variable among sites (Fig. 4.1f), but because of high within-site variability among the three replicate samples at two sites (KP and KL), site differences were not statistically significant (Fig. 4.1f, $F_{5,13}$=2.10 p=0.13). AMF abundance was weakly correlated with site productivity (rho=0.16), soil phosphorus (rho=0.18), net nitrification (rho=0.2), and nitrogen mineralization (rho=0.28), but more strongly negatively correlated with soil moisture (rho=-0.46).

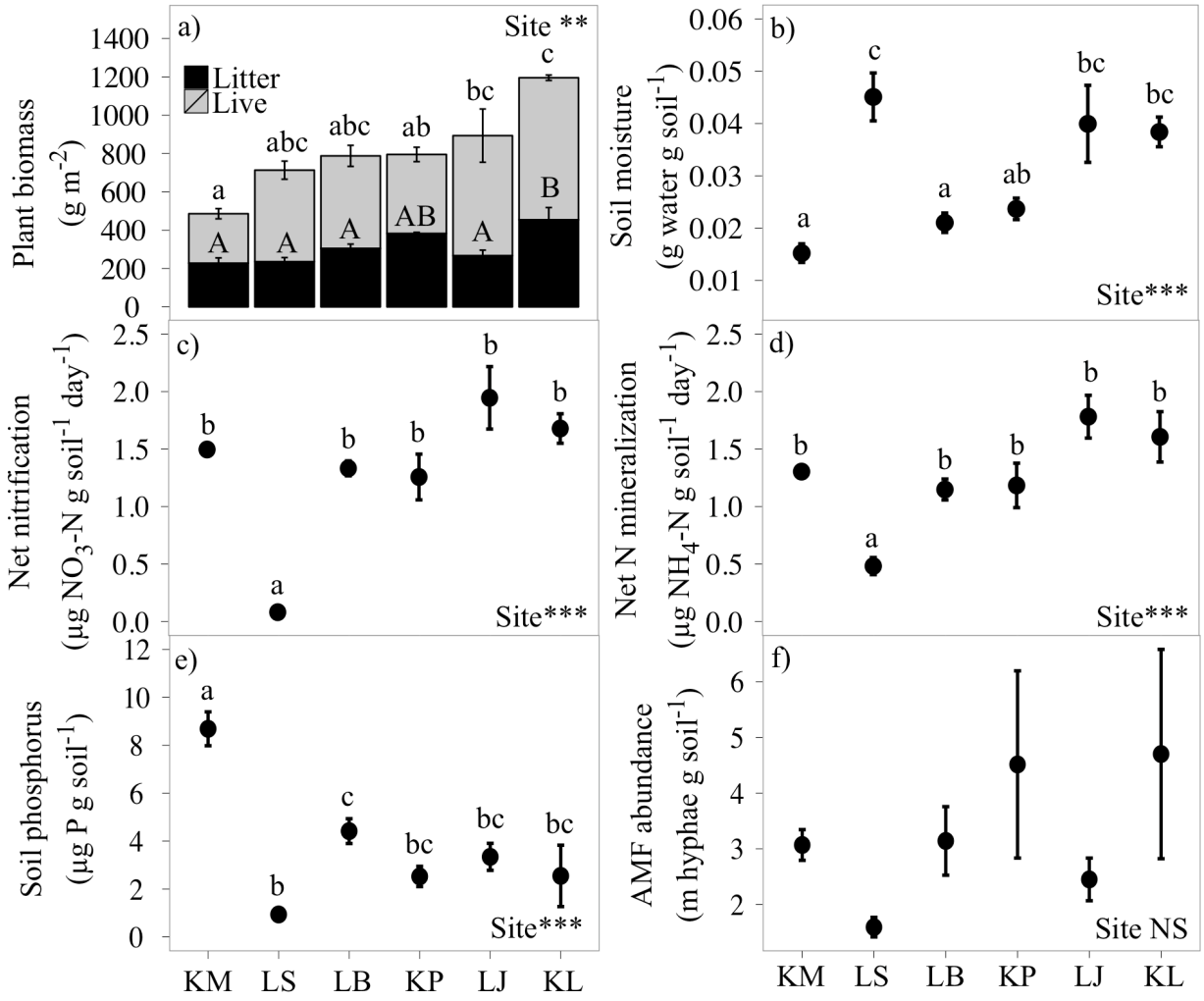


Fig. 4.1: Results of 2007 site characterization. a) Contributions of litter (black bars) and standing live biomass (gray bars) to total aboveground plant biomass. b) Gravimetric soil moisture in late June. c) Net nitrification in lab incubations. d) Net nitrogen mineralizations in lab incubations. e) Water-extractable soil phosphorus. f) Abundance of arbuscular mycorrhizal fungi (AMF) hyphae in the bulk soil. Values are means (±1 SE, n=3-4). Statistical significance of site differences is indicated: $p<0.001$***, $p<0.01$**, $p<0.05$*, $p>0.05$ NS.

Treatment effects on the biotic and abiotic environment

The light addition treatment changed many aspects of the abiotic environment. The treatment increased light availability at sites LS, KP, and KL (Fig. 4.2a; interaction $F_{5,24}=4.60$ $p=0.004$) in July 2008 and at all sites (KM, LS, LB, and KP) in September 2009 (Fig. 4.2b; treatment $F_{1,16}=157.71$ $p<0.001$; interaction $F_{3,16}=3.78$ $p=0.03$). The treatment also increased

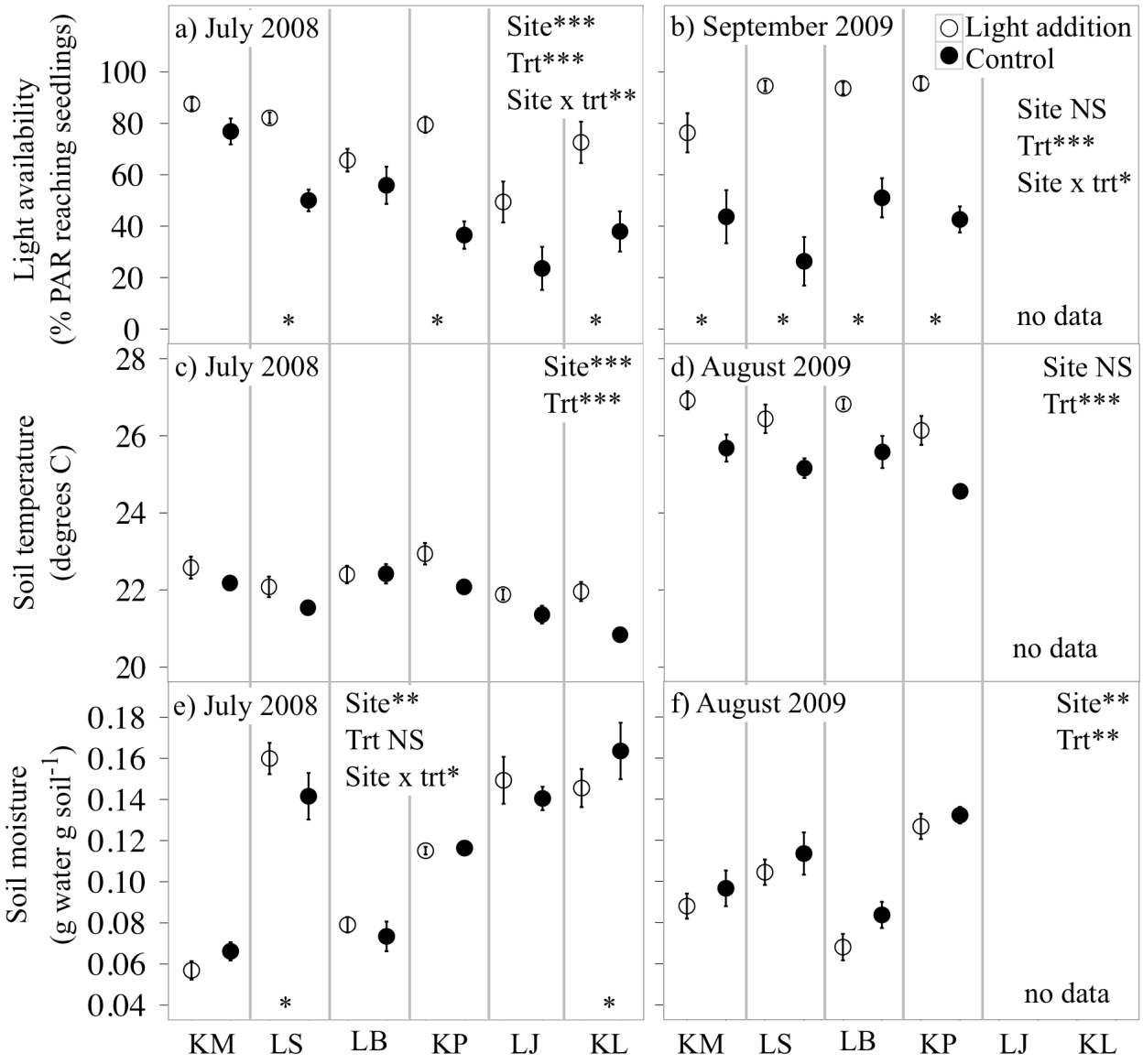


Fig. 4.2: Light addition treatment effects on light availability in a) July 2008 and b) September 2009, measured as percent PAR at the seedling level, soil temperature in c) July 2008 and d) August 2009, and gravimetric soil moisture in e) July 2008 and f) August 2009. Open symbols indicate treated plots and closed symbols indicate control plots; values are means (±1 SE, n=5). Statistical significance of site differences and treatment effects is indicated: $p<0.001$***, $p<0.01$**, $p<0.05$*, $p>0.05$ NS. For analyses where the site x treatment interaction term was significant, stars along the x-axis indicate sites where the treatment had a significant ($p<0.05$) effect. Sites are listed in rank order of plant biomass (see Fig. 4.1a).

soil temperature by a degree or two (Fig. 4.2c,d) in July 2008 ($F_{1,24}=127.11$ $p<0.001$), August 2008 (data not shown; $F_{1,24}=22.83$ $p<0.001$), and August 2009 ($F_{1,16}=65.86$ $p<0.001$). The

treatment affected soil moisture (Fig. 4.2e,f) in July 2008 (interaction $F_{5,24}$=3.37 p=0.02) and August 2009 (treatment $F_{1,16}$=14.27 p=0.002) but not in August 2008 (data not shown; treatment $F_{1,24}$=2.12 p=0.16). The treatment did not affect nitrogen availability, measured with resin strips (Fig. 4.3a; $F_{1,16}$=2.42 p=0.14), but it slightly increased water-extractable soil phosphorus (Fig. 4.3b; $F_{1,16}$=8.42 p=0.01).

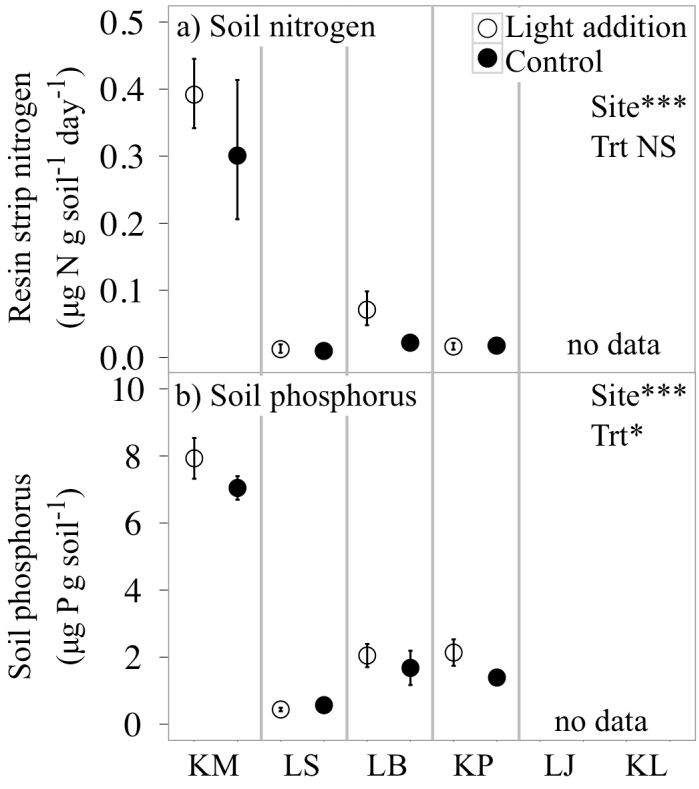


Fig. 4.3: Light addition treatment effects on a) nitrogen availability in July 2009 and b) soil phosphorus availability in August 2009. Open symbols are treatment plots and closed symbols are control plots. Values are means (±1 SE, n=5); nitrogen values were square-root transformed and backtransformed. Statistical significance of site differences and treatment effects is indicated: p<0.001***, p<0.01**, p<0.05*, p>0.05 NS. Sites are listed in rank order of plant biomass (see Fig. 4.1a).

Two consecutive years of treatment application also affected the biotic environment in the plots. The treatment decreased plant aboveground biomass in 2009 by decreasing the amount of litter (Fig. 4.4a; $F_{1,16}$=40.5 p<0.001), dead biomass ($F_{1,16}$=14.77 p=0.001), and, at some sites, *B. inermis* live biomass (interaction $F_{3,16}$=13.80 p<0.001). The treatment did not affect the

growth of AMF into the buried bags at any site (Fig. 4.4b; interaction $F_{3,16}$=3.66 p=0.04, no significant post-hoc tests).

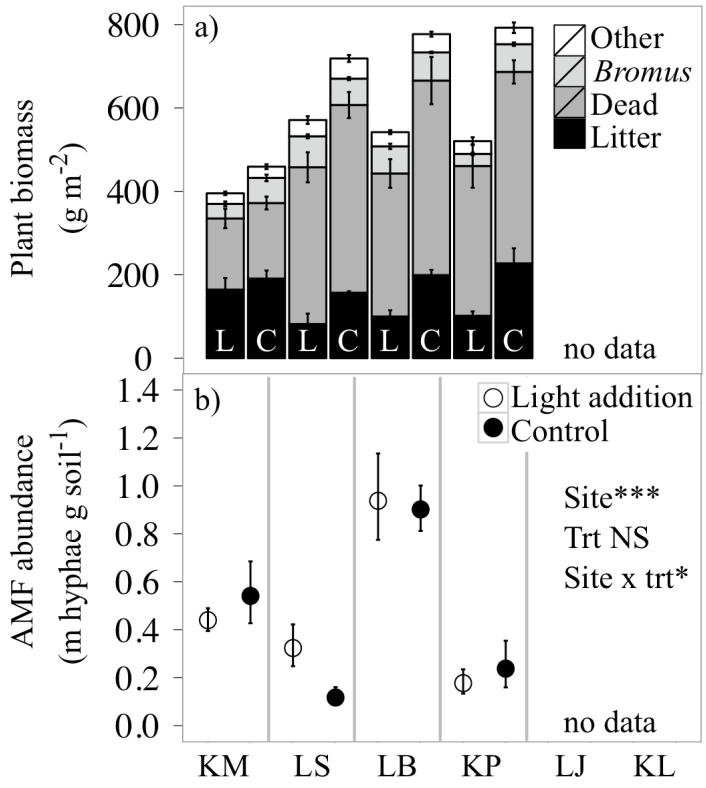


Fig. 4.4: Light addition treatment effects on a) aboveground plant biomass and b) the abundance of AMF that grew into buried bags in a year. Aboveground plant biomass is partitioned into litter (black bars), standing dead biomass (dark gray bars), live biomass of *B. inermis* (light gray bars), and live biomass of other species (white bars). In a), light addition treatment plots are indicated with L; control plots are indicated with C. In b), open symbols are light addition treatment plots and closed symbols are control plots. Values are means (±1 SE, n=5); AMF abundance values were log transformed and backtransformed. Statistical significance of site differences and treatment effects is indicated: p<0.001***, p<0.01**, p<0.05*, p>0.05 NS. Sites are listed in rank order of plant biomass (see Fig. 4.1a).

