

Integrating physiological, community, and evolutionary perspectives on the arbuscular mycorrhizal symbiosis¹

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Abstract: Our knowledge of arbuscular mycorrhizal (AM) function is largely based on results from short-term studies in controlled environments. While these have provided many important insights into the potential effects of the symbiosis on the two symbionts and their communities, they may have also inadvertently led to faulty assumptions about the function of the symbiosis in natural settings. Here we highlight the consequences of failing to consider the AM symbiosis from the perspectives of community ecology and evolutionary biology. Also, we argue that by distinguishing between physiological and evolutionary viewpoints, we may be able to resolve controversies regarding the mutualistic vs. parasitic nature of the symbiosis. Further, while most AM research has emphasized resource transfers, primarily phosphate and carbohydrate, our perceptions of parasitism, cheating, bet-hedging, and partner choice would most likely change if we considered other services. Finally, to gain a fuller understanding of the role of the AM symbiosis in nature, we need to better integrate physiological processes of plants and their AM fungi with their naturally occurring temporal and spatial patterns. It is our hope that this article will generate some fruitful discussions and make a contribution toward this end.

Key words: arbuscular mycorrhiza, mutualism, parasitism, physiology, evolution, community ecology.

Résumé : Notre connaissance de la fonction des mycorhizes arbusculaires (MA) repose largement sur des résultats provenant d'études à court terme conduites sous des conditions contrôlées. Bien que celles-ci aient fourni plusieurs informations importantes sur les effets potentiels de la symbiose sur les symbiotes et leurs communautés, elles ont possiblement conduit par inadvertance à de fausses perceptions au sujet du fonctionnement de la symbiose en conditions naturelles. Les auteurs soulignent les conséquences de ne pas considérer la symbiose MA dans la perspective de l'écologie des communautés et de la biologie évolutive. Ils avancent également qu'en distinguant entre les points de vue physiologiques et évolutifs, on peut résoudre les controverses portant sur la nature mutualiste vs. parasitaire de la symbiose. De plus, alors que la plupart des recherches sur les MA ont mis l'emphase sur les transferts de ressources, surtout phosphates et glucides, notre perception du parasitisme, de la tricherie, le bet-edging et du choix des partenaires serait très probablement modifiée en considérant les autres services. Finalement, afin d'obtenir une compréhension plus complète du rôle de la symbiose MA en nature, nous devons mieux intégrer les processus physiologiques des plantes et leurs champignons MA à leurs patrons de distribution spatio-temporelle naturels. Les auteurs espèrent que cet article générera des discussions fertiles et constituera une contribution à cette fin. [Traduit par la Rédaction]

Mots-clés : mycorhize arbusculaire, mutualisme, parasitisme, physiologie, évolution, écologie des communautés.

Introduction

The arbuscular mycorrhizal (AM) symbiosis has been studied by scientists trained in a variety of traditions since it was first described in the middle to late 19th century (Koide and Mosse 2004). Most scientists have taken a physiological or autecological approach. Fewer have studied the symbiosis in the context of natural communities or ecosystems. Fewer still, perhaps, have considered the symbiosis in an evolutionary context. Our opinion is that while each of these approaches has produced important insights into the AM symbiosis, the balkanization of our science has led to some misunderstanding and confusion. We feel that a more synthetic approach in which we integrate knowledge of physiological mechanisms, patterns of community structure, and an appreciation for evolutionary processes will yield greater insight into the symbiosis. Such an approach may help us to reconcile models of AM function that, at first glance, appear contradictory.

For a variety of reasons, a great deal of what we think we know about the AM symbiosis derives from greenhouse experiments. Unfortunately, the symbiosis did not evolve in a greenhouse where plants are grown singly in soil that has been sieved, autoclaved or irradiated, mixed with large amounts of sand, placed in plastic pots and warmed to unnatural temperatures in the sun, kept perpetually moist, inoculated with single isolates of AM fungus that may not interact with the host in nature, fertilized with reagent-grade salts, illuminated by 1000 W metal halide lamps, and given no exposure to other members of natural communities including other plant species, herbivores, or pathogens. We are poking fun here, obviously, but the point we are making is simply that many of the experiments we cite when we want to provide evidence for certain phenomena such as mutualism, parasitism, cheating, and bet-hedging are quite artificial. Thus, we question some of the dogma that has become established concerning the function of the symbiosis in natural communities. When consid-

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ering the AM symbiosis, one needs to be cognizant of the fact that the symbiosis evolved in natural communities. We are not the first ones to point this out, of course (e.g., see [Johnson et al. 1997](#); [Read 2002](#)), but we have taken some care here to discuss the consequences of failing to do so.

In this article we attempt to reconcile disparate views, critically examine some long-held assumptions, and suggest ways in which insights from community ecology and evolutionary biology can inform our understanding of the interactions between symbionts that determine the outcome of the symbiosis. It is not within the scope of this paper to provide an exhaustive review of the literature that is relevant to the topics we discuss. It is our hope, however, that we will provoke some critical thinking and generate fruitful discussion of the benefits of a more integrative approach in future AM research.

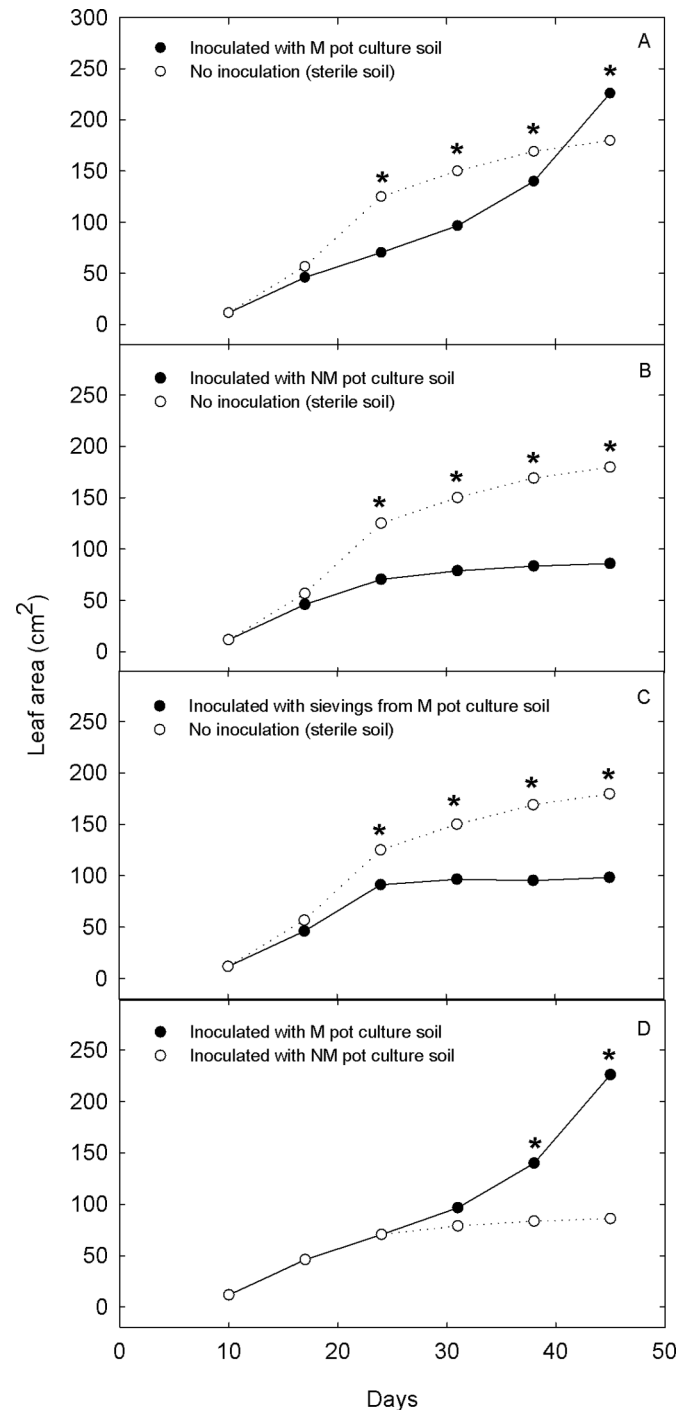
Comparing mycorrhizal and nonmycorrhizal plants: the problem with artifacts

Our understanding of the physiological, ecological, and evolutionary consequences of the AM symbiosis to plants stems largely from comparisons of colonized (mycorrhizal) and uncolonized (nonmycorrhizal) plants grown in the greenhouse. Classically, colonized plants are larger owing to enhanced P content as a consequence of transfer of P from fungus to plant. But a nontrivial proportion of trials have produced colonized plants that are either smaller than or no larger than uncolonized plants ([Smith et al. 2009](#)). For convenience, we assume for the moment that the only benefit of colonization to the plant is increased P content. We shall discuss other benefits later.

It is relatively easy to produce colonized and uncolonized plants. It is not easy to produce plants that are the same in every respect except AM status, but that is necessary if we are to determine the real effects of mycorrhizal colonization on plant performance. Frequently, colonized plants are grown in a semi-sterile (autoclaved, steamed, or irradiated) soil that has been inoculated with AM fungal pot culture soil containing spores, hyphae, and roots. Uncolonized control plants are often grown in the same semi-sterile soil either given no inoculum or inoculated with sievings from the AM fungal inoculum ([Nagy et al. 2009](#); [Pearson and Jakobsen 1993](#)). Sievings are produced by mixing the AM fungal inoculum with water and passing this through a sieve that selectively removes particles larger than 30 μm or so, eliminating AM fungi while allowing other microorganisms to pass. We demonstrate in the following paragraphs that this combination of treatments can produce the impression that AM fungi cause a growth depression in the plant when, in fact, the depression arises from the use of an inappropriate control.

Such a growth depression associated with AM colonization is shown for *Helianthus annuus* L. plants in [Fig. 1A](#) (results redrawn from [Koide and Li 1989](#)). In this case, colonization by AM fungi was not the cause of the growth depression, as has often been assumed. To demonstrate this, [Koide and Li \(1989\)](#) produced nonmycorrhizal (NM) pot cultures under the very same conditions as pot cultures containing AM fungi. They discovered that inoculation of sterile soil with a small amount of NM pot culture soil produced a plant growth depression relative to sterile soil ([Fig. 1B](#)) similar to the early growth depression observed with mycorrhizal (M) pot culture soil ([Fig. 1A](#)). Sievings from the M pot culture soil also produced a growth depression ([Fig. 1C](#)). These results indicate that something other than AM fungi can depress plant growth and that these depressions are of the same magnitude as those assumed in many studies to be caused by mycorrhizal colonization ([Smith et al. 2009](#)). When [Koide and Li \(1989\)](#) compared colonized plants produced by inoculation with M pot culture soil and uncolonized plants produced by inoculation with NM pot culture soil, there was no growth depression, not even a transient one

Fig. 1. Growth of *Helianthus annuus* plants in autoclaved soil given various types of inoculants. Asterisks indicate significant differences ($P \leq 0.05$) between pair-wise means. (A) Plants inoculated with mycorrhizal (M) pot culture soil (mycorrhizal whole-soil inoculum) vs. uninoculated plants. (B) Plants inoculated with nonmycorrhizal (NM) pot culture soil (nonmycorrhizal whole-soil inoculum) vs. uninoculated plants. (C) Plants inoculated with sievings from mycorrhizal (M) pot culture soil (see text) vs. uninoculated plants. (D) Plants inoculated with mycorrhizal (M) pot culture soil vs. plants inoculated with nonmycorrhizal (NM) pot culture soil. See [Koide and Li \(1989\)](#) for details.



(Fig. 1D), presumably because whatever caused the growth depression was present in equal abundance in both inoculants.

Some have suggested that growth depressions associated with mycorrhizal colonization may be caused by pathogenic or parasitic (nonmycorrhizal) fungi present in the inoculum (Kloepper and Schroth 1981). The roots in the Koide and Li (1989) study did not appear diseased, and the fact that both *Sinapis alba* L. (Brassicaceae) and *H. annuus* (Asteraceae) reacted in the same way to the NM pot culture soils suggests that the growth depression was not caused by a species-specific plant pathogen. It is possible, however, that non-specific pathogens or parasites could have caused the growth depression, but the stained roots revealed no evidence of them.