Treatment effects on A. gerardii seedlings

The *A. gerardii* seedling transplants generally responded positively to the treatment (Fig. 4.5), but the effect was not always significant. The number of seedlings surviving did not differ between treatment and control plots in either 2008 (p=0.6) or 2009 (p=0.4). Seedling biomass was greater in treated plots at site KP in 2008 (Fig. 4.5a; treatment $F_{1,24}$=24.18 p<0.001, interaction $F_{5,24}$=2.92 p=0.03). Mean seedling biomass also differed across sites in 2008 ($F_{5,24}$=7.15 p<0.001). The treatment increased seedling biomass (treatment $F_{1,12}$=69.20

p<0.001) at all three sites with more than three surviving seedlings in 2009 (sites KM, LS, and LB; Fig. 4.5b).

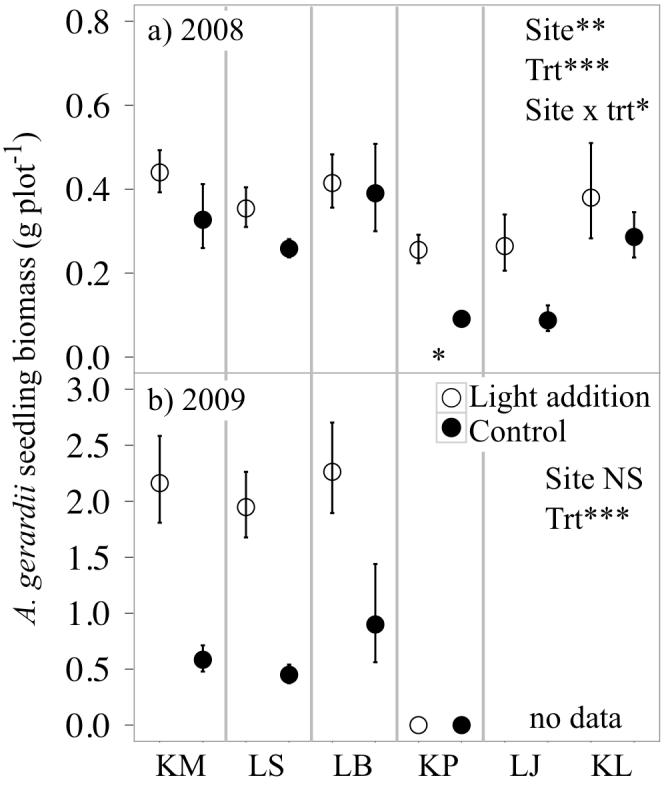


Fig. 4.5: Light addition treatment effect on *A. gerardii* seedling biomass in a) 2008 and b) 2009. Open symbols are treatment plots and closed symbols are control plots. Values are log transformed and backtransformed means (±1 SE, n=5). Statistical significance of site differences and treatment effects is indicated: p<0.001***, p<0.01**, p<0.05*, p>0.05 NS. For analyses where the site by treatment interaction term was significant, stars along the x-axis indicate sites where the treatment had a significant (p<0.05) effect. Sites are listed in rank order of plant biomass (see Fig. 4.1a).

Drivers of seedling response to the treatment across the sites

Seedling biomass responded to the light addition treatment at some sites. However, other potential determinants of seedling biomass also responded to the treatments, including soil moisture, soil temperature, soil phosphorus, and plant aboveground biomass. Seedling biomass also differed among sites, and sites differed in many characteristics including plant aboveground biomass, nitrification, and nitrogen mineralization, soil phosphorus, and soil moisture. Partial least squares regression (PLSR) allowed me to determine which of these potential drivers had the greatest effect on seedling biomass.

For the analysis of 2008 seedling biomass, I compared the effects of site-level variables collected in 2007 prior to the establishment of the treatment (productivity, nitrogen mineralization, net nitrification, water-extractable phosphorus, and AMF abundance) and plot-level variables collected in 2008 (soil moisture in July and August, soil temperature in July and August, and light availability). The first PLSR component explained only 19% of variation in seedling biomass and loaded most heavily on light and soil temperature (Table 4.1; Fig. 4.6a), thus indicating the effect of the treatment. However, PLSR could not separate out the effects of the treatment via changes in light from effects via changes in temperature because they were too highly correlated (rho=0.51 and rho=0.58 for July and August temperatures, respectively). The second component explained an additional 13% of variation in seedling biomass and loaded most heavily on site productivity, soil moisture, and light (Table 4.1, Fig. 4.6b). Therefore, in 2008, the most important variables determining seedling biomass were light, soil temperature, site productivity, and soil moisture.

For the analysis of 2009 seedling biomass in the three sites remaining in the experiment with more than three surviving seedlings, I compared the same site-level variables collected in 2007 and plot-level variables collected in 2009 (light, soil temperature and moisture in August, resin strip nitrogen, water-extractable phosphorus, and the abundance of AMF). The first PLSR component explained 48% of variation in seedling biomass. Three predictors had the strongest loading weights: light availability, soil temperature, and litter biomass. Thus, the first component appeared to function mainly to separate out control plots from light addition plots (Fig. 4.6c). The second component explained an additional 9% of variation in seedling biomass and separated the sites along site-level (2007) variables such as net nitrification, nitrogen mineralization, soil moisture, soil phosphorus, and AMF abundance (Table 4.1, Fig. 4.6d).

Table 4.1: Loading weights for components of partial least squares regressions in 2008 and 2009. Bolded entries had squared loading weights greater than 0.1. Site level variables were measured in the 2007 site characterization plots; plot level variables were measured in the year of analysis in each of the replicate light addition and control plots. ND indicates that a variable was not collected in that year and so was excluded from that analysis.

	2008: 6 sites		2009: 3 sites	
Variable	1st comp	2nd comp	1st comp	2nd comp
Site productivity	-0.12	**0.64**	0.05	0.25
Site N mineralization	-0.20	-0.01	0.12	**-0.37**
Site net nitrification	-0.15	-0.07	0.13	**-0.36**
Site phosphorus	0.26	-0.07	0.07	**-0.38**
Site soil moisture	-0.18	**0.39**	-0.12	**0.37**
Site soil AMF abundance	-0.07	0.03	0.15	**-0.34**
Plot light	**0.53**	**0.43**	**0.49**	0.10
Plot July soil temperature	**0.44**	0.16	ND	ND
Plot Aug soil temperature	**0.46**	0.05	**0.62**	0.24
Plot July soil moisture	-0.26	**0.31**	ND	ND
Plot Aug soil moisture	-0.25	**0.34**	-0.26	0.06
Plot nitrogen	ND	ND	0.01	-0.29
Plot phosphorus	ND	ND	0.06	-0.25
Plot neighbor abundance	ND	ND	-0.28	0.05
Plot litter	ND	ND	**-0.34**	-0.20
Plot soil AMF abundance	ND	ND	0.21	-0.09
Cumulative R^2 seedling biomass	19	32	48	57

Surprisingly, site productivity was not among the variables with the greatest loading weights in the second component. Therefore, the most important variables determining 2009 seedling biomass were light, soil temperature, litter biomass, and secondarily site-level differences in soils.

Seedling response to hypothesized drivers

PLSR indicated that seedlings responded to light, soil temperature, soil moisture, site productivity, and nitrogen and phosphorus availability. Therefore, it was useful for determining which site differences and treatment-induced environmental changes were most closely

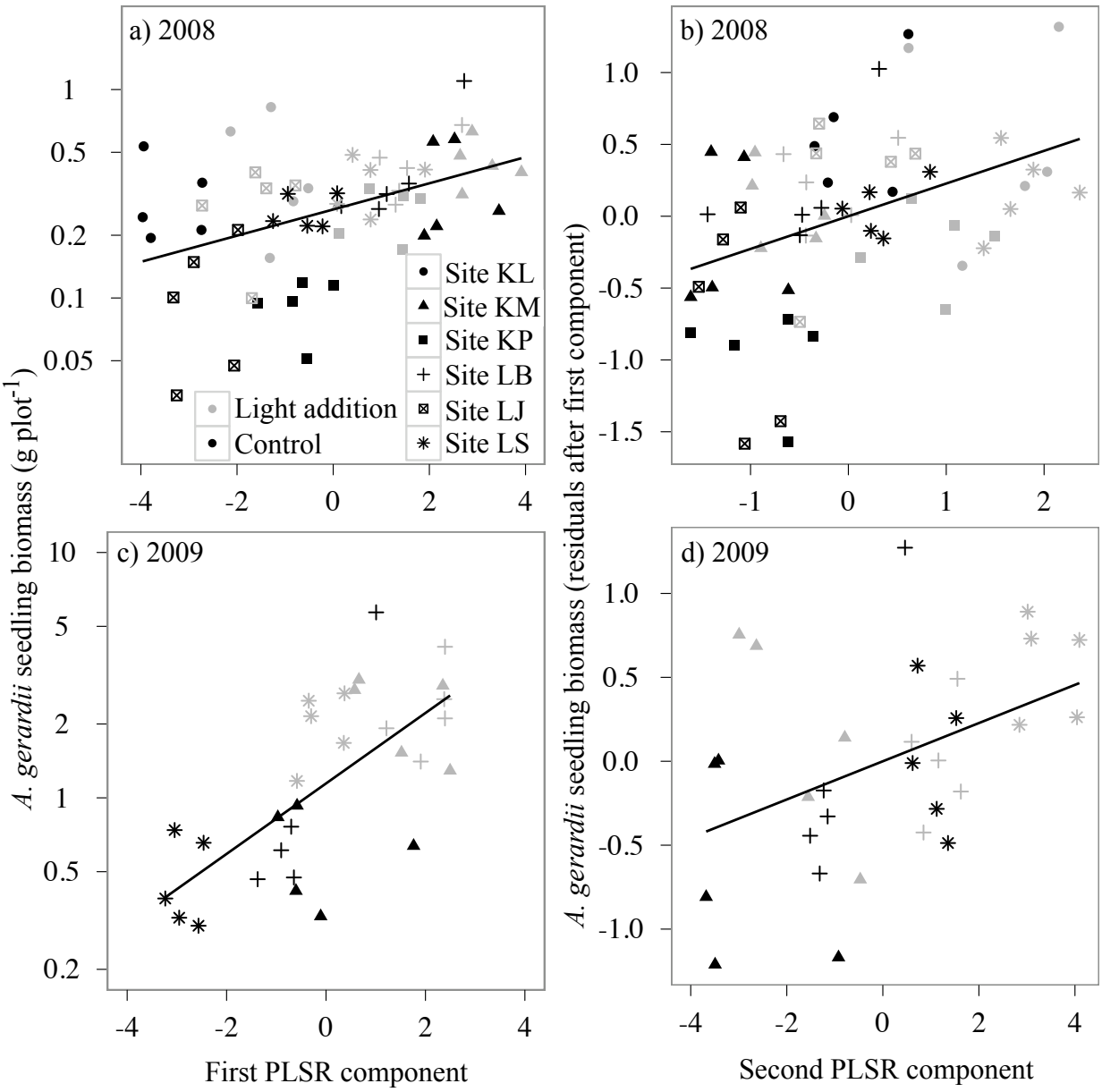


Fig. 4.6: Results of partial least squares regressions (PLSR): relationship between the first component and seedling biomass in a) 2008 and c) 2009, and relationship between remaining variation in seedling biomass (residuals) and the second component in b) 2008 and d) 2009. Sites are indicated by their two-letter code (see Fig. 4.1 for site characterization). Control plots are indicated in black text and treated plots in gray. Note log-scale y-axis in (a) and (c).

associated with seedling biomass. However, it cannot test for interactions among those variables. Therefore, I conducted specific analyses to test my hypotheses that seedlings were

light limited, that site productivity affected seedling light limitation, and that AMF would benefit seedlings more when light was abundant.

To directly test the hypothesis that light would increase seedling biomass, I conducted ANCOVA to determine the interactive effects of site and measured light availability on seedling biomass. In 2008, both site and light availability affected seedling biomass (Fig. 4.7a,c,e,g,i,k; site $F_{5,24}=6.14$ $p<0.001$, light $F_{1,24}=9.39$ $p=0.005$); the relationship between light availability and seedling size was the same at all sites. In 2009, there were only three sites with sufficient seedlings surviving for the analysis and the range in productivity among those sites was smaller (Fig. 4.1a). However, the results were the same: light availability increased seedling biomass (Fig. 4.7b,d,f; $F_{1,12}=21.94$ $p<0.001$) but seedling biomass was the same across all three sites and the effect of light did not differ across sites.

I more explicitly tested for an interaction between site productivity (measured in 2007) as a continuous predictor and the light addition treatment using ANCOVA. The treatment increased seedling biomass in 2008 ($F_{1,28}=17.60$ $p<0.001$), but there was no effect of site productivity and the effect of the treatment was the same across the productivity gradient. In 2009, including only the three sites with more than three surviving seedlings, the results were identical: only the treatment increased seedling biomass ($F_{1,13}=64.88$ $p<0.001$). I also compared the effect of measured light availability across the productivity gradient using multiple regression. Again, productivity had no effect on seedling biomass and did not interact with light availability, but light availability did increase seedling biomass, both in 2008 ($F_{1,28}=19.08$

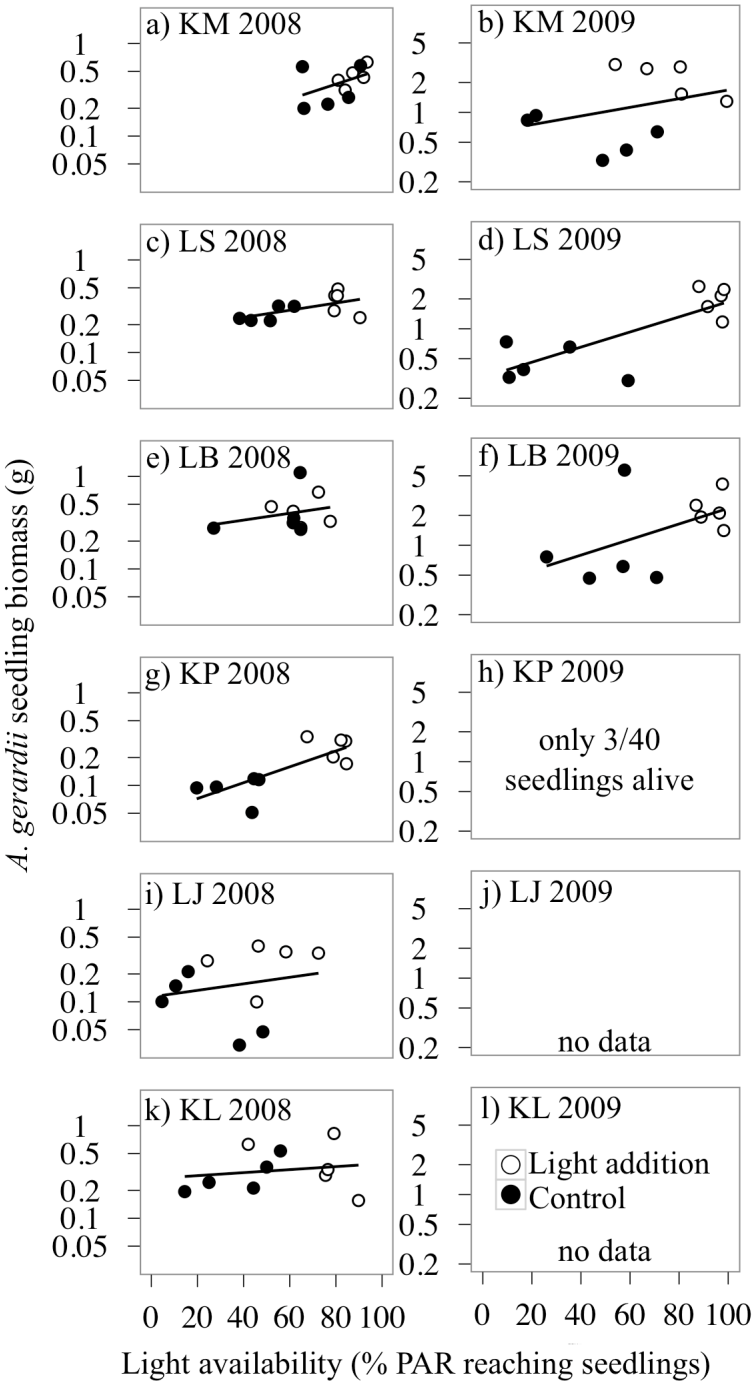


Fig. 4.7: The relationship between light availability and *A. gerardii* seedling biomass at each site in a,c,e,g,i,k) 2008 and b,d,f,h,j,l) 2009. Open symbols are treatment plots and closed symbols are control plots. Sites are shown vertically in rank order of plant biomass (see Fig. 4.1a). Note log-scale y-axes.