Because NM pot culture soil and sievings from M pot culture soil contain a well-developed nonmycorrhizal microbial community, one possible explanation for growth depression is microbial immobilization of N, P, or some other essential plant nutrient, which could result in plant nutrient deficiency. Wilson et al. (1988) reached a similar conclusion. Thus, some transient depressions in growth or nutrient uptake in AM plants (inoculated with M pot culture soil) compared with non-AM plants (growing in semi-sterile soil) may be caused by nutrient immobilization by the nonmycorrhizal microbial community, which makes it more difficult for the plant to take up nutrients (Barber 1978; Hetrick et al. 1987). This has been observed before, especially when microbes are inoculated into sterile soil (Bünemann et al. 2012; Shen et al. 1984).

Many studies have employed sievings from M pot culture soil as the control, and while this was probably appropriate as the control inoculant in Koide and Li (1989), sievings may not always be appropriate control inoculants. The size of the microbial community in sievings could vary markedly from that in the M pot culture soil depending on how the sievings are prepared (the relative amounts of soil and water, the size of the mesh, etc.).

We recommend that researchers take greater care to produce mycorrhizal and nonmycorrhizal plants that are comparable in every way except for their mycorrhizal status. This could be done by inoculating M plants with M pot culture soil and NM plants with NM pot culture soil (that has been inoculated with soil sievings devoid of AM fungi), or by inoculating M plants with AM fungal spores only and adding nothing to NM plants, for example. In any case, one point we wish to make clear is that some of what we think we know about plant growth depressions caused by colonization of roots by AM fungi, about parasitism or about cheating in this symbiosis may be on slightly shaky ground as a consequence of the use of inappropriate controls.

Growth depressions: The parasitism–mutualism continuum and hidden P uptake

We do not take the position that AM colonization per se can never reduce plant growth. Klironomos (2003), for example, provided an excellent example of growth responses to AM ranging from negative to positive in which appropriate nonmycorrhizal controls were used. The cause of the growth reductions in his study is unknown, but the data strongly suggest that AM fungi can, on occasion, reduce plant growth, at least under the greenhouse conditions employed. These occasional growth reductions have been interpreted in two different ways: (1) carbohydrate–phosphate trade imbalance and (2) phosphate limitation. These correspond to the parasitism–mutualism and hidden P uptake perspectives.

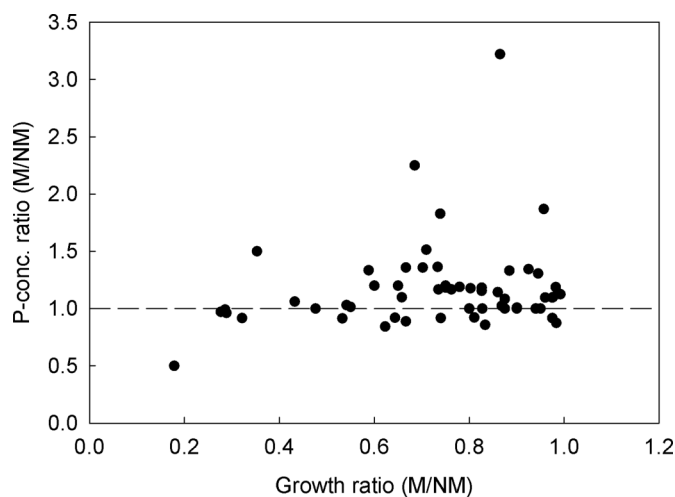
In the parasitism–mutualism continuum (Johnson et al. 1997), the overall effect of mycorrhizal colonization on plant performance is the net result of independent “costs” and “benefits”. If costs to the plant outweigh benefits, parasitism is the result. If benefits to the plant outweigh costs, mutualism is the result. The

biotrophic fungus is assumed to always derive a net benefit from the association. Carbohydrate transfer to the fungus is often considered to be the major cost of the symbiosis to the plant, while phosphate transfer to the plant is frequently considered the major benefit (Smith and Read 2008). Unfortunately, in most cases it is difficult to determine whether carbohydrate transfer does, in fact, represent a cost with any consequence. Plants certainly require C for growth, defense, and reproduction, and one might reason that allocating up to 30% of the total photosynthate to AM fungi (Drigo et al. 2010) would reduce what is available to the plant and cause growth depressions. The complication here, however, is that the C economy of AM plants is not necessarily a zero sum game. Smith and Smith (2012) point out that the plant may bear no net C cost if it reduces allocation to roots or if photosynthesis is sink-regulated. Indeed, the increase in photosynthesis due to mycorrhizal colonization may more than offset the carbohydrate cost of the fungus borne by the plant (Kaschuk et al. 2009), and growth reductions are not necessarily observed even when the fungus imposes large carbohydrate demands in return for very little phosphate (Lendenmann et al. 2011). Even if significant carbohydrate is transferred to the fungus in the absence of sink-regulated photosynthesis or reduced root allocation, it may still have no consequence to the plant if the activities of the plant are not limited by carbohydrate. Large growth depressions have been observed in relatively poorly colonized root systems, and it seems unlikely that carbohydrate transfer to the AM fungi under these conditions would be sufficient to substantially reduce plant growth (Smith and Smith 2012). Nevertheless, imbalances in carbohydrate and phosphate transfer may occur in some cases (Douds et al. 1988).

Hidden P uptake is thought to occur when AM fungi, while transferring phosphate to the root, somehow impair or eliminate direct, root-mediated phosphate uptake, making the phosphate flux from fungus to host unaccountable by simply comparing the P contents of AM and non-AM plants (Smith et al. 2011; Smith and Smith 2011, 2012). In the hidden P uptake perspective, growth depressions are hypothesized to arise not from plant C limitation but from P deficiency, owing to a transfer of phosphate from fungus to plant that is insufficient to make up for the loss of phosphate uptake via the direct, root-mediated pathway. In some cases it has been proposed that host P deficiency and resultant growth depression can occur when roots are only poorly colonized by mycorrhizal fungi (Smith and Smith 2012). However, it is difficult for us to envision a physiological mechanism or an evolutionary strategy in which low colonization by AM fungi inactivates the entire root system’s phosphate uptake mechanism (Smith et al. 2004; Smith et al. 2009). Moreover, if P deficiency caused the growth depressions, one would expect plant P concentrations to be lower in mycorrhizal than in nonmycorrhizal plants. This does not often appear to be the case. We extracted data from trials exhibiting AM fungus-induced growth depressions (i.e., $M_{\text{Growth}}:NM_{\text{Growth}} < 1$) from Table 1 in Smith et al. (2009) and other references in the text referring to such (see Supplementary Table S1 for publications included).² The average P-concentration ratio ($M_{\text{P-conc.}}:NM_{\text{P-conc.}}$) was < 1 in only 24% of the trials (Fig. 2). And, in fact, the P-concentration ratio was significantly ($p < 0.001$, paired *t*-tests) greater (average 75% greater) than the growth ratio (Supplementary Table S1). Thus, in most cases, AM plants had a higher shoot P concentration than uncolonized plants. It is interesting to note, too, that only a few studies (e.g., Hetrick et al. 1994, 1996) used what we would consider to be appropriate controls (comparable nonmycorrhizal microbial communities in M and NM pots) in the trials from which we extracted these data (Supplementary Table S1). Nevertheless, while there may still be some

²Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjcb-2013-0182>.

Fig. 2. Relationship between the growth ratio ($M_{\text{Growth}}:NM_{\text{Growth}}$) and P-concentration ratio ($M_{\text{P-conc.}}:NM_{\text{P-conc.}}$) of mycorrhizal and nonmycorrhizal plants. All studies included were from Table 1 or the text in Smith et al. (2009) and showed a growth reduction associated with AM fungal inoculations (i.e., $M_{\text{Growth}}:NM_{\text{Growth}} < 1$, Supplementary Table S1²).



degree of uncertainty about the underlying cause of growth depressions, the work by Smith et al. has clearly shown that AM fungi can provide the vast amount of P required by plants.

We showed in the previous section that growth depressions associated with AM colonization may be artifacts of larger microbial populations, possibly as a result of nutrient immobilization. The immobilization of N or P or other nutrients by nonmycorrhizal microbes, in fact, may explain some cases of “parasitism” by AM fungi (Johnson et al. 1997), as well as some cases of impaired direct phosphate uptake (Smith et al. 2004, Smith et al. 2009; Smith and Smith 2011).

Physiological versus evolutionary perspectives

In the continuum perspective, parasitism and mutualism are regarded as net results of costs and benefits (Johnson et al. 1997). To Smith and Smith (2011), however, mutualism is not a net effect; mycorrhizal fungi are always mutualists (and never parasites) as long as they deliver some phosphate to their host plant, even if their net effect on the plant is neutral or negative. This difference has generated some confusion and debate (e.g., Johnson and Graham 2013; Smith et al. 2009), but we believe that the two perspectives view the symbiosis differently and are therefore not comparable. They are not different answers to the same question but, instead, appear to us to be bases for different questions. The hidden P uptake perspective is a physiological one in which transfers of resources are emphasized, independent of net effects. In contrast, the continuum perspective is essentially an evolutionary one in which the net effects are of primary interest.

Mutualism is usually regarded as the case in which both symbionts benefit from their association. From an evolutionary perspective, benefits must be net effects, as natural selection can only act on the overall result. For example, nature can gain no selective traction to favor one fungal symbiont over another unless their net effects on host fitness are different. From an evolutionary perspective, therefore, it is difficult to consider fungi as mutualists if they merely deliver *some* P to the plant when their overall effect is negative. No one would regard a guest at a hotel as a mutualist if he paid less than the actual cost of his stay, even if he did pay something.

While the net effects perspective is essential in an evolutionary context, accounting for costs and benefits that lead to a net effect

is challenging, to say the least. Johnson et al. (1997) wrote about costs and benefits (from the plant’s perspective) in terms of carbohydrate and phosphate transfers, but these are not comparable currencies that can be summed to a net effect. That may be why Johnson et al. (1997) ultimately suggested using fitness as a common currency for costs and benefits. With a common currency the various costs and benefits can be summed to arrive at the net result, either mutualism (if the net result is an increase in plant fitness) or parasitism (if the net result is a decrease in fitness). While this approach is instructive in a theoretical way, the difficulty is simply that one cannot measure fitness in the way one measures fluxes of phosphate or carbohydrate. One might argue that various proxies for fitness could be used that *are* measurable. For example, size, nutrient status, reproductive output, survival, and other traits that can be measured have been used as proxies for fitness. But they are not necessarily correlated with fitness in the expected way. Larger plants, for example, are sometimes more apparent to herbivores and thus suffer from greater herbivory than smaller plants (Castagneyrol et al. 2013), plants of higher nutrient status are sometimes more disease-prone than more deficient ones (Talukder et al. 2005), and plants with greater reproductive output may be subjected to greater rates of frugivory (Sallabanks 1992). Thus the environmental context may determine whether various proxies for fitness relate to fitness in a positive or a negative way.

A purely physiological approach emphasizing resource transfers may not be useful in an evolutionary context. But much of the research on the AM symbiosis has been performed using cultivated plants for which natural selection is meaningless and, for them, the purely physiological approach may be useful and appropriate. In contrast, if we are ever to fully understand the nature and consequences of the symbiosis for species of natural communities, we must take an evolutionary approach. While fitness may not be a practical currency with which to quantify costs and benefits, the parasitism–mutualism continuum, which makes clear the relationships among costs, benefits, and net effects, is clearly helpful in at least a theoretical sense. In any case, it is essential to maintain an evolutionary perspective when we consider the symbiosis with respect to various concepts such as cheating, bet-hedging, and partner choice.

The concept of cheating

Thus far we have largely concerned ourselves with studies involving single plant–fungus combinations. In nature, however, most plants associate with multiple fungal taxa, and one fungus can colonize multiple plants, resulting in complicated interconnections via common mycorrhizal networks (Smith and Read 2008). Some have called these networks “potentially dangerous” (Selosse et al. 2006) because the AM symbiosis allows for an open, many-to-many set of interactions in which cheating becomes a possibility. Essentially, why would any symbiont provide potentially costly resources if benefits could be obtained through the hard work of others?

In the context of the AM symbiosis, cheating is frequently defined as the case in which one symbiont receives a benefit from the other *without reciprocating* (Johnson et al. 1997). Indeed, Egger and Hibbett (2004) suggested that the mycorrhizal symbiosis would best be viewed not as a mutualism but as mutual exploitation, the case in which partners essentially give as little as they can get away with. Of course if a resource is not limiting, there is no advantage in being sparing with it. But if the resource is costly, and especially if there are alternative symbionts, there may be some advantage to cheating (see review by Kiers and van der Heijden 2006).

This “without reciprocating” definition of cheating has, at its core, the assumption of mutualism (benefit exceeds cost). Obviously if the expectation were parasitism, not reciprocating could

hardly be considered cheating! Cheating, then, must be defined in relation to an expectation. If the expectation is mutualism, then anything less is parasitism. In that case, parasitism and cheating are synonymous, as suggested by Smith and Smith (2012). However, even mutualistic fungi could be considered cheats. Consider a plant that can be colonized by three fungi. Assume that all three fungi receive the same amount of carbohydrate. Further assume that they are all mutualistic but that they benefit the plant to different extents because they differ in the amount of phosphate they transfer to the plant. Even though they all promote plant growth, the two that transfer less phosphate than the third could easily be considered cheats because for the amount of carbohydrate received, they transfer less phosphate than the other. Here, cheating and parasitism are not the same.

Do fungi cheat?

That is a very difficult question to address. If cheating is simply being less beneficial than others, fungi that cheat plants almost certainly exist. Indeed, results from controlled greenhouse experiments show drastic differences in the effects on plant growth among fungal taxa (e.g., Bever 2002; Munkvold et al. 2004; Pringle and Bever 2008; Smith et al. 2004) and in the ratio of phosphate delivered to plant and carbohydrate transferred to fungus (Lendenmann et al. 2011; Pearson and Jakobsen 1993). Because experiments are frequently performed under conditions that differ markedly from those in nature, labeling an AM fungus a cheat should be done with caution. In the greenhouse, for example, pathogen pressure may be low. But in the field, pathogens may be very important and the fungus that is poor at transferring phosphate may be superior in providing pathogen protection (Newsham et al. 1995; see bet-hedging, below). Also, while short-term greenhouse experiments may suggest the *potential* for cheating under very specific conditions, the real laboratory is the natural community in which time and space are very much expanded and in which plant populations struggle for survival over multiple generations during which environmental conditions are changing constantly. The importance of adopting a lifetime fitness view of symbiotic functioning was recently shown in an ant-*Acacia* symbiosis in which symbionts that appeared to be cheats at one life-stage were mutualistic at a different life-stage and contributed to increased lifetime fitness (Palmer et al. 2010). The same issue continues to be discussed and researched in regards to orchid mycorrhiza, which provide carbohydrate to germinating seedlings and may (Cameron et al. 2006) or may not (Rasmussen and Rasmussen 2009) be repaid by adult plants.

Even if we narrowly focus on cheating in terms of carbohydrate and phosphate transfers, physiological mechanisms may exist that reduce cheating in the AM symbiosis. For example, Fitter (2006) suggested that plants can identify and selectively allocate carbohydrate to regions of locally high phosphate concentration in the root, such as are presumably produced at the arbuscule, the site of phosphate exchange. If the amount of carbohydrate transferred is regulated by the concentration of phosphate in the patch (positive feedback), this mechanism could serve to reduce the likelihood of phosphate cheating by the fungus.

However, as Fitter also acknowledged (Fitter 2006), if a cheat placed itself in close proximity to a beneficial fungus, it might be able to absorb carbohydrate intended for the other fungus without having to transfer phosphate itself. Thus, despite a mechanism such as that proposed by Fitter (2006), phosphate cheating could still exist given certain physical arrangements of the fungi within the root. Visual observations indicate that multiple fungal taxa can occupy the same root fragment (Bennett and Bever 2009). Molecular surveys of field-grown roots suggest that many 0.5 cm root segments are dominated by single taxa (Rosendahl and Stukenbrock 2004), but larger (2.5–5 cm) segments can harbor 2–4 taxa (Aldrich-Wolfe 2007; Clapp et al. 1995), and whole root systems have anywhere from 1 to 15 taxa (Bidartondo et al. 2002;

Lekberg et al. 2013a). Thus, depending on the spatial precision of carbohydrate transfer, some phosphate cheating seems possible even with a phosphate patch mechanism in place.

Given this possibility, is there experimental evidence to suggest that plants reward good mutualists over cheats? Results are mixed. For example, while Kiers et al. (2011) showed that *Medicago truncatula* Gaertn. allocates more carbohydrate to the more cooperative fungus, Bever et al. (2009) indicated that the “superior competitor” fungus, which was the poorer mutualist, could win over the better mutualist (Bever et al. 2009; Bennett and Bever 2009). Visual observations of stained roots by Bennett and Bever (2009) indicated that while the two fungal taxa co-occurring within root fragments occupied different sides of the stele or different layers of the cortex, they were apparently not sufficiently separated to allow the plant to selectively reward the more beneficial one. In contrast, the fungi observed by Kiers et al. (2011) must have been sufficiently spatially separated to allow selective rewards. The ability to discriminate among alternative fungal partners may also differ among plant species because plants differ in their ability to detect phosphate patches (Robinson 1994). Also, plants that are more dependent on AM fungi may be less discriminatory (Grman 2012), and it would be interesting to know whether this has anything to do with the degree of precision with which carbohydrate is delivered to the fungi. Assuming that the trade-off between competitiveness and host growth promotion extends to more taxa than those investigated by Bennett and Bever (2009), it seems critical to learn which plant-AM fungus combinations are susceptible to cheats, as this could determine the nature of the symbiosis.

Positive and negative feedback – can we have both?