$p<0.001$) and 2009 ($F_{1,13}=24.39$ $p<0.001$). Thus, site productivity had no direct effect on seedling biomass and did not affect the degree of seedling light limitation.

To test the hypothesis that AMF would benefit *A. gerardii* seedlings more in high light environments, I conducted ANCOVA to determine the interactive effects of AMF abundance

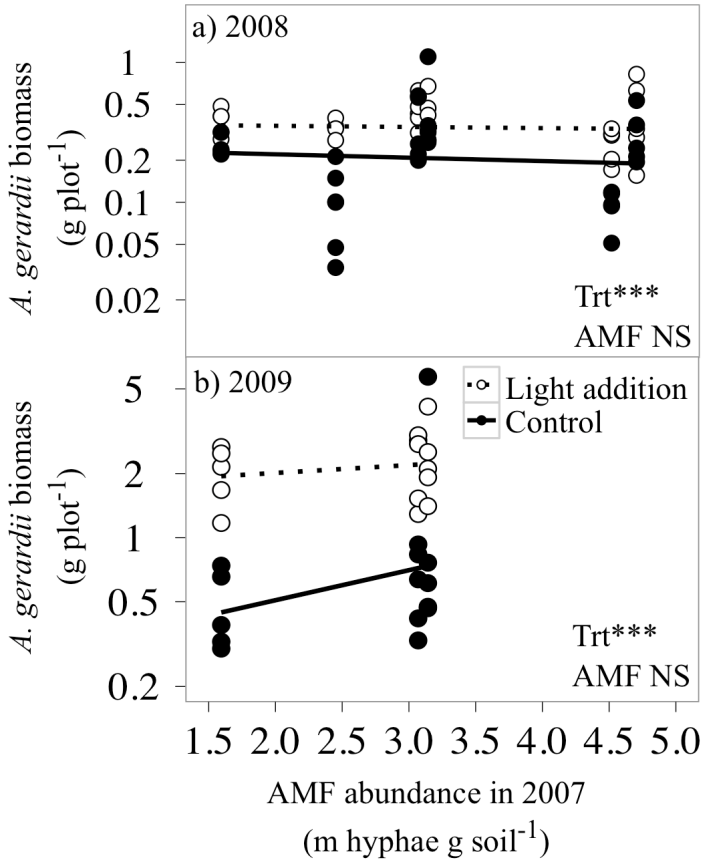


Fig. 4.8: The relationship between the abundance of AMF measured in 2007 and seedling biomass in a) 2008 and b) 2009. Open symbols are light addition treatment plots and closed symbols are control plots. Statistical significance of effect is indicated: $p<0.001$***, $p<0.01$**, $p<0.05$*, $p>0.05$ NS. Note log-scale y-axis.

(measured in 2007) and the light addition treatment. Seedling biomass did not respond to the soil hyphal abundance of AMF in either control or treated plots in either 2008 or 2009 (Fig. 4.8). I also conducted multiple regression to compare the effect of AMF abundance (in 2007) and measured light availability. The result was the same: light availability did not determine seedling response to AMF abundance in 2008 or 2009.

Discussion

Seedling establishment may determine plant community composition (Goldberg and Miller 1990; Tilman 1993), so it is important to understand the factors affecting seedling

establishment. In particular, it is important to identify the resource limiting seedling establishment and growth because it can affect seedling interactions with other organisms, especially competitors (Hautier et al. 2009) and mutualists such as arbuscular mycorrhizal fungi (AMF) (Johnson 2010). In this study, I asked whether light affected seedling establishment, whether light limitation was stronger in higher productivity old-fields, and whether light availability would affect seedling response to the natural abundance of AMF.

Are A. gerardii seedlings light limited in the field?

Because they are small and easily shaded by neighboring plants, seedlings and other small-statured plants are prone to light limitation (Foster and Gross 1998; Hautier et al. 2009). I expected to find light limitation in transplanted *A. gerardii* seedlings in this field study: in some sites in some years, light available to the seedlings in the control plots was 350-550 $\mu m/m^2/sec$, well below the 800-1000 $\mu m/m^2/sec$ at which *A. gerardii* displays light limitation of photosynthetic rates (Awada et al. 2003). My light addition treatments, which prevented neighbors from shading the target seedlings, increased light available to the seedlings by 14-253%. Unsurprisingly, I did detect light limitation in transplanted *A. gerardii* seedlings: the light addition treatment increased seedling biomass in some sites in some years (Fig. 4.5). Measured light availability was also associated with greater seedling biomass (Fig. 4.7).

Although the light addition treatment affected other environmental properties, both biotic and abiotic, I argue that seedling responses to the treatment reflect their degree of light limitation. The partial least squares regression (PLSR) analysis supports this argument: light was associated with increased seedling biomass (Table 4.1). However, the role of light could not be completely separated from soil temperature because the treatment affected both. Thus, PLSR

suggested that both light and temperature were important drivers of seedling response to the treatment. In contrast, none of the additional abiotic or biotic factors that responded to the treatment were associated with seedling biomass, except litter biomass.

Litter has previously been shown to reduce seedling establishment and growth (Foster and Gross 1998; Foster 1999). In fact, litter generally affects seedlings negatively, and these effects can be as strong as the effects of competition or predation (Xiong and Nilsson 1999). The light addition treatment decreased litter biomass in 2009, probably because of the treatment's probable effect on aboveground biomass in 2008. Despite this, there are several reasons why litter responses to the treatment are an unlikely explanation for seedling responses. First, prior field experiments have shown that negative effects of litter on *A. gerardii* are much weaker at the seedling stage than at the germination stage (Foster and Gross 1998; Foster 1999). I transplanted seedlings after they were most likely to be inhibited by litter. Second, in those studies, Foster reasoned that the effects of litter were likely driven by light reduction (Foster and Gross 1998; Foster 1999). My treatment involved pulling litter back from seedlings and preventing shading, so this mechanism of litter-induced seedling suppression was likely minor. Third, other mechanisms of litter effect on seedlings, such as moisture retention, nitrogen immobilization, or temperature reductions, would have been detected by my direct measurements of those environmental factors, although other effects such as protection from frost or herbivores or allelopathy would not have been detected (Xiong and Nilsson 1999).

This study provides support for the idea that light limits seedling establishment in grasslands. Other studies have also found that light can limit the growth or establishment of seedlings or other small-statured plants (Foster and Gross 1998; Dickson and Foster 2008;

Hautier et al. 2009; Dickson and Foster 2011). This study thus confirms the importance of light limitation in grassland plant communities.

Does A. gerardii light limitation increase with site productivity?

A central hypothesis to explain changes in plant community composition across fertility gradients is that competition, and therefore resource limitation, transitions from chiefly below-ground in low productivity, infertile sites to at least partially aboveground in high productivity, fertile sites (Tilman 1988; Wilson and Tilman 1993). This increase in the strength of light limitation in high productivity sites is thought to reduce the abundance of small-statured understory plants in high productivity sites, driving reductions in species richness and diversity (Goldberg and Miller 1990; Tilman 1993). Many studies have shown that the growth and establishment of seedlings and small-statured species increases with light availability in highly productive or fertile soils (Foster et al. 2004; Hautier et al. 2009), but others find that the effect is weak or inconsistent (Dickson and Foster 2011 and refs therein).

I found no pattern of stronger light limitation in high productivity grasslands than in low productivity grasslands. Three pieces of evidence support this finding. First, variation across sites in seedling response to the light addition treatment was not related to site productivity. In 2008, one-year-old seedling biomass responded positively to the treatment in a site of intermediate productivity (site KP) but not at a higher productivity site (site KL). In 2009, two-year-old seedling biomass responded positively to the treatment in all three sites with more than three individuals surviving (sites KM, LS, and LB, all low productivity sites). Second, the positive relationship between measured light availability and seedling biomass did not differ among sites in either 2008 or 2009 (Fig. 4.7), despite the fact that sites differed in productivity

(Fig. 4.1a). Finally, neither the effect of the treatment nor the effect of light availability on seedling biomass depended on site productivity. Although replication was low and data were noisy in this study, I argue that low statistical power is not the only reason I did not detect stronger light limitation in high productivity sites. Observed patterns, statistically significant or not, were simply not indicative of stronger light limitation in sites of higher productivity (Fig. 4.5, Fig. 4.7). What can explain differences in this result between this study and others?

One potential explanation is that the productivity gradient in this study was too narrow to observe a transition from soil nutrient limitation to light limitation. Other studies have found that the effect of fertility gradients on seedling establishment depend on the magnitude of the gradient (Foster 1999). Foster (1999) found that an aboveground productivity gradient of about 200-1200 g/m^2 was insufficient to detect increasingly negative effects of aboveground productivity on the growth of *A. gerardii* seedling transplants, but he did detect the predicted effect with a larger gradient of about 80-2000 g/m^2. He concluded that sites of very low productivity drove the pattern. The sites in the present study fall well within the smaller range of aboveground productivity values in those studies (Fig. 4.1a): 460-1200 g/m^2 in 2008 and 460-800 g/m^2 in 2009, possibly explaining why I did not observe the expected effect of productivity.

A second explanation is that productivity was not the most important difference among the sites. Although the sites differed in productivity (Fig. 4.1a), and were consistent in terms of resident plant community composition, they did differ in other aspects. Seedlings responded to these site differences (Fig. 4.5; Fig. 4.7a,c,e,g,i,k; Table 4.1); other site differences beyond productivity could account for these site effects. The PLSR in 2009 indicated positive effects of site-level differences such as lower rates of nitrogen mineralization and net nitrification, higher

soil moisture, and lower abundance of AMF, but not productivity. Increased nitrogen availability is frequently associated with decreased *A. gerardii* growth, but in other studies this seems to be an indirect effect, mediated through shading or litter of competitors (e.g., Foster 1999). However, increased moisture may benefit seedlings directly; in fact seedling response to the availability of soil nutrients and light may depend on water availability (Dickson and Foster 2008; Dickson and Foster 2011).

Other unmeasured important site differences may also have played a role. For example, site KP seemed to have high levels of rodent herbivory, but I did not quantify this across sites. Soil texture also varied across sites and may have affected seedlings in ways that my measurements of resource availability did not detect (gravimetric soil moisture, nitrogen mineralization and nitrification, resin strip nitrogen, and water-extractable phosphorus). Another possibility is differences across sites in the community composition of AMF. I did not investigate the identity of the AMF at the different sites, but other studies have shown that community composition of AMF depends on disturbances, soil fertility, and other factors (Bever et al. 2003; Egerton-Warburton et al. 2007; Schnoor et al. 2011) and may affect the establishment of some plant species (Smith et al. 1998; Bever et al. 2003). Thus, other measured or unmeasured site differences could have been more important than productivity in determining seedling response to light.

Does light availability affect A. gerardii seedling response to the abundance of AMF in the field?

The identity of the limiting resource is thought to be an important determinant of plant responses to AMF (Smith and Read 1997; Johnson 2010). Interactions between AMF and seedlings may be particularly vulnerable to light limitation (Johnson et al. 1997). I expected to

find that *A. gerardii* seedlings would respond more positively to increases in AMF when light was abundant. In a greenhouse experiment (Chapter 3), I did not detect any effect of light on *A. gerardii* seedling response to AMF, perhaps because the 30% light manipulations in that study were too slight. In this field study, light increases were sometimes much more dramatic (Fig. 4.2a,b), but I still found no effect of light availability on seedling response to AMF (Fig. 4.8). In fact, I detected no seedling response to variation in AMF abundance, suggesting that this study was not a good test of this hypothesis.

Why did variation in the natural abundance of AMF have no detectable effect on *A. gerardii* seedling establishment? One potential reason is that variation across sites in AMF abundance was too slight to cause measurable effects on *A. gerardii* seedlings. Although sites did not differ significantly in AMF abundance (Fig. 4.1f), there was substantial variation across plots: hyphal lengths ranged from 1.3-8.4 m/g soil. However, it is unclear whether this range of variability is large enough to drive differences in seedling size.

Another potential reason is that increases in AMF abundance across natural gradients may not indicate increases in mutualistic functioning. Other studies have found that seedlings, especially of highly mycorrhizal species like *A. gerardii*, generally respond positively to increased abundance of AMF (Johnson 1998; Lekberg and Koide 2005; Hoeksema et al. 2010). Similarly, I had assumed that old-fields dominated by weakly mycorrhizal species such as *B. inermis* (Wilson and Hartnett 1998; Chapter 3) would support only small populations of AMF and thus that any increases in AMF abundance would benefit *A. gerardii* seedlings. However, it is difficult to compare AMF abundance in these sites to published values because accurate and consistent detection of AMF hyphal lengths in soil is a difficult technical problem. Furthermore, AMF abundance should depend on a variety of site-specific factors including soil texture, the

history of soil disturbance, the composition of the plant community, and soil fertility (Miller et al. 1995; Treseder and Allen 2002; Wilson et al. 2009; Bach et al. 2010). However, given this uncertainty, studies using relatively similar extraction procedures report hyphal lengths in bulk soil of 0.1 to well over 100 m/g soil (Miller et al. 1995; Johnson et al. 2003a; Bingham and Biondini 2009; Wilson et al. 2009). AMF hyphal lengths in this study were at the low end of this range, 1.3-8.4 m/g soil (Fig. 4.1f). These data suggest that *A. gerardii* seedlings should have responded positively to increases in the natural abundance of AMF.

A third explanation is that although they were not abundant, relative to other sites, AMF may have been abundant enough at all of the sites to be effective. In a mathematical model (Chapter 2), I showed that the relative sizes of plants and AMF can affect critical aspects of the mutualism, including plant and fungal allocation to uptake of light and soil nutrients and the negotiated exchange ratio of carbon for nutrients. Seedlings in this study were small: even after two years of growth, only three seedlings produced more than 3 g aboveground biomass. It is possible that AMF were relatively abundant enough to cause the magnitude and benefit of trade to be limited by the *A. gerardii* seedlings' carbon contributions. However, few empirical data can address the idea that the relative sizes of mutualists can determine the outcome of trade. Whether AMF are sufficiently abundant at these sites to support *A. gerardii* seedlings is therefore unclear.

A fourth explanation why seedling biomass did not respond to natural variation in AMF abundance is that the effect of AMF should depend on the availability of soil nutrients. Greenhouse experiments have shown that soil nutrients affect how strongly AMF benefit plants (Chapter 3; Hoeksema et al. 2010). Furthermore, soil nutrient availability likely interacted with light availability to determine seedling response to AMF (Chapter 2; Johnson 2010). Other

differences among sites, such as soil texture, herbivory, or differences in AMF community composition, may also have affected these interactions. Because of limitations imposed by the study design and sampling scheme, I could not test for these interactive effects. However, this study represents the first attempt of which I am aware to examine the effects of light on AMF interactions with seedlings in the field. It is therefore a critical first step in understanding these complex interactions.

Resource limitation of seedling establishment affects communities and ecosystems

Understanding the limits on seedling establishment and growth is important for understanding plant community structure and ecosystem function (Foster et al. 2004). Seedling establishment is particularly important in a restoration context, where native prairie species such as big bluestem must compete against other native weeds or non-native species such as *B. inermis*. Understanding the identity of the limiting resource and how it affects interactions with other organisms such as AMF thus has clear implications for the restoration of native plant communities. However, this study also indicated that other site characteristics are also important, further complicating our understanding of the controls on seedling establishment.

CHAPTER FIVE

Resource availability and imbalance affect plant-mycorrhizal interactions: A field test of two stoichiometric hypotheses

with Todd M P Robinson

Abstract

Ecological stoichiometry, the study of how ecological processes depend on the relative abundances of limiting resources, can explain many species interactions such as competition and herbivory. Ecological stoichiometry may also explain major shifts in plant interactions with arbuscular mycorrhizal fungi (AMF) across fertility gradients, but major gaps in its explanatory power still remain. Fertilization increases the ratio of availability of soil nutrients to photosynthetically fixed carbon, which, according to stoichiometric theory, is hypothesized to cause plants to reduce their allocation to AMF and consequently reduce AMF abundance. However, fertilization sometimes causes increases in allocation to AMF. Here we explore two additional stoichiometric hypotheses explaining these increases. First, we hypothesized that AMF might be nitrogen limited in very nitrogen poor soils, which would explain increases in AMF abundance with nitrogen fertilization. In support of this hypothesis, we found that AMF abundance did increase along a gradient of nitrogen availability. Second, we hypothesized that the N:P ratio of fertilization would affect plant allocation to AMF. We also found support for this hypothesis: plants receiving fertilization at very low N:P ratio had higher allocation to AMF than plants receiving balanced N:P fertilization. However, these two hypotheses did not operate identically in two different grass species (*B. inermis* and *A. gerardii*). Results from this field

study therefore suggest that both stoichiometry and species-specific factors jointly determine how AMF respond to changes in nutrient availability.

Introduction

Understanding the factors that control the distribution and abundance of arbuscular mycorrhizal fungi (AMF) is a critical problem for ecologists because of the key role they play in many terrestrial ecosystems. By associating with the majority of terrestrial plant species (Wang and Qiu 2006), AMF can be important determinants of plant nutrition and productivity (Smith and Read 1997) and plant community composition (Urcelay and Diaz 2003; Klironomos et al. 2011). AMF also support important ecosystem functions, including nutrient retention (van der Heijden 2010), soil stability (Rillig and Mummey 2006), and soil carbon storage (Treseder et al. 2007). However, ecologists do not yet understand the controls on the abundance of AMF. For example, eutrophication, a global change that can profoundly impact communities and ecosystems (Vitousek 1994), has inconsistent effects on AMF: fertilization can increase or decrease AMF abundance and the magnitude of the effect is highly variable across systems (Johnson et al. 2003a; Treseder 2004).

Many community and ecosystem responses to eutrophication can be explained with insights from ecological stoichiometry, the branch of ecology that considers the effect of the relative abundances of resources (Sterner and Elser 2002). Resource stoichiometry may be especially important in explaining AMF responses to fertilization because the relative abundance of soil nutrients and photosynthetically fixed carbon are key drivers of interactions between plants and AMF (Johnson 2010). AMF provide plants with soil nutrients such as nitrogen and

phosphorus because their hyphae, much smaller in diameter than plant roots, enable them to access additional pools of soil nutrients beyond what roots can access (Smith and Read 1997). As a result, when plants are nutrient-limited, AMF can strongly benefit plant growth (Hoeksema et al. 2010). In exchange for these limiting soil nutrients, plants provide AMF with photosynthetically-fixed carbon, which they have in abundance (Johnson 2010). In high nutrient soils, however, the abundance of light is lower, relative to soil nutrients, and plants are more limited by fixed carbon (Bloom et al. 1985; Sterner and Elser 2002). Plant roots are also more able to take up sufficient nutrients for plant growth in high nutrient soils. This high ratio of soil nutrients to light should therefore drive plants to reduce carbon allocation to AMF and suppress AMF abundance because AMF have no alternative carbon source (Johnson 2010).

In accordance with this stoichiometric hypothesis, many field studies do find reductions in different metrics of plant-mycorrhizal interactions with fertilization (Egerton-Warburton and Allen 2000; Johnson et al. 2003a; Treseder 2004; Blanke et al. 2005). Most studies focus on the proportion of plant root systems that were colonized by AMF and many see reduced root colonization with fertilization (Treseder 2004). Reductions in root colonization are typically interpreted as reductions in carbon allocation to AMF, even though empirical evidence of this link is often weak and root colonization is probably jointly determined by traits of the plant and of the fungi (Wilson and Hartnett 1998; Johnson et al. 2003a; Maherali and Klironomos 2007). Fewer studies have measured changes in the abundance of AMF in response to fertilization (Treseder 2004). One indicator of AMF abundance is the length of AMF hyphae in soils; some studies have also found that fertilization can reduce AMF abundance (Johnson et al. 2003a; Treseder 2004). A better metric of plant allocation to AMF than root colonization may be the relative population sizes of AMF and plants, but few studies report this measure.

However, regardless of the measure of plant-mycorrhizal interaction used, there is wide variation in the effect of fertilization: AMF abundance may increase or decrease in response to fertilization (Johnson et al. 2003a; Treseder 2004). This diversity of responses does not disprove the stoichiometric hypothesis that increases in the ratio of nutrients:light should decrease plant allocation to AMF. Instead, it suggests that the factors determining plant-mycorrhizal interactions are more complex and that the hypothesis requires further refinement. Here, we test two additional stoichiometric hypotheses to explain the effects of fertilization on plant-mycorrhizal interactions.

Hypothesis 1: AMF abundance may be nutrient limited in very infertile soils.

Treseder and Allen (2002) suggested that positive AMF responses to fertilization might be caused by direct nutrient limitation in very nutrient-poor soils. In other words, extremely low nutrient:light ratios could cause increases in AMF abundance in response to fertilization. Because of their inferior nutrient uptake efficiencies, plants would remain nutrient limited at higher nutrient availabilities than those that alleviate AMF nutrient limitation, and would therefore maintain high allocation to AMF. In even higher nutrient soils (higher nutrient:light ratios), however, fertilization would alleviate plant nutrient limitation and cause plants to suppress AMF abundance. This hypothesis therefore predicts a hump-shaped relationship between AMF abundance and nutrient availability: a positive AMF response at low nutrients and a negative response at higher nutrients.

This hypothesis has only occasionally been tested and the results to date have been mixed. In support of their prediction, Treseder and Allen (2002) found that nitrogen addition increased AMF hyphal length in a nitrogen-poor site, and that phosphorus addition increased

hyphal length in a phosphorus-poor site but decreased it in a phosphorus-rich site. Most other field studies include only two levels of nitrogen or phosphorus addition, so they cannot test for a nonlinear response to fertilization. Some field studies have measured fungal abundance across natural or artificial fertility gradients, and most report no relationship or monotonic declines (Johnson et al. 1992; Egerton-Warburton and Allen 2000; Blanke et al. 2005; Powers et al. 2005; Chapter 4; Grman, unpublished). However, it is possible that these studies have not included soils where fertility is low enough for AMF to be nutrient-limited. Further tests of this hypothesis could help resolve whether AMF abundance increases in response to fertilization in very infertile soils.

Hypothesis 2: N:P ratio determines plant allocation to AMF.

Johnson et al. (2003a) hypothesized that AMF response to fertilization with one soil nutrient might depend on whether another soil nutrient was also limiting to plants. Specifically, they suggested that nitrogen fertilization should decrease plant allocation to AMF only where plants are primarily nitrogen-limited and phosphorus is abundant. In other words, nitrogen additions should decrease allocation to AMF only where soil N:P is low (Johnson et al. 2003a). On the other hand, plants might increase allocation to AMF in response to nitrogen addition in phosphorus poor soils where N:P is high and plants are phosphorus limited (Johnson et al. 2003a; Johnson 2010). Therefore, soil N:P ratio should affect whether plants increase or decrease allocation to AMF in response to fertilization. Johnson et al. (2003) initially presented this hypothesis to explain results of a field study where nitrogen fertilization increased AMF abundance at a site with very high N:P (Konza Prairie). Other field and greenhouse experiments

have also indicated that N:P affects AMF response to fertilization (Sylvia and Neal 1990; Corkidi et al. 2002; Blanke et al. 2005).

However, this hypothesis does not take into account a key difference between nitrogen and phosphorus that may affect whether plants allocate to AMF in nutrient-limited environments. Specifically, nitrogen and phosphorus have different mobility in soils, and this may affect whether AMF provide uptake benefits to plants. Phosphate is relatively immobile in soil and plant phosphorus uptake is frequently diffusion-limited (Smith and Read 1997). As a result, plant phosphorus uptake can be greatly increased by investing in AMF hyphae that can grow towards phosphorus-rich patches of soil (Smith and Read 1997). In contrast, rates of diffusion for nitrate and ammonium are much higher than phosphate, so AMF hyphae may not provide nitrogen uptake advantages over plant roots (Johnson 2010). Indeed, plant growth benefits from association with AMF under nitrogen-limiting conditions have been difficult to document (Reynolds et al. 2005; Johnson et al. 2010), despite clear evidence that AMF do take up and transfer nitrogen to plants (Govindarajulu et al. 2005). Therefore, we hypothesized that although N:P ratio would affect plant allocation to AMF (Johnson et al. 2003a), nitrogen limited plants (in low N:P soils) would allocate to AMF less than phosphorus limited plants (in high N:P soils).

Testing two stoichiometric hypotheses in two dominant plant species

Both stoichiometric hypotheses have potential to explain variation in mycorrhizal response to changes in soil fertility. The first proposes that AMF response to changes in soil fertility is driven by the relative abundance of soil nutrients and light. The second proposes that the relative abundance of nitrogen and phosphorus is a critical driver. Importantly, the hypotheses make predictions about different aspects of plant-mycorrhizal interactions. The first

hypothesis makes predictions about how AMF *abundance* should respond to changes in resource stoichiometry, while the second hypothesis makes predictions about plant *allocation* to AMF. The hypotheses are not mutually exclusive, and both may contribute to explaining variation in AMF response to fertilization.

However, these stoichiometric hypotheses may not operate identically in all species. Different plant species may be respond differently to the same ratio of resource availabilities, even in the same environment, perhaps because of different requirements for the resources or different inherent abilities to take up the resources (Tilman 1982; Sterner and Elser 2002). Furthermore, plant species differ in the strength of association with AMF (Wilson and Hartnett 1998) and may differ in how strongly they reduce allocation to AMF when fertilized (Graham and Eissenstat 1994; Chapter 3). For example, big bluestem (*Andropogon gerardii*) is a native C_4 grass that associates strongly with AMF (Wilson and Hartnett 1998; Chapter 3) and that does not strongly reduce its allocation to AMF when fertilized (Johnson et al. 2008; Chapter 3). In contrast, smooth brome (*Bromus inermis*) is an introduced C_3 grass that associates only weakly with AMF, and in fact may gain little benefit from the association even in low nutrient soils (Wilson and Hartnett 1998; Chapter 3). When fertilized with phosphorus, however, it effectively suppresses its allocation to AMF (Chapter 3). Both grass species are present in midwestern old-fields and grasslands. Because of these species differences, we asked whether the two stoichiometric hypotheses would control AMF abundance when AMF associated with either species.

To test these hypotheses, we set up a fertilization experiment in an old-field in southwest Michigan with these two dominant grass species, *A. gerardii* and *B. inermis*. The results support

both hypotheses, confirming that a stoichiometric framework is essential for understanding plant-mycorrhizal interactions.

Methods

Site selection, preparation, and maintenance: We set up the experiment in a 12-15 year old field in southwest Michigan, USA, in the W. K. Kellogg Biological Station's Lux Arbor Reserve (site NP in Fig. 1.2). The site is dominated by *Solidago* spp., *Rubus* spp., *Phleum pratense*, *Dactylis glomerata*, and *Poa* spp. The soil is clayey, with pH 5.8, 0.0 μg nitrogen as nitrate, 7.2 μg nitrogen as ammonium, and 26 μg Bray 1 phosphorus per g soil in May 2007. To prepare the site, we applied glyphosate herbicide four times to kill existing vegetation in June-July 2007. In September-October 2007, we set up eight replicate blocks of 38 plots (75 cm x 75 cm) separated by 1-m wide aisles. Into these plots, we transplanted nine two-month-old seedlings of either big bluestem (*Andropogon gerardii*) or smooth brome (*Bromus inermis*) that had been started in peat moss in the greenhouse. In spring 2008, we replaced over-winter transplant mortality with new seedlings grown in the greenhouse in peat moss and sieved soil from the field site. We continued replacing dead seedlings until each plot contained 7-9 seedlings. In June 2008, transplanted seedlings were covered with plastic cups and plots were sprayed with glyphosate to control vigorous regrowth of weeds. In summer 2008 and 2009, plots were weeded twice yearly to maintain dominance by the planted species, either *A. gerardii* or *B. inermis*. Common weeds included *Trifolium* spp., *Elymus repens*, *Poa* spp., and *Rumex acetosella*. We also mowed the aisles throughout the summers to reduce weed spread into the plots.

Nutrient treatments: To test the two hypotheses that the availability (Hypothesis 1) and the relative abundance (Hypothesis 2) of nitrogen and phosphorus would affect plant-mycorrhizal interactions of each dominant species, in each block we established one control plot and 18 different treatment combinations where we manipulated nitrogen, phosphorus, and N:P ratio (Fig. 5.1a). In total, there were 304 plots (19 treatments per species in 8 blocks). We created eight levels of phosphorus addition, ranging from 0-57.6 g P/m^2/year added as triple super phosphate, and four levels of nitrogen addition, ranging from 0-24 g N/m^2/year added as urea. We also included two carbon addition treatments intended to reduce nitrogen availability through microbial uptake (Blumenthal et al. 2003; Suding et al. 2004), one with added phosphorus and one at control phosphorus levels. Each carbon addition treatment received 624 g sawdust/m^2/year and 624 g sucrose/m^2/year. We included two gypsum ($CaSO_4$) addition treatments (190 g gypsum/m^2/year) to reduce phosphorus availability (Suding et al. 2004), one with added nitrogen and one without (Fig. 5.1a). In 2008 and 2009, we applied nitrogen and phosphorus fertilizers twice yearly (June and August) and carbon and gypsum additions three times yearly (June, July, and August).

Soil sampling and analysis: To measure soil nitrogen, phosphorus, and pH, we collected and composited four 10-cm deep, 2-cm diameter soil cores from each plot in late September 2009. We weighed the wet soil collected for determination of root biomass (described below). Within 36 hours, we sieved soil samples with 2 mm sieves, retaining roots for determination of root biomass and root colonization by mycorrhizal fungi. We determined soil moisture gravimetrically on fresh soils (Robertson et al. 1999). To measure soil inorganic N, we extracted fresh soils in 1M KCl, filtered the supernatant, and froze the extracts until analysis on an O. I.