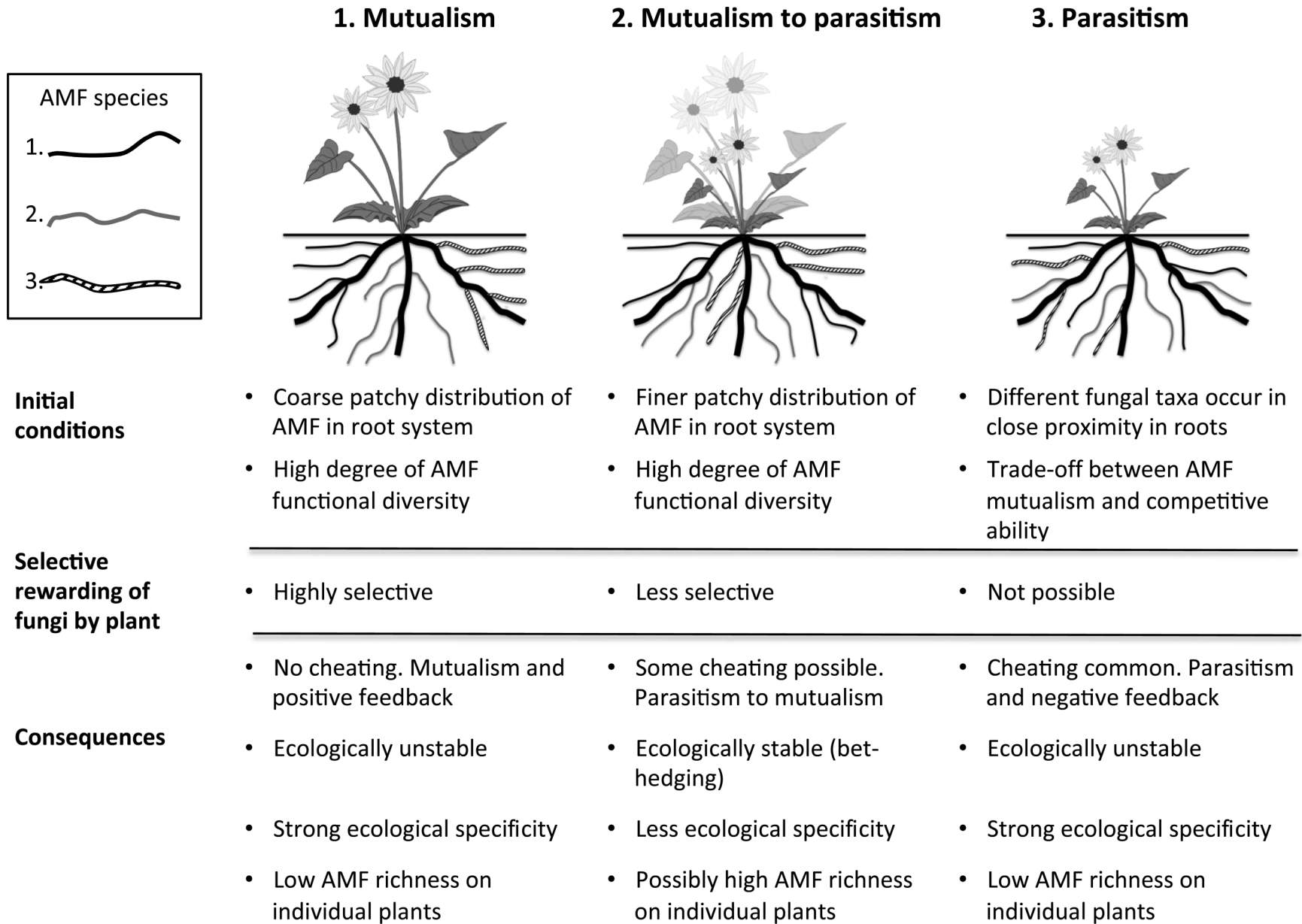
Selective allocation by the plant to the most beneficial AM fungus (Fitter 2006; Hammer et al. 2011; Kiers et al. 2011; Lekberg et al. 2010) is a mechanism that, on the surface, appears to potentially eliminate parasitism (Johnson et al. 1997) and negative feedback, in which the worst mutualist increases in abundance (Bennett and Bever 2009; Bever et al. 2009). We propose three contrasting scenarios in Fig. 3 that may help explain how the nature of AM symbiosis can range from parasitism to mutualism.

Scenarios 1 and 3 represent extreme cases in which the plant’s ability to selectively reward good mutualists is either very high (scenario 1) or very low (scenario 3) because the spatial distribution among co-occurring fungi is either very coarsely patchy (scenario 1) or overlapping (scenario 3). In scenario 1, the best mutualist receives all the carbohydrate, resulting in positive feedback and a mutualism, but only under environmentally stable conditions (not likely in nature). Under less stable conditions the symbiosis might prove to be less than mutualistic because the most mutualistic fungi may not be present. Therefore, we expect scenario 1 to be ecologically unstable in most circumstances.

In scenario 3, the best competitor will receive all the carbohydrate, but via a different mechanism (inherent competitiveness). If the best competitor were not the best mutualist, this would result in negative feedback and possible parasitism. If plant fitness is consequently reduced, we expect scenario 3 also to be ecologically unstable. In both scenarios 1 and 3, we anticipate the AM fungal richness on the individual plant to be reduced over time because either the best mutualist or the best competitor will receive the vast majority of the carbohydrate, although if there is a high degree of functional redundancy among AM fungal taxa, richness may not be strongly affected.

Scenario 2, the intermediate between the two extremes, maintains a greater diversity of partners owing to either plant choice (bet-hedging) or imprecise C allocation within the roots. Because a greater AM fungal functional diversity is supported, we propose that this scenario is ecologically stable under realistic, changing environmental conditions.

Fig. 3. Three hypothetical scenarios of different physical co-occurrences of arbuscular mycorrhizal (AM) fungi and abilities of plants to distinguish and selectively reward better mutualists, and their consequences for AM function, ecological stability, and AM fungal richness. The underlying reasons for these relationships are discussed in the text. AMF, arbuscular mycorrhizal fungi.



There are some indications that both positive and negative feedback operate in the field. Johnson et al. (2010) demonstrated that plants perform best with their “home AMF community”. Helgason et al. (2002) showed that *Acer platanoides* L. associates predominantly with *Glomus hoi* in the field, which was the only fungus that colonized and increased P uptake in this plant under controlled conditions. While these two examples are consistent with positive feedback, negative and positive feedback could both be operating within the same field (discussed in Bever 2002). Overall, to assess whether the AM symbiosis is likely to be mutualistic as a result of positive feedback, or is more likely driven by negative feedback, more studies like the ones above are sorely needed. We also need more studies assessing the small-scale AM fungal colonization patterns within root systems. It is remarkable that the patterns that were so nicely described on stained roots long ago (Abbott 1982; Clapp et al. 1995; Merryweather and Fitter 1998) have remained largely unexplored using appropriate molecular methods (Rosendahl and Stukenbrock 2004).