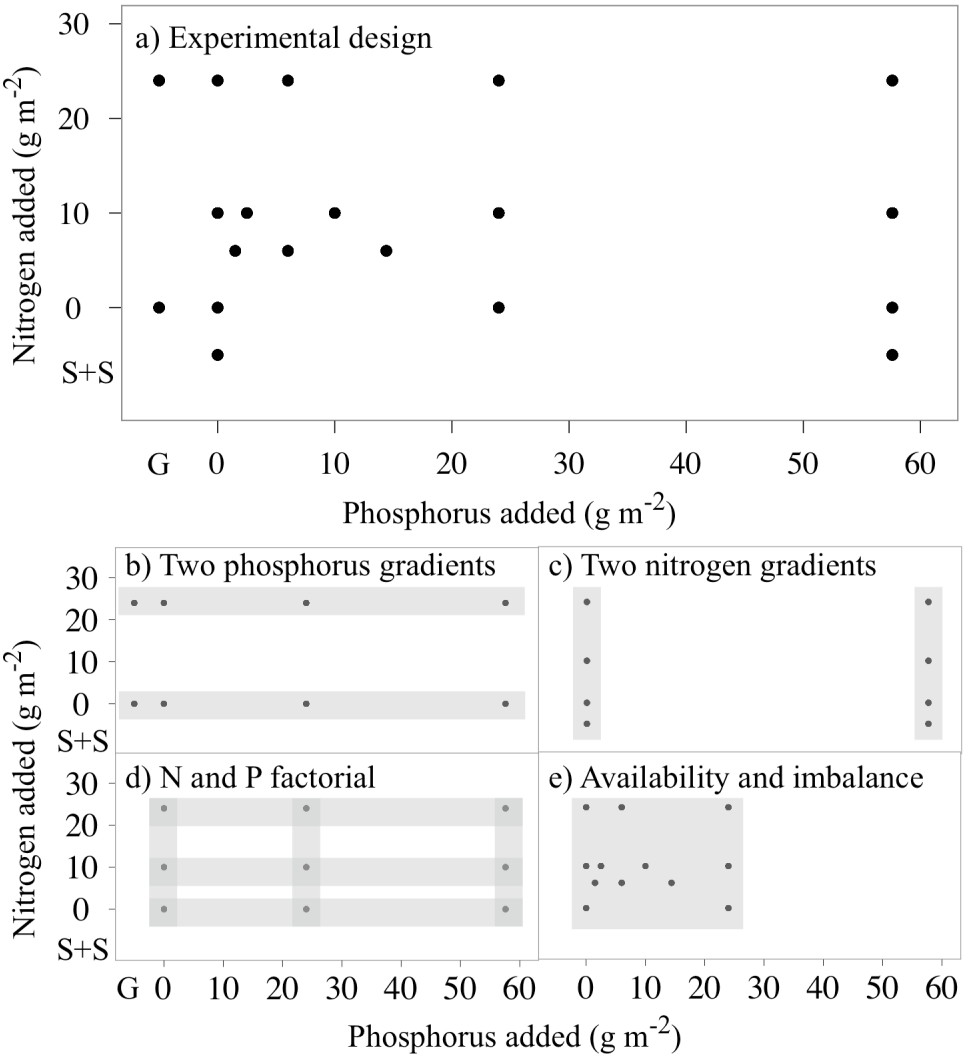


Fig. 5.1: Experimental design showing a) different combinations of nitrogen and phosphorus manipulations. We subset the data to test specific hypotheses. We tested Hypothesis 1 by evaluating plant and fungal responses to b) two phosphorus gradients (at low and high nitrogen) and c) two nitrogen gradients (at low and high phosphorus) in each dominant plant species. We tested Hypothesis 2 using d) a nitrogen and phosphorus addition factorial design in each dominant species. We tested both Hypothesis 1 and 2 using e) a fourth subset of the treatments in each species and a statistical technique to separate resource "availability" from "imbalance." Values of "G" below zero on the phosphorus addition axis indicate gypsum addition treatments, which were intended to reduce phosphorus availability below control levels. Similarly, values of "S+S" below zero on the nitrogen addition axis indicate carbon (sugar and sawdust) additions intended to reduce nitrogen availability below control plots.

Analytical Flow Solutions IV analyzer (O. I. Analytical, College Station, TX; Robertson et al.

1999). To measure soil inorganic P, we air-dried soils, later extracting with water, filtering, and

freezing extracts (modified from Olsen and Sommers 1982). We later analyzed extracts for phosphate content using malachite green on a spectrophotometer (SpectraMax M5, Molecular Devices, Sunnyvale, CA, USA; modified from D'Angelo et al. 2001). We measured soil pH on air-dried samples with a VWR sympHony pH meter (VWR, Batavia, IL; Robertson et al. 1999).

Plant sampling: To measure aboveground plant biomass production in response to the treatments, we clipped *A. gerardii* and *B. inermis* plants 2-4 cm above the soil surface in October 2009. We harvested both spring growth of *B. inermis*, present as standing dead material in October, and fall regrowth, present as green plant material. We sorted biomass to discard weeds and retained only the biomass of the two target species. Aboveground plant biomass was dried at 65 degrees C for 48-72 hours and weighed. To estimate belowground plant biomass, we saved roots removed from soil samples during sieving. We washed the roots in water to remove soil, rocks, and litter, then dried samples at 65 degrees C for 48 hours and weighed them. We calculated the mass of root per g soil by dividing root mass by the total wet mass of soil collected.

Fungal sampling: We measured AMF abundance both within plant roots and in the soil. To measure AMF abundance inside plant roots, we haphazardly chose subsamples of each dried root sample and cleared the roots in 2.5% KOH, rinsed in water, and stained with 5% Shaeffer black ink in white vinegar (modified from Vierheilig et al. 1998). To quantify root colonization, we mounted roots on microscope slides and inspected at 100x using the gridline intercept method (Giovannetti and Mosse 1980).

To measure the growth of AMF outside plant roots, we used a modified buried bag technique. In September 2008, we created two holes per plot with 2 cm diameter soil corers. Into each hole, we placed a bag made of 250 μm mesh fabric, 5 cm long and approximately 2 cm

in diameter, and filled with play sand. The bags were positioned so that the top edge was flush with the soil surface. We retrieved the bags in September 2009, allowing for a full year of fungal growth into the bags. To extract fungi that grew into the bags, we composited the contents of both mesh bags from a plot and removed the roots from a 5-g subsample. We stirred and sonicated the subsample in 20 g/L sodium hexametaphosphate solution and filtered it through 20 μm mesh. We stained the mesh and the hyphae trapped on it in trypan blue stain, then resuspended the stained hyphae and re-filtered them onto a nitrocellulose filter. We then quantified AMF hyphae by mounting the nitrocellulose filter on a microscope slide and examining 100 haphazardly chosen fields at 200x, counting the points of contact between AMF hyphae and the lines in a grid reticle (modified from Miller et al. 1995). We used AMF hyphal length (m/g soil) as the measure of AMF abundance. To calculate plant allocation to AMF, we divided hyphal length per g soil by plant aboveground biomass per m^2. High values of this metric, indicating high AMF abundance relative to plants, would indicate high plant allocation to AMF. We got qualitatively similar answers when we repeated the analysis with a slightly different metric of plant allocation to AMF (hyphal length per g soil divided by plant root biomass per g soil); we do not present those results.

Statistical analysis: To test the two hypotheses in two different dominant grass species, we analyzed four different subsets of the data (Fig. 5.1b-e). To test Hypothesis 1, we chose plots along the largest phosphorus gradient (Fig. 5.1b) with each dominant species. This phosphorus gradient was created with two levels of phosphorus fertilization, control plots with no amendments, and plots with gypsum addition to reduce phosphorus availability. We also tested the hypothesis along the largest nitrogen gradients (Fig. 5.1c) in each dominant species, which contained two levels of nitrogen fertilization, control plots, and plots amended with a mixture of

sugar and sawdust intended to reduce nitrogen availability. We used ANCOVA to examine the response of plants and AMF to resource availability along these gradients. We used the log of measured available nutrient as an independent variable to maximize our ability to detect responses at low nutrient availabilities and to allow inclusion of the nitrogen and phosphorus reduction treatments. We tested for both linear and quadratic relationships to determine whether AMF response to nutrient availability was indeed hump-shaped as predicted. Each phosphorus gradient was available at two nitrogen fertilizer levels (Fig. 5.1b) and with two dominant species; we included nitrogen and dominant plant species as discrete factors in the analysis of the phosphorus gradient. Similarly, the nitrogen gradient was present at two levels of phosphorus fertilization (Fig. 5.1c) and two dominant species; we included phosphorus and species as discrete factors in this analysis.

To test Hypothesis 2, we chose a third subset of data (Fig. 5.1d) and conducted ANCOVA to determine plant and AMF responses to a fully factorial (three by three) design of nitrogen and phosphorus fertilizer additions. The amounts of nitrogen and phosphorus added were treated as continuous variables. A significant interaction between nitrogen addition and phosphorus addition would suggest that N:P ratio affected plant-mycorrhizal interactions. However, testing for a significant interaction was not a direct test of the role of N:P. It was not possible to explicitly test for N:P ratio using this approach because N:P was confounded with nitrogen and phosphorus additions.

To more explicitly test both hypotheses, the role of N:P ratio and of the abundance of soil nutrients, we used a fourth subset of the data (Fig. 5.1e) and modified an approach developed by Cardinale et al. (2009). We statistically separated resource "availability" (a) from "imbalance" (θ). Resource availability (a) is not a direct measure of the abundance of either resource

independently, but is a combined measure of availability of both nitrogen and phosphorus and thus represents a test of Hypothesis 1. Resource imbalance (θ) is a measure of the relative abundance of the two nutrients added to the plots and in this case is analogous to N:P ratio, so it allows us to test Hypothesis 2. Zero values of θ, indicating perfectly balanced resources, were achieved in plots where both resources were added in equal proportions (0 g nitrogen and 0 g phosphorus, 6 g nitrogen and 6 g phosphorus, 10 g nitrogen and 10 g phosphorus, and 24 g nitrogen and 24 g phosphorus). Although plants and AMF require approximately 15 times more nitrogen than phosphorus (according to N:P ratios in tissue; Cleveland and Liptzin 2007; Johnson 2010), and although equal additions of nitrogen and phosphorus do not necessarily cause equivalent increases in availability, we used 1:1 ratios of N:P to indicate "balanced" resource addition. Negative values of θ indicate that plots received relatively more phosphorus than nitrogen (e.g., 6 g nitrogen and 14.4 g phosphorus; low N:P) and positive values indicate that plots received relatively more nitrogen than phosphorus (e.g., 24 g nitrogen and 6 g phosphorus; high N:P). We conducted ANCOVA to compare the effects of resource availability (*a*) and imbalance (θ) on plant-mycorrhizal interactions with two dominant grass species.

For all analyses, we log-transformed the following dependent variables to achieve homoscedasticity: aboveground plant biomass, root biomass, AMF hyphal length, AMF:aboveground plant biomass ratio. Although analysis of ratios such as AMF:aboveground plant biomass present notorious problems (Jasienski and Bazzaz 1999; Garcia-Berthou 2001), we use them here because we are interested in testing proportional changes. All analyses also included a random block factor. One plot was excluded from all analyses because all the plants in it died. Two samples of AMF hyphal lengths were mislabeled and thrown out. We used marginal sums of squares in all analyses because of slight imbalance in the data (7-8 replicates

per treatment combination per species) and possible covariance among continuous predictor variables. Following Crawley (2007), we removed all non-significant interactions. If we found significant interactions, we investigated the effect of one factor at each single level of the other factor using ANCOVA. We performed ANCOVA in R 2.12.2, library nlme. For any significant quadratic terms, we used a Mitchell-Olds and Shaw (MOS) test (Mitchell-Olds and Shaw 1987) to determine whether the maximum or minimum of the curve fell within the range of predictor variables examined.

Results

Effects of nutrient treatments on soil nitrogen, phosphorus, and pH

The treatments strongly affected available soil nitrogen and phosphorus (Figs. 5.2-5.3). Two years of nitrogen and phosphorus addition increased KCl-extractable inorganic nitrogen (Fig. 5.2a; $F_{1,229}$=331.96 $p<0.001$ and $F_{1,229}$=37.34 $p<0.001$, respectively). Sugar and sawdust addition reduced inorganic nitrogen more than 66% (Fig. 5.3a; $F_{1,23}$=20.32 $p<0.001$) but gypsum addition had no effect on nitrogen. Phosphorus fertilization increased water-extractable soil phosphorus (Fig. 5.2b; $F_{1,229}$=679.86 $p<0.001$), and sugar and sawdust additions increased it by 88% ($F_{1,23}$=13.36 $p=0.001$). Gypsum addition decreased water-extractable phosphorus by nearly 60% (Fig. 5.3b; $F_{1,23}$=25.45 $p<0.001$). Additions of nitrogen and, to a lesser degree, phosphorus reduced soil pH ($F_{1,228}$=49.55 $p<0.001$ and $F_{1,228}$=18.98 $p<0.001$, respectively); the

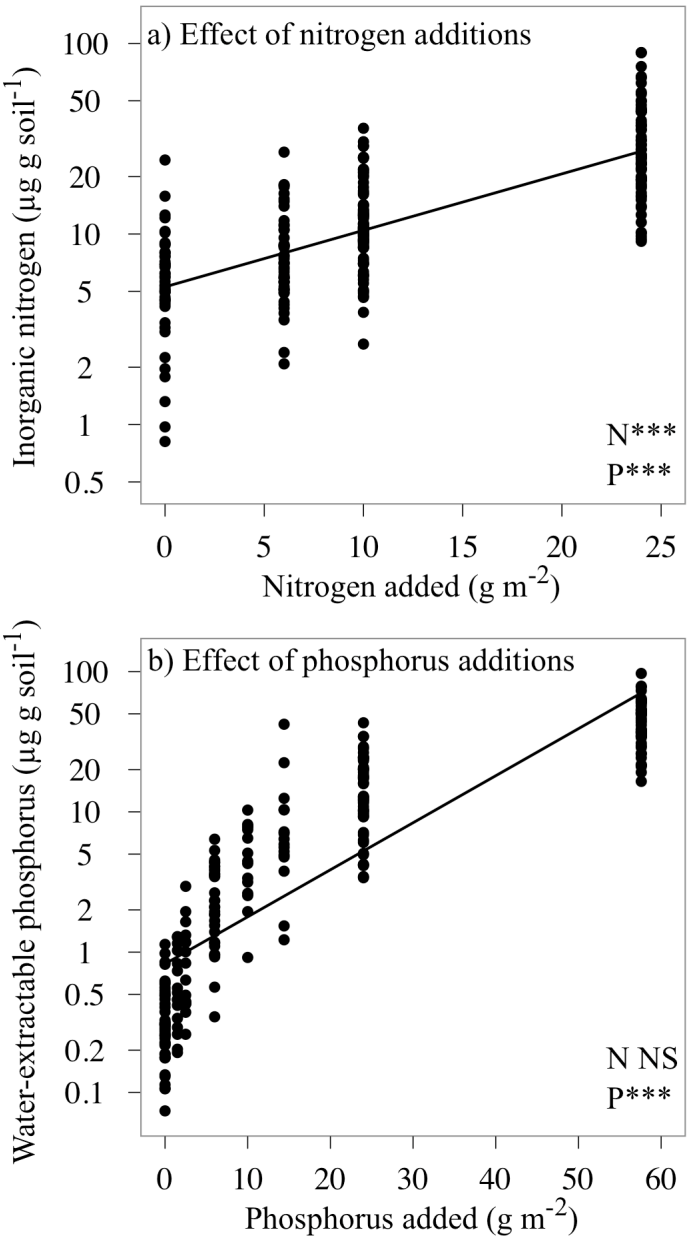


Fig. 5.2: Effect of nitrogen and phosphorus fertilization treatments on a) inorganic soil nitrogen and b) water-extractable soil phosphorus. Note log-scale y-axes. Statistical significance of responses to each predictor variable are indicated: $p<0.001$***, $p<0.01$**, $p<0.05$*, $p>0.05$ NS.

largest difference between treatment means was 0.46 units. Neither sugar and sawdust nor gypsum affected soil pH.

Effects of nitrogen and phosphorus availability on plant and AMF abundance

To test Hypothesis 1, that extremely low nutrient availability would cause AMF to increase in response to nutrient addition, we analyzed two subsets of the dataset (Fig. 5.1b,c).

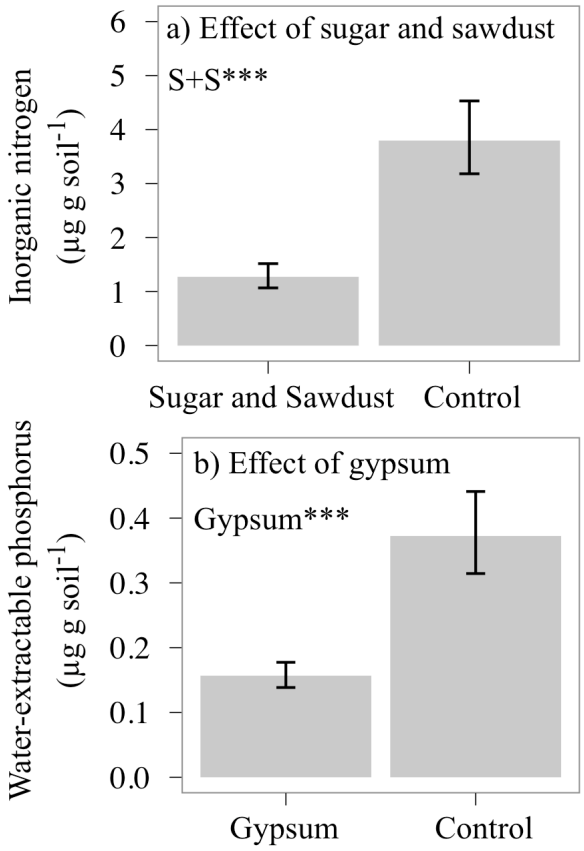


Fig. 5.3: Effect of a) carbon (sugar and sawdust, S+S) additions on inorganic soil nitrogen and of b) gypsum additions on water-extractable soil phosphorus. Values are log transformed and backtransformed means (±1 SE, n=16). Statistical significance of responses to each predictor variable are indicated: p<0.001***.

The first subset established two gradients of phosphorus availability, one at low nitrogen and one at the highest level of nitrogen addition (Fig. 5.1b), in each dominant species. The phosphorus gradients had no effect on the length of AMF extra-radical hyphae in the soil, or plant aboveground or belowground biomass (Fig. 5.4a,b,e,f). However, root colonization decreased along the phosphorus gradient (Fig. 5.4c,d; $F_{1,116}$=6.18 p=0.01) and *B. inermis* had lower root colonization in the high-nitrogen than in the low-nitrogen phosphorus gradient (Fig. 5.4c,d; species interaction $F_{1,116}$=17.79 p<0.001). The phosphorus gradients at high and low nitrogen did not differ in AMF abundance, but they did differ in plant aboveground biomass (Fig. 5.4a,b) and root biomass, indicating plant nitrogen limitation, with larger responses to nitrogen in *B.*

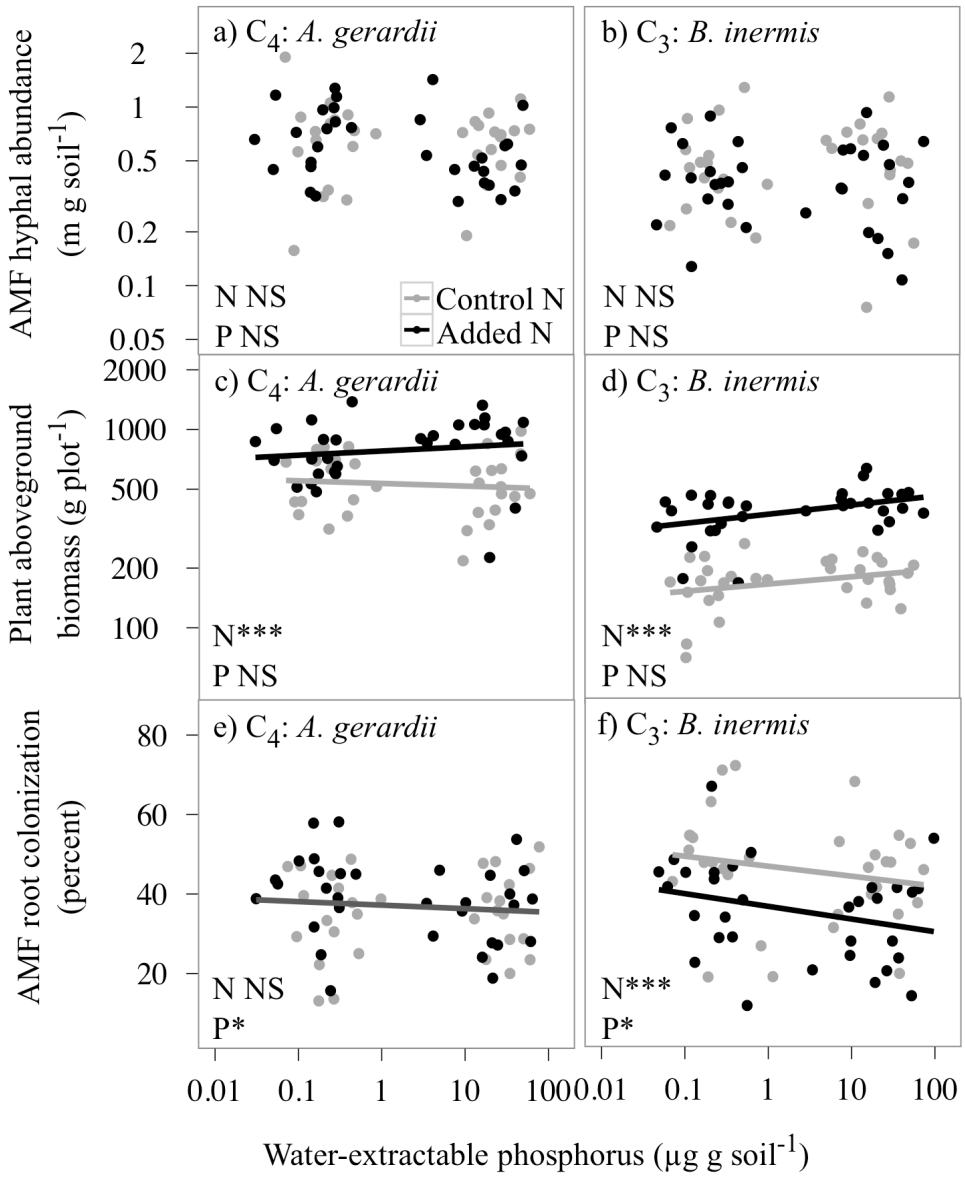


Fig. 5.4: Effects of variation in phosphorus availability at high nitrogen (black) and low nitrogen (gray), on a-b) aboveground biomass, c-d) percent root colonization by AMF, and e-f) AMF length for a,c,e) *A. gerardii* and b,d,f) *B. inermis*. See Fig. 5.1b for experimental treatments included in this analysis. Note log-scale axes. Statistical significance of responses to each predictor variable are indicated: $p<0.001$***, $p<0.01$**, $p<0.05$*, $p>0.05$ NS.

inermis than *A. gerardii* (interactions $F_{1,116}=14.82$ $p<0.001$ and $F_{1,116}=1.15$ $p=0.001$, respectively).

We also tested Hypothesis 1 using a second subset of data: two gradients of nitrogen availability, one at low phosphorus and one at the highest level of phosphorus (Fig. 5.1c), in each

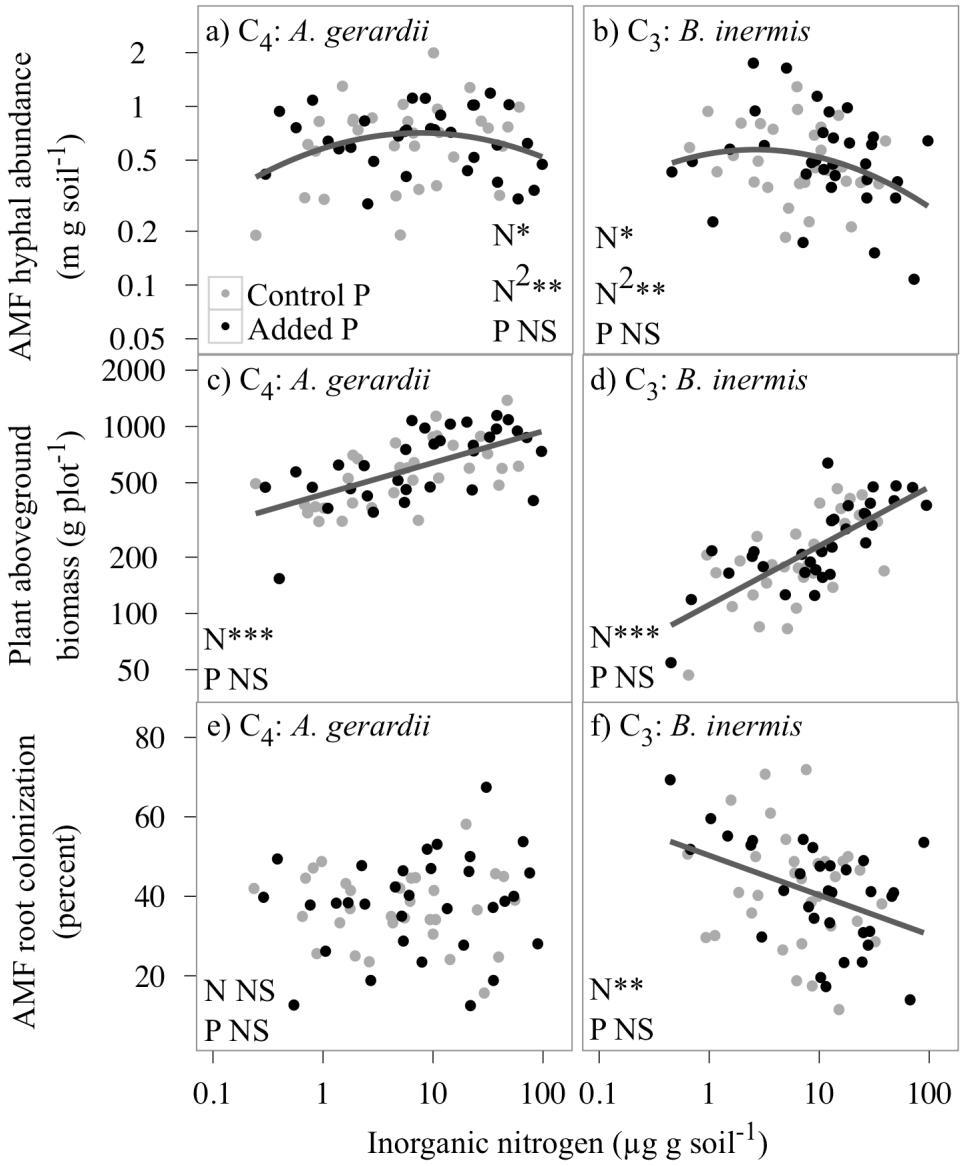


Fig. 5.5: Effects variation in nitrogen availability at high phosphorus (black) and low phosphorus (gray) on a-b) aboveground biomass, c-d) percent root colonization by AMF, and e-f) AMF length for a,c,e) *A. gerardii* and b,d,f) *B. inermis*. See Fig. 5.1c for experimental treatments included in this analysis. Note log-scale axes. Statistical significance of responses to each predictor variable are indicated: $p<0.001$***, $p<0.01$**, $p<0.05$*, $p>0.05$ NS.

dominant species. Both plants and AMF responded to the nitrogen availability gradients. Nitrogen availability affected the abundance of AMF in the expected hump-shaped pattern (Fig. 5.5e,f; linear term $F_{1,116}=5.00$ $p=0.03$; quadratic term $F_{1,116}=7.65$ $p=0.007$, MOS test significant). This hump-shaped pattern persisted, even when using ANCOVA to remove the

effect of plant aboveground biomass (linear term $F_{1,115}$=3.82 p=0.05; quadratic term $F_{1,116}$=7.51 p=0.007, MOS test significant). In contrast, plant biomass increased linearly across the nitrogen gradients (Fig. 5.5a,b); *B. inermis* responded more strongly than *A. gerardii*, both aboveground (interaction $F_{1,116}$=9.30 p=0.003) and belowground (interaction $F_{1,115}$=27.20 p<0.001). The species also differed in the degree to which they suppressed root colonization along the two nitrogen gradients: nitrogen reduced root colonization of *B. inermis* (Fig. 5.5d) but not *A. gerardii* (Fig. 5.5c; interaction $F_{1,116}$=10.99 p=0.001). The two nitrogen gradients were similar in most respects, indicating little effect of phosphorus on plant and fungal growth. There was one exception: nitrogen increased root biomass of both species more strongly when phosphorus was low (interaction $F_{1,115}$=4.57 p=0.03). However, phosphorus did not affect plant aboveground biomass, root colonization, or AMF abundance.

The second approach to testing Hypothesis 1 used a fourth subset of our plots (Fig. 5.1e). After statistically separating resource "availability" (*a*) from "imbalance" (θ; Cardinale et al. 2009), resource imbalance was only weakly negatively correlated with resource availability (r=-0.26). We could therefore test for the effects of resource availability on AMF abundance, independent of the identity of the limiting resource. There was no relationship, either linear or hump-shaped, between resource availability (*a*) and the abundance of AMF with either dominant plant species (Fig. 5.6).

Effects of N:P ratio on plant allocation to AMF

To test Hypothesis 2, that N:P ratio would affect plant allocation to AMF, we used two approaches. The first approach was a traditional nitrogen-by-phosphorus factorial design (Fig.

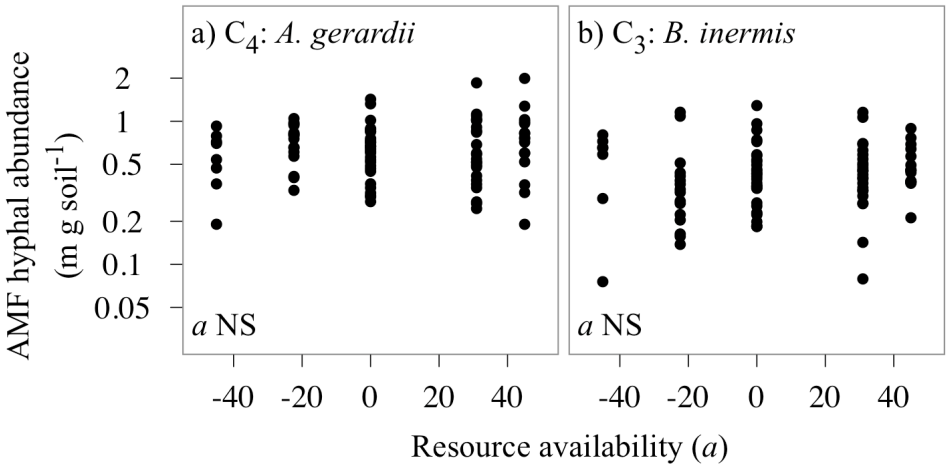


Fig. 5.6: Effects of resource availability (*a*) on AMF hyphal lengths in soil under a) *A. gerardii* and b) *B. inermis*. This measure of resource availability considers both nitrogen and phosphorus availability; see text for details. See Fig. 5.1e for experimental treatments included in this analysis. Note log-scale y-axis. Statistical significance of responses to resource availability is indicated: p>0.05 NS.