Do plants cheat?

It is clear that plants differ in their AM dependency and how much carbohydrate they transfer to AM fungi (e.g., Grman 2012; Wilson and Hartnett 1998). Cheating by plants, therefore, may occur when a single fungus colonizes more than one plant and when, for a given amount of phosphate, one plant (the cheat) transfers less carbohydrate than the other. Perhaps the most drastic example of cheating by plants is described by Bidartondo et al. (2002), in which a non-photosynthetic plant delivers no carbon to the fungus but instead obtains reduced carbon from the fungus by consuming its tissue. Since this is a very specific case and may not be representative of the great majority of plant–fungus associations, it will not be discussed in more detail here.

AM fungi may have ways to identify and selectively reward good symbionts. For example, more phosphate was allocated to a host plant that provided more carbohydrate (Lekberg et al. 2010), and when the fungus did not have access to a good host, it accumulated phosphate in spores and hyphae (Hammer et al. 2011). This was also recently shown in a system where adult plants and seedlings were connected via a mycorrhizal network (Merrild et al. 2013). The adult plants, which contributed more carbohydrate to the fungi than seedlings, also received more phosphate, which amplified the asymmetric competition between adults and seedlings in close proximity. Likewise, the benefit from colonization to seedlings appeared to be greatest when they were grown in the absence of an adult plant, because competitive interactions outweighed the benefit from a subsidized fungal network (Pietikäinen and Kytöviita 2007).

In at least one documented case, AM fungi appeared to allocate more resources to the host that provided fewer benefits (Walder et al. 2012). This has the appearance of plants cheating, but significant benefits other than resource transfer, such as overwintering protection (Kabir et al. 1997), are difficult to assess and must also be factored into the cheating equation. Moreover, apparent cheating may also be an artifact of measuring costs and benefits at different, and inappropriate, time scales.

Mechanisms to significantly reduce cheating, either by a plant or by a fungus, are not likely to arise as a consequence of natural selection simply because cheating happens on occasion. There may only be selection against it if it happens frequently enough to result in a net fitness cost to the plant or fungal population, not just the individual. Moreover, what appears to be cheating could actually be a strategy by both plants and fungi to associate with many partners, even those that may temporarily be poor mutualists, to maximize their lifetime fitness. This has been referred to as bet-hedging.

Bet-hedging

Greenhouse experiments are usually characterized as environmentally benign and constant. Under such conditions, a single isolate of mycorrhizal fungus may be the most beneficial to the host. In nature, however, soil moisture, nutrient availability, pathogen and herbivore pressure, local plant density, the amount of light available to the plant, etc., are in constant flux. Different fungal species and families differ in their growth patterns (Hart and Reader 2002), which may result in different abilities to provide benefits. For example, the extensive extraradical mycelium of many members of the Gigasporaceae may be advantageous for phosphate acquisition, whereas the greater intraradical colonization by members of the Glomeraceae may help provide pathogen protection (Maherali and Klironomos 2007; Sikes et al. 2009). From the fungal perspective, the phenology of root production varies with plant species, as do a number of other traits that may influence their quality as hosts. Given this variation, and because the degree of benefit both partners experience is context-dependent (Hoeksema et al. 2010; Koide 1991), bet-hedging may be advantageous. Bet-hedging is a strategy favored in varying and unpredictable environments that sacrifices mean fitness to decrease variation in fitness (Stearns 1976). For the AM symbiosis, this may mean that plants and fungi support multiple partners despite some of the partners being significantly less beneficial than others under the current circumstances.

Although bet-hedging is a phenomenon that is best investigated in natural communities, one can find results that are consistent with bet-hedging even under controlled conditions. For example, Lekberg et al. (2010) found that while the fungus allocated more phosphate to the host that provided more carbohydrate, AM colonization did not differ between the good and poor hosts (although the abundance of arbuscules was higher in the good host). One interpretation of these results is that selection has favored fungi that optimize their likelihood of acquiring carbohydrate across space and time by colonizing multiple plants. Also, perhaps the biggest surprise in the research of Kiers et al. (2011) was not that the better phosphate-transferring fungus received more carbohydrate than the worse phosphate-transferring fungus, but that the latter received as much carbohydrate as it did. From a bet-hedging perspective, it is possible that selection has favored plants that associate with fungi that range in the benefits they provide. For example, the worse phosphate-transferring fungus may provide other benefits that become more important under different conditions (as carefully pointed out by Kiers et al. 2011). For example, the behavior of the fungus they termed a “hoarder” may serve to store phosphate during winter months when plants are inactive (Merryweather and Fitter 1998), thus preventing loss of important resources from the system.

When interpreting results from greenhouse experiments, we need to be aware that greenhouse conditions offer selective pressures and filtering mechanisms that are quite different from those found in nature, that they are incapable of revealing all the consequences of mycorrhizal colonization, and that they may produce outcomes from mycorrhizal colonization that do not frequently occur in natural settings. This may be especially true for long-lived plants, which might reasonably be expected to experience an even wider range of circumstances in nature than short-lived plants.

It is also important to point out that bet-hedging should not simply be inferred as an explanation when no other strategies are observed. It is quite possible that some fungi simply cannot distinguish good hosts from bad, and that some plants cannot distinguish beneficial fungi from less beneficial ones. To unequivocally demonstrate bet-hedging, we would need to show that temporarily poor mutualists contribute to plant fitness in the long term (similar to the plant–*Acacia* system described by Palmer et al.

2010), and not just for a single plant but for the entire population of plants.

Partner choice

While bet-hedging may favor associations between many different plants and fungi that provide a range of benefits, non-random distributions, or “ecological specificity” (McGonigle and Fitter 1990; Zhu et al. 2000), among plants and fungal taxa are often observed in surveys of natural communities. These are not necessarily inconsistent with bet-hedging because bet-hedging can also be non-random. For example, if the fungal species that are preferentially colonizing the root happen to comprise a mixture of fungi that transfer phosphate and inhibit pathogens, then both ecological specificity and bet-hedging may occur. We do not discuss strict genetic specificity here because it does not appear to exist in the AM symbiosis.