5.1d). Significant nitrogen by phosphorus interaction terms would indicate that the effect of one nutrient depended on the abundance of the other, indirect evidence for the role of N:P ratio in determining response to nutrient additions. Aboveground plant biomass increased in response to phosphorus (Fig. 5.7a,b; $F_{1,132}$=11.43 p<0.001) but root biomass did not respond (Fig. 5.7c,d); nitrogen did not affect either response to phosphorus. Nitrogen addition increased *B. inermis* biomass more than *A. gerardii*, both aboveground (interaction $F_{1,132}$=11.74 p<0.001) and belowground (interaction $F_{1,132}$=9.28 p=0.003). Root colonization also decreased in response to nitrogen addition, but only in *B. inermis* (Fig. 5.7e,f; interaction $F_{1,132}$=8.70 p=0.004) and equally at all phosphorus levels. AMF hyphal lengths decreased in response to nitrogen additions, but only at the highest phosphorus level (Fig. 5.7g,h; interaction $F_{1,131}$=6.82 p=0.01). Plant allocation to AMF, measured as the ratio of AMF hyphal length to plant aboveground biomass, also decreased in response to nitrogen, but again only at the highest phosphorus level

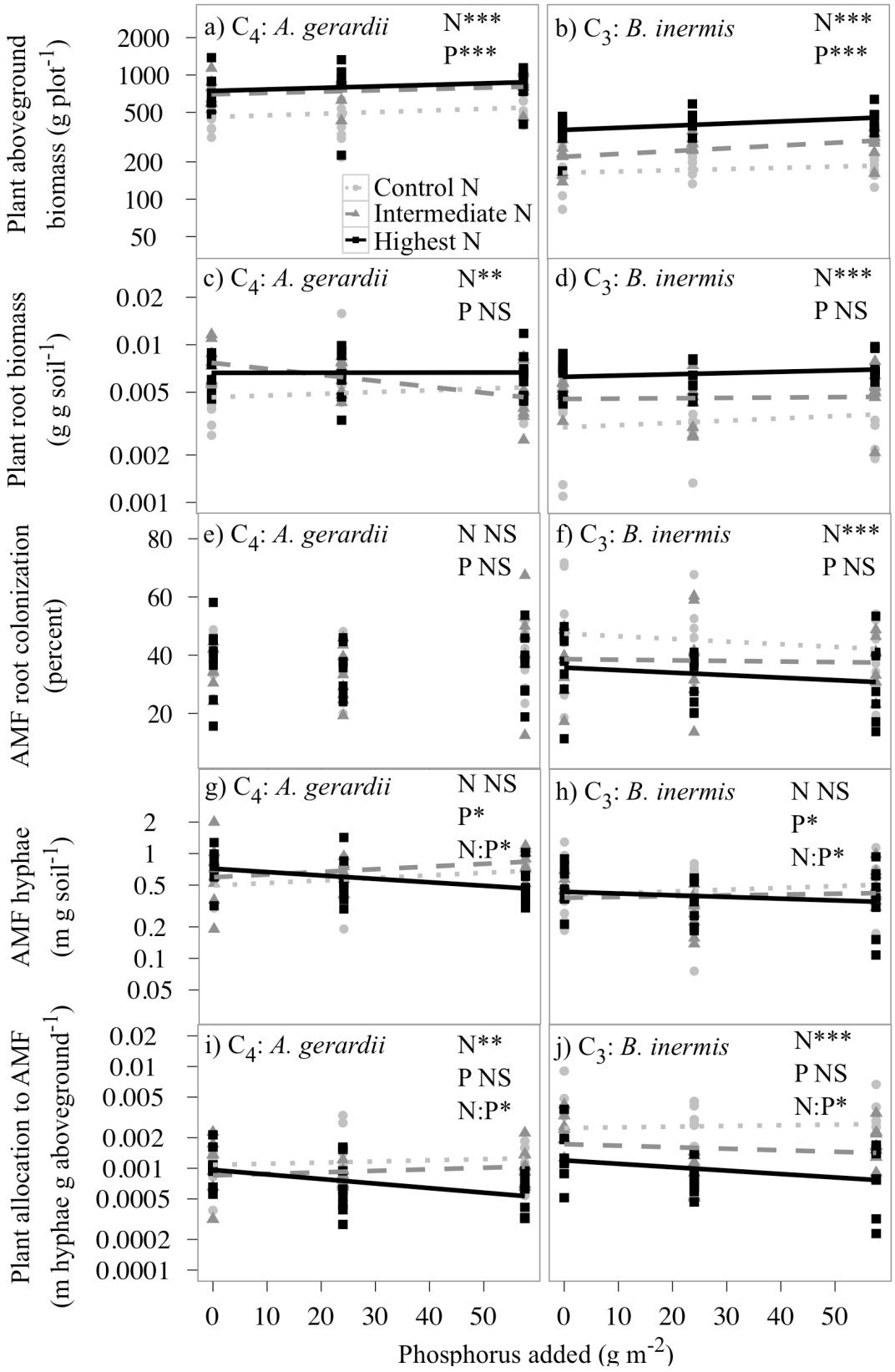

a) C4: A. gerardii
N***
P***
b) C3: B. inermis
N***
P***
Control N
Intermediate N
Highest N
Plant aboveground biomass (g plot-1)
2000
1000
500
200
100
50
c) C4: A. gerardii
N**
P NS
d) C3: B. inermis
N***
P NS
Plant root biomass (g g soil-1)
0.02
0.01
0.005
0.002
0.001
e) C4: A. gerardii
N NS
P NS
f) C3: B. inermis
N***
P NS
AMF root colonization (percent)
80
60
40
20
g) C4: A. gerardii
N NS
P*
N:P*
h) C3: B. inermis
N NS
P*
N:P*
AMF hyphae (m g soil-1)
2
1
0.5
0.2
0.1
0.05
i) C4: A. gerardii
N**
P NS
N:P*
j) C3: B. inermis
N***
P NS
N:P*
Plant allocation to AMF (m hyphae g aboveground-1)
0.02
0.01
0.005
0.002
0.001
0.0005
0.0002
0.0001
0
10
20
30
40
50
Phosphorus added (g m-2)

Fig. 5.7

Fig. 5.7 (cont'd): Interactive effects of phosphorus (x-axis) and nitrogen additions (line color and symbol) on plant a-b) aboveground biomass, c-d) root biomass, e-f) percent root colonization, g-h) AMF hyphal length in soil, and i-j) plant allocation to AMF for a,c,e,g,i) *A. gerardii* and b,d,f,h,j) *B. inermis*. See Fig. 5.1d for experimental treatments included in this analysis. Note log-scale y-axes. Statistical significance of responses to each predictor variable are indicated: $p<0.001$***, $p<0.01$**, $p<0.05$*, $p>0.05$ NS.

(Fig. 5.7i,j; interaction $F_{1,130}=6.14$ $p=0.01$). The species differed in the degree to which they reduced allocation to AMF in response to increasing nitrogen: *B. inermis* reduced allocation by 63% while *A. gerardii* reduced allocation by only 35% (Fig. 5.7i,j; interaction $F_{1,130}=5.55$ $p=0.02$).

The second approach to determining whether N:P ratio affected plant-mycorrhizal interactions was a more direct test. Using the fourth subset of our plots (Fig. 5.1e) and statistically separating resource "availability" (*a*) from "imbalance" (θ; Cardinale et al. 2009), we could test for the independent effect of N:P ratio on plant allocation to AMF (Fig. 5.8). AMF:plant aboveground biomass ratios were highest at low N:P (negative θ) and decreased when resource additions were more balanced (linear term $F_{1,176}=8.66$ $p=0.004$, MOS test significant at low θ). The smallest AMF:plant aboveground biomass ratios occurred when added nitrogen was slightly more abundant than added phosphorus (θ=22) and did not increase significantly with further increases in N:P (quadratic term $F_{1,176}=3.90$ $p<0.05$, MOS test NS at high θ). However, the effect disappeared when we repeating the analysis using a "balanced" N:P ratio of 15:1 to correspond with the ratio in which plants and fungi require the two nutrients (Cleveland and Liptzin 2007; Johnson 2010), likely because the fertilizer addition treatments were not designed to test that hypothesis (Fig. 5.1a).

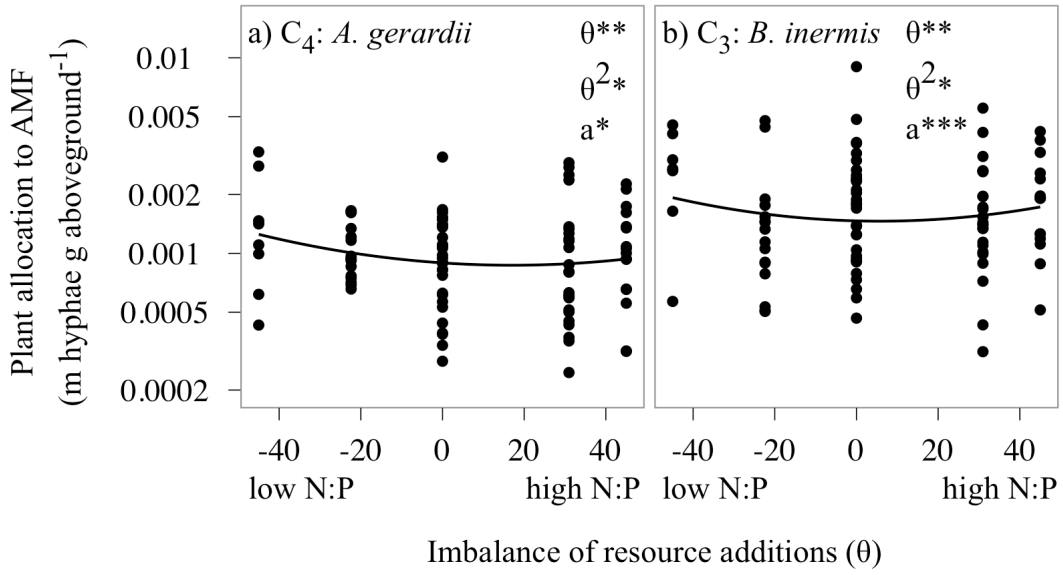


Fig. 5.8: Effects of the imbalance of resource additions (θ) on plant allocation to AMF for a) *A. gerardii* and b) *B. inermis*. Low values of θ indicate low N:P ratio, high values indicate high N:P ratio, and *a* indicates the effect of overall resource availability; see text for details. See Fig. 5.1e for experimental treatments included in this analysis. Note log-scale y-axis. Statistical significance of responses to each predictor variable are indicated: $p<0.001$***, $p<0.01$**, $p<0.05$*, $p>0.05$ NS.

Dominant species effects on AMF abundance

Although the availability and imbalance of nitrogen and phosphorus were important determinants of plant-mycorrhizal interactions, we also found that the identity of the plant species affected interactions. Two-year-old *A. gerardii* plants produced much more biomass than *B. inermis*, both aboveground (e.g., Fig. 5.7a,b; $F_{1,132}=260.24$ $p<0.001$) and belowground (Fig. 5.7c,d; $F_{1,132}= 25.90$ $p<0.001$). Mean AMF hyphal lengths were also greater in *A. gerardii* plots (Fig. 5.7g,h; $F_{1,131}= 26.07$ $p<0.001$). These species differences combined to cause *A. gerardii* to have lower AMF:plant aboveground biomass ratios than *B. inermis* (Fig. 5.7i,j; $F_{1,130}= 32.55$ $p<0.001$). Root colonization was also lower in *A. gerardii* than *B. inermis* (Fig.

5.7e,f; $F_{1,132}$= 9.82 p=0.002), but this was probably an artifact of the difficulty in quantifying colonization in the darkly pigmented roots of *A. gerardii*.

Discussion

Ecological stoichiometry predicts that plant allocation to arbuscular mycorrhizal fungi (AMF), and consequently AMF abundance, should decline with increases in the ratio of soil nutrients to light (Johnson 2010). Many studies find evidence of this (Treseder 2004; Johnson 2010). However, many studies also find no pattern or the opposite pattern (Treseder and Allen 2002; Johnson et al. 2003a; Treseder 2004). Refining and expanding this stoichiometric hypothesis might help explain the differences in response to fertilization. We found evidence that two additional stoichiometric hypotheses played an important role in determining how plant-mycorrhizal interactions depended on resource availability.

Hypothesis 1: AMF are nutrient limited in very infertile soils.

Hypothesis 1 proposes that in very infertile soils, where the ratio of soil nutrients to photosynthetically fixed carbon is very low, both plants and AMF should be nutrient limited. Increases in nutrients should therefore increase AMF abundance. This increase, combined with the decrease in AMF abundance with fertilization in moderately fertile soils, should lead to a hump-shaped curve over broad gradients of soil fertility (Treseder and Allen 2002). Our two tests of Hypothesis 1 revealed that in this grassland system, nitrogen availability does affect AMF abundance in the predicted hump-shaped curve, but that phosphorus availability does not.

At very low nitrogen availabilities in the nitrogen gradients, AMF abundance increased with increases in nitrogen (Fig. 5.5e,f), supporting the hypothesis that AMF may be nitrogen limited. It is not surprising that we detected evidence of direct nitrogen limitation of AMF. Grasslands are frequently strongly nitrogen limited (LeBauer and Treseder 2008), and in this grassland we saw strong positive responses to nitrogen in plant above and belowground biomass in both dominant species. Four lines of evidence suggest that AMF might not be immune to this nitrogen limitation. First, because nitrogen is mobile in the soil, AMF may not outcompete plants for nitrogen uptake (Hodge et al. 2010), increasing AMF susceptibility to nitrogen limitation (Johnson 2010). Second, AMF tissue is much more nitrogen-rich than plant tissue, suggesting that AMF have greater nutritional requirements for nitrogen (Hodge and Fitter 2010). Third, AMF hyphae proliferate in patches of decomposing organic material, likely in response to direct nitrogen limitation of the fungus (Hodge and Fitter 2010; Hodge et al. 2010). Finally, Treseder and Allen (2002) detected (marginally significant) increases in AMF abundance in response to nitrogen fertilization in a very nitrogen-poor site. These lines of evidence, together with our result that nitrogen increases AMF abundance in low-nitrogen soils, indicate that nitrogen limitation may be a key driver of AMF abundance in natural systems. An alternative explanation for increases in AMF abundance with nitrogen fertilization is that larger fertilized plants may have allocated more carbon to AMF and this alleviation of AMF carbon limitation could have driven increases in AMF abundance. We cannot rule out this alternative hypothesis, but it is unlikely to explain our result because the hump-shaped response of AMF persisted even when statistically removing the effect of plant biomass. Further tests of plant and AMF responses to nitrogen additions will be required to determine definitively whether AMF nitrogen limitation is ecologically important.

However, we detected no evidence of phosphorus limitation in AMF. Compared to plants, AMF have a superior ability to take up phosphorus from soils, even when it is scarce (Smith and Read 1997), so it is unlikely that phosphorus levels in this experiment were low enough to cause phosphorus limitation of AMF. Evidence for direct phosphorus limitation by AMF has previously been found in very phosphorus poor Hawaiian soils (Treseder and Allen 2002) and mine tailings (Noyd et al. 1995). AMF are also less abundant in the world's most phosphorus-impoverished soils (Lambers et al. 2008). Although direct phosphorus limitation of AMF may occur in these extremely phosphorus-poor habitats, phosphorus limitation is unlikely to determine AMF abundance in soils of more moderate fertility such as those in midwestern grasslands and old-fields. We did detect weak phosphorus limitation in plants in one analysis (Fig. 5.7a,b), and plants seemed to invest in AMF to alleviate phosphorus limitation: we observed reductions in root colonization (Fig. 5.4c,d) in response to phosphorus additions in another analysis. Grogan and Chapin (2000) have suggested that the arbuscular mycorrhizal symbiosis functions to alleviate grassland plant phosphorus limitation. Therefore, evidence that plants invested in AMF for phosphorus uptake is further indication that AMF could access enough phosphorus to meet both their own requirements and plant requirements.

Hypothesis 2: N:P ratio affects plant allocation to AMF

Johnson et al. (2003) hypothesized that N:P ratio would determine AMF response to fertilization: nitrogen additions should exacerbate plant phosphorus limitation at high N:P sites and increase plant reliance on AMF. The results of their field study support this hypothesis: among five grassland sites with different N:P ratios, nitrogen fertilization decreased AMF abundance in sites with low N:P ratios, but increased it in a site with a high N:P ratio (Johnson et

al. 2003a). A few greenhouse studies have also suggested that soil N:P can determine the response of AMF root colonization to fertilization (Sylvia and Neal 1990; Corkidi et al. 2002; Fraser and Feinstein 2005). However, because AMF increase plant access to phosphorus but may not increase plant access to nitrogen (Hodge et al. 2010), it is not clear how soil N:P should determine plant-mycorrhizal responses to fertilization.

Johnson et al. (2003) hypothesized that limitation by either nutrient would cause plants to allocate to AMF, but we posited that phosphorus limited plants (in high N:P soils) would allocate more strongly to AMF than nitrogen limited plants (in low N:P soils) because AMF may not provide growth benefits to nitrogen-limited plants (Hodge et al. 2010; Johnson 2010; Johnson et al. 2010). Surprisingly, we found the opposite response: plants increased allocation to AMF when we added resources at a low N:P ratio but not when resources were imbalanced in the opposite direction (Fig. 5.8). However, these findings make sense given that plants were strongly nitrogen limited across all fertilizer treatments and were not strongly phosphorus limited in any treatment (Fig. 5.4a,b, Fig. 5.5a,b, Fig. 5.7a,b). Even in high N:P ratio treatments, plants were likely not phosphorus limited, so allocation to AMF should not have differed from a balanced ratio of fertilization. However, in low N:P ratio treatments, plants were very strongly nitrogen limited and increased their allocation accordingly, supporting Johnson et al.'s (2003) hypothesis. Further work will be required to determine whether plant allocation to AMF in nitrogen-limiting conditions is optimal (Johnson 2010).

Our results generally support the hypothesis that limitation by either nitrogen or phosphorus would induce allocation to AMF (Johnson et al. 2003a; Johnson et al. 2010). Strongly nitrogen limited plants increased allocation to AMF (Fig. 5.8). A second piece of evidence confirms this idea: plant allocation to AMF was lowest when nitrogen and phosphorus

were both added at their highest levels (Fig. 5.7i,j). Addition of either nutrient singly did not affect allocation to AMF, supporting the idea that limitation by either nutrient would cause plants to invest in AMF. The stoichiometry of nitrogen and phosphorus in soils thus seems to be an important determinant of plant allocation to AMF.

Dominant species affect AMF response to fertilization

Although the two stoichiometric hypotheses explained many aspects of plant-mycorrhizal interactions in this field study, there were also differences between the two dominant species. AMF were consistently less abundant in soils under *B. inermis* than under *A. gerardii* (Fig. 5.4e,f; Fig. 5.5e,f; Fig. 5.6; Fig. 5.7g,h). This result is not surprising: *A. gerardii*, a native C_4 grass, typically benefits more from interacting with AMF than *B. inermis*, an introduced C_3 grass (Wilson and Hartnett 1998; Chapter 3) and should therefore allocate more carbon and promote AMF abundance. Other studies have also found that AMF hyphal lengths and spore numbers are greater when plant communities are dominated by C_4 grasses such as *A. gerardii* than by C_3 grasses such as *B. inermis* (Johnson et al. 1992; Miller et al. 1995).

There were also interesting differences between the dominant species in how interactions with AMF changed with resource stoichiometry. *Bromus inermis* always reduced AMF root colonization in response to increases in nitrogen more than did *A. gerardii* (Fig. 5.4c,d; Fig. 5.5c,d; 5.7e,f). Root colonization is thought to be an index of plant participation in the symbiosis or plant carbon allocation to AMF, but the correlation is often weak (Wilson and Hartnett 1998). In this field experiment, reductions in root colonization were not associated with reductions in AMF abundance, perhaps because of variability inherent in field studies. Alternatively,

reductions in root colonization may not have resulted in reductions in carbon flows to AMF. Other field studies (Johnson et al. 1992; Johnson et al. 2003a) have also not detected differences in dominant species effects on AMF in response to fertilization, but greenhouse studies have suggested that plant species' ability to reduce allocation to AMF can be important determinants of AMF abundance (Noyd et al. 1995; Chapter 3). Thus, the relative abundance of limiting resources likely interacts with the identity of the dominant species in determining AMF abundance, but further study is required to document this in the field.

Stoichiometry explains important aspects of plant-mycorrhizal interactions

In this field study, we found support for two stoichiometric hypotheses explaining plant-mycorrhizal interactions. Nitrogen increased AMF abundance in low fertility soils, in line with the idea that very low ratios of soil nutrients to photosynthetically fixed carbon could cause nutrient limitation of AMF (Treseder and Allen 2002). The ratio at which we fertilized with nitrogen and phosphorus also determined whether plants increased their allocation to AMF, supporting the idea that N:P ratio affects the outcome of plant-mycorrhizal interactions (Johnson et al. 2003a). These modifications of stoichiometric theory increase the predictive power of the hypothesis that plant-mycorrhizal interactions are based on the ratios of limiting resources. However, the identity of the dominant plant species also affected AMF abundance. Across the field of ecological stoichiometry, these idiosyncrasies are common (Sterner and Elser 2002). Thus, the abundance and functioning of arbuscular mycorrhizal symbiosis has two important, and potentially interacting, drivers: the identity of the dominant plant species and the relative abundances of limiting resources.

CHAPTER SIX

Conclusions and future directions

Summary of key findings

The results of this work generally support the idea that the relative abundance of limiting resources determines the outcome of plant-mycorrhizal interactions (Fig. 6.1). Stoichiometric theory predicts that increases in soil nutrients should decrease plant benefit, plant allocation, and fungal benefit. However, there were important exceptions.

First, our model (Chapter 2) showed that although differences in the relative abundance of limiting resources affected plant-mycorrhizal interactions, differences in how strongly plants and AMF benefitted from the interaction were interpretable only with additional information about the organisms. Specifically, plants can take up carbon (through photosynthesis), soil nutrients, or both; the degree to which they specialized on carbon uptake depended on the abundance of limiting resources and affected how beneficial the interaction was for plants and AMF. Similarly, the negotiated ratio of exchange of carbon for the soil nutrient varied with the abundance of the limiting resources and determined whether shifts in resource availability increased, decreased, or had no effect on plant and fungal benefit. Furthermore, the traits of the organisms were as important as resource availability in determining plant and fungal benefit. Given this variability, resource availability did affect plant-mycorrhizal interactions: both plant and fungal benefit might increase or show a hump-shaped response (Fig. 6.1).

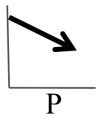
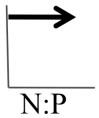
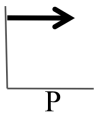
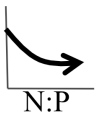
	Prediction	Model	greenhouse (P)	field (P)	field (N)	N:P ratio field
Plant benefit				X	X	X
Plant allocation: root colonization		X				
Plant allocation: proportional abundance		X				
Fungal benefit						

Fig. 6.1: Summary of key results in this dissertation. I measured the response of four different metrics of plant-mycorrhizal interactions (plant benefit, plant allocation measured as root colonization, plant allocation measured as proportional abundance, and fungal benefit) to changes in the availability of soil nutrients. Stoichiometric theory predicts that all metrics should decrease with increases in soil nutrients. In the model (Chapter 2), I simulated responses to increases in phosphorus availability. In the greenhouse, I measured responses to increases in phosphorus (Chapter 3). In the field, I measured responses to increases in phosphrous, nitrogen and N:P ratio (Chapter 5). Solid gray arrows show results for the mutualism with C_4 grasses and dashed gray arrows show results with C_3 grasses. Black arrows indicate responses common across all species investigated.

Second, the predictions of stoichiometric theory were supported by results of a greenhouse experiment, but only in some species of grass (Fig. 6.1). Two C_4 grasses experienced declines in benefit with increases in phosphorus, as predicted, but the two C_3 grasses did not. All species reduced allocation to fungi with increases in phosphorus, as expected, but the two C_3 grasses did so more strongly than the two C_4 grasses. Finally, only

fungi grown with the two C_3 grasses showed the predicted reduction in fungal benefit with increases in phosphorus (Fig 6.1). Again, stoichiometry was only a partial determinant of the outcome of plant-mycorrhizal interactions. Inherent differences in plant species determined whether increases in fertility would induce reductions in allocation to fungi, suppression of fungal benefit, and avoidance of reduced plant benefit.

Third, stoichiometry successfully explained plant and AMF response to fertilization in the field (Chapter 5), but responses again differed between plant species and also differed between soil nutrients (Fig. 6.1). Nitrogen was a much stronger force affecting the outcome of plant-mycorrhizal interactions than phosphorus, likely because of strong nitrogen limitation in the field. Nitrogen limitation of the fungi was apparent: nitrogen increased fungal abundance in very low nitrogen:light ratios. Nitrogen limitation of the plants also affected their allocation to fungi: very low soil nitrogen:phosphorus ratios exacerbated plant nitrogen limitation and increased plant allocation to AMF.

Finally, stoichiometric theory did not explain plant response to a natural productivity gradient or to increasing AMF abundance (Chapter 4). In the cross-site light addition experiment (Chapter 4), other site factors beyond productivity were more important in explaining seedling establishment success and response to the light addition treatment.

Overall, these studies support the idea that stoichiometry controls the outcome of plant-mycorrhizal interactions, including plant benefit from the interaction, plant allocation to AMF, and AMF abundance. However, it also suggests that inherent species differences affect their response to changes in resource availability.

Unanswered questions

This work suggests many avenues for future research. Several are discussed in the individual chapters. Here I highlight four additional questions arising from Chapters 2-5.

1) *What are the long-term consequences of the short-timescale interactions we modeled?* In Chapter 2, we modeled allocation and trade over very short, behavioral timescales and asked how trade affected instantaneous growth rates. However, we were not able to determine how these instantaneous growth rates, combined with resource depletion, would affect population growth or equilibrium population sizes. There are likely complex feedbacks between plant and AMF population sizes, resource availabilities, and the degree to which each partner gains from trade, so extrapolating from our short-timescale model to longer timescale population dynamics is not realistic. However, it would be very interesting to compare results of a longer timescale model that incorporates these feedbacks to empirical patterns of plant and fungal abundance across gradients of resource availability.

2) *What are empirical exchange ratios of carbon for phosphorus and carbon for nitrogen? How do they compare to the tradeoffs plants face in adjusting allocation to independently take up carbon and soil nutrients?* Our model (Chapter 2; Fig. 2.2) and other theoretical work (Schwartz and Hoeksema 1998) has suggested that association with AMF will be beneficial to plants whenever expenditures of carbon bring in more phosphorus (or nitrogen) through trade than plants would gain by allocating more to direct uptake of

phosphorus (or nitrogen). This idea is especially interesting in light of Johnson et al.'s (2010) finding that nitrogen-limited plants had high allocation to AMF *even though the interaction was not beneficial*. One interpretation of this result is that plants were trading outside the range of beneficial exchange ratios: nitrogen cost more carbon through trade than through direct root uptake. Nevertheless, plants maintained the interaction, raising the question of how often plants reduce allocation to AMF at the appropriate exchange ratio threshold.

3) *Do plant species differences in ability to reduce allocation to AMF in high-nutrient environments determine species persistence in eutrophic communities?* In the greenhouse experiment (Chapter 3), I found that *B. inermis* and *E. repens* were better able to reduce carbon allocation to AMF than the other two species, and that *A. gerardii* experienced parasitism in high phosphorus soils. I therefore expect that nutrient enrichment should cause stronger declines in the abundance of *A. gerardii* than *B. inermis* and *E. repens*. This prediction is supported by evidence that nutrient rich old-fields are dominated by *B. inermis* and *E. repens* and that *A. gerardii* seedlings establish very poorly there (Foster 1999). However, in Michigan grasslands, increases in productivity are more likely driven by increases in nitrogen than phosphorus (Chapters 4-5) and it is not clear that plants and AMF should respond identically to increases in both nutrients, so further tests of this idea are necessary. Johnson et al. (2008) pursued this idea, comparing two "winner" species that typically responded positively to long-term nitrogen fertilization in the field (*E. repens* and *Panicum virgatum*) to two "loser" ecotypes that typically declined in abundance with nitrogen fertilization (both ecotypes

were of a single species, *A. gerardii*). They found that the "winners" were more effective in reducing root colonization by AMF than the "losers"—partial support for the hypothesis. However, they were able to include only a few species, and they did not measure AMF biomass so their measure of allocation to AMF is imperfect. To test this hypothesis in a different experimental system, I am planning to conduct a greenhouse experiment in collaboration with Jen Lau, Tomomi Suwa, and Rachel Prunier. We will select many different species of legumes for which we have data on long-term response to nitrogen fertilization in grassland LTER sites. We will grow the legumes with and without rhizobia, with and without added nitrogen, and test the hypothesis that the species lost with fertilization are those less likely to reduce carbon allocations to rhizobia and more likely to suffer parasitism in high nitrogen environments.

4) *Should AMF increase the growth of nitrogen-limited plants?* Although there is clear evidence that AMF take up nitrogen and transfer it to plants (Govindarajulu et al. 2005), most greenhouse studies find that nitrogen-limited plants do not benefit from the interaction (e.g., Reynolds et al. 2005; Johnson et al. 2010). It is not clear whether this results from artifacts of the experimental design, such as low light availabilities or the lack of an established network of AMF. It may also result from fundamental differences between nitrogen and phosphorus movement in the soil. Some authors have suggested that nitrogen's greater mobility in the soil, combined with greater fungal nitrogen requirements, negate the advantages over plant roots provided by fungal hyphae (Hodge et al. 2010; Johnson 2010). This question could be addressed with a mathematical model that compared the different movement of nitrogen and phosphorus in the soil (diffusion

or mass flow) with key differences between plants and AMF: different carbon costs of root and hyphal construction and metabolism, different requirements for nitrogen and phosphorus, different uptake kinetics, and possibly differential access to mineralizing organic material. If parameterized with realistic values, this model could indicate whether we are likely to see nitrogen-limited plant growth benefits from association with AMF.

APPENDIX

APPENDIX

Calculations of parameter values for mathematical model

Values not listed were presented directly or involved only simple calculations. Dashes indicate range of values, not subtraction.

f_{NP}: 0.9–42 fmol P cm root^{-1} s^{-1} (Sanders and Tinker 1973; Smith 1982; McGonigle and Fitter 1988) * 10^{-15} mol P fmol P^{-1} * 31 g P mol P^{-1} * 100 cm m^{-1} * 61–230 m g root^{-1} (Miller et al. 1995; Craine et al. 2002) * 2 g root g plant C^{-1} = 3.4–600*10^{-10} g P g plant C^{-1} s^{-1}

V_{CP}: 9–20 µmol C fixed m leaf^{-2} s^{-1} (Awada et al. 2003; Allred et al. 2010) * 10^{-6} mol C µmol C^{-1} * 12 g C mol C^{-1} * 10^{-4} m leaf^{2} cm leaf^{-2} * 45–140 cm leaf^{2} g leaf^{-1} (Awada et al. 2003; Allred et al. 2010) * 2 g leaf g plant C^{-1} = 9.7–67.2*10^{-7} g C fixed g plant C^{-1} s^{-1}

f_{NF}: 0.6–50 fmol P cm hyphae^{-1} s^{-1} (Jakobsen et al. 1992; Schweiger and Jakobsen 1999; Smith et al. 2000; Smith et al. 2004) * 10^{-15} mol P fmol P^{-1} * 10^{-4} cm µm^{-1} * 31 g P mol P^{-1} * 0.08–0.1 µm biovolume^{3} µm hyphae^{-1} (Miller et al. 1995) * 4 cm^{3} g hyphae^{-1} (Miller et al. 1995)* 10^{12} µm^{3} cm^{-3} * 2 g hyphae g fungal C^{-1} = 1.2–124 * 10^{-6} g P g fungal C^{-1} s^{-1}

P: 1500–4300 g plant m^{-2} * 0.455–0.519 g C g $plant^{-1}$ (Craine et al. 2002; Mahaney et al. 2008) = 686–2129 g plant C m^{-2}

F: 0.12–0.9 m g $soil^{-1}$ (extra-radical fungi extracted from 1-year incubations of ingrowth bags; Chapters 4-5) * 1.6 g soil cm^{-3} (KBS LTER website) * 10^{6} µm hyphae m^{-1} * 0.08–0.1 µm $biovolume^{3}$ µm $hyphae^{-1}$ (Miller et al. 1995) * 0.25 g fungi cm^{-3} fungi (Miller et al. 1995) * 10^{-12} cm^{3} μm^{-3} * 20 cm depth * 10^{5} cm^{2} m^{-2} * 0.5 g fungal C g $fungi^{-1}$ * 1.5–10 g total fungi g^{-1} extra-radical fungi = 0.006–0.36 g total fungal C m^{-2}

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