Ecological specificity is often referred to as host preference, which may be a misleading term because it implies that plants, not fungi, choose their partners and that those partners are good. Based on earlier discussions (fungal cheats), it seems equally plausible that the most abundant fungus is as good a competitor as it is a mutualist. Nonetheless, here we refer to ecological specificity resulting from positive feedback as partner choice, and we predict that this relationship is favored under the following four conditions (modified from Hoeksema 1999): (1) large variation in benefits conferred by potential partners, (2) an ability to distinguish among alternative partners, (3) stability of benefits in time and space, and (4) a predictable co-occurrence between plants and fungi across time and space. Condition 1 appears to exist. Greenhouse experiments, at least, demonstrate the potential for different plant–fungus combinations to generate large differences in benefit to either partner (Bever et al. 1996, 2009; Klironomos 2003; Smith et al. 2000). Obviously we need to be cautious here. The large differences that are observed occur under particular circumstances. If the circumstances change, the rankings among the fungal taxa may change (see bet-hedging, above).

Controlled environment experiments lend some support to condition 2; both plants and fungi appear to be able to distinguish and selectively reward better symbionts (e.g., Hammer et al. 2011; Kiers et al. 2011; Lekberg et al. 2010), although this ability may differ among plant species, depend on the spatial distribution of co-occurring fungi (Bever et al. 2009), and be moderated by bet-hedging. One could argue that condition 3 (stability of benefits in time and space) also exists, at least at some level. For example, the different growth patterns (Hart and Reader 2002) and functional diversity among fungal families (Maherali and Klironomos 2007; Sikes et al. 2009) may be functionally complementary with certain root traits (Koide 2000). Indeed, a coarse-rooted plant was shown to benefit most from a fungus that provisioned phosphate, while a plant with a finely branched root system benefited most from a fungus that protected it against pathogens (Sikes et al. 2009). This functional complementarity may explain why there appear to be no universally parasitic AM fungi; one that is a poor mutualist on one plant may be a good mutualist on another (Klironomos 2003, but see Smith and Smith 1996). This also suggests that differences in fungal communities are more likely to occur among plants with functionally distinct root systems (but see Sykorova et al. 2007). Indeed, an annual, shallow-rooted invasive grass and a perennial, deep-rooted invasive forb harbored the most dissimilar AM fungal communities (Lekberg et al. 2013a). Tracking relationships between root architecture and field distributions of AM fungal taxa could be very informative, although other traits may also prove to be important. On the other hand, condition 3 does not seem highly probable because environmental conditions in the field are constantly changing. Thus for any particular host, the most beneficial fungal taxon may change repeatedly during

the host’s lifetime (Husband et al. 2002) and (or) across seasons (Dumbrell et al. 2011).

Condition 4 (predictable co-occurrences between plants and fungal taxa) probably occurs in some — but not all — situations, and may depend on the relative differences in edaphic conditions among the sites included, because they likely exert a stronger habitat filter than host plant identity (Dumbrell et al. 2010a). For example, consistent patterns in host preference were recently described for *Lolium perenne* L. and *Trifolium repens* L. across numerous grasslands in Ireland (Hazard et al. 2013), but in a comparison of thermal and nonthermal grasslands in Yellowstone National Park, USA, some plant communities responded strongly to soil temperature, while the AM fungal communities responded mainly to pH (Lekberg et al. 2011).

Given that the four conditions occur in at least some circumstances, it is not surprising to find evidence for partner choice (Husband et al. 2002; Vandenkoornhuysen et al. 2003). It may be, however, that ecological specificity operates on the basis of function rather than taxonomic identity. For example, Öpik et al. (2009) found differences in fungal communities between forest specialists and generalists. Also, while partner choice was not evident among highly colonized forb species in a Danish grassland (Stukenbrock and Rosendahl 2005), fungal communities differed significantly between *Dianthus deltooides* L., a species often considered to be nonmycorrhizal, and one of the highly colonized forbs (Y. Lekberg, unpublished data). Thus, taxonomic specificity may only occur when functional variation corresponds reliably to taxonomic variation.

Coupling physiological processes and community patterns

Most studies involving physiological processes in the AM symbiosis are performed under controlled conditions, and there is seldom an attempt to assess their relevance in natural ecosystems. Likewise, many community ecology studies describe patterns in the field without much discussion of underlying mechanisms. We would learn much from coupling physiological mechanisms to ecological patterns through interdisciplinary collaborations, a plea that was made more than a decade ago by David Read (Read 2002) and more recently for the ectomycorrhizal symbiosis (Koide et al. 2011). For example, many rank abundance distributions show a few very abundant and many rare fungal taxa in communities (Dumbrell et al. 2010b). Knowing whether the most abundant taxa are the best mutualists or superior competitors, or whether they are simply those that are best adapted to the physical conditions of the environment, would help us better understand AM fungal function in the field.

There are a few exceptions in the literature in which physiological processes and community patterns have been coupled, and these have been informative. Helgason et al. (2002) combined inoculation experiments on local AM fungal isolates and plant species with field surveys and concluded that *A. platanooides* is colonized by the best mutualist in the field (indicative of positive feedback). In contrast, intensive work by Bever’s group in a North Carolina grassland showed that both negative and positive feedback may operate within the same community (Bever et al. 1996, 2001, 2009; Bever 2002). Also, by combining field surveys and inoculation experiments, Lekberg et al. (2007) showed that the field distribution of Gigasporaceae and Glomeraceae was driven by restrictions in their fundamental and realized niches.

Methods continue to be developed (e.g., Allen and Kitajima 2013; Whiteside et al. 2009), allowing for more sophisticated functional studies in the field. However, we already have a suite of underutilized tools available to us. For example, stable isotope probing allows for both seasonal and spatial tracking of plant C flow to AM fungi (Drigo et al. 2010; Lekberg et al. 2013b; Olsson and Johnson 2005), and P-isotope labeling can estimate P allocation

patterns and reciprocal reward from AM fungi to plants (e.g., Chiariello et al. 1982; Lekberg et al. 2010; Smith et al. 2004). The use of fungicides, although problematic, has been informative (Helgason et al. 2007), as have in-growth cores that allow for in situ functional studies of AM (Johnson et al. 2001).

To conclude, we believe that AM research will benefit from a broader view of the symbiosis that includes services other than resource transfers, and from explicit consideration of physiological, community, and evolutionary processes. It is our sincere hope that this article will make a contribution toward this end.

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