
More than a Carbon Economy: Nutrient Trade and Ecological Sustainability in Facultative Arbuscular Mycorrhizal Symbioses

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Research review

More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses

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Summary

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Symbiosis is well recognized as a major force in plant ecology and evolution. However, there is considerable uncertainty about the functional, ecological and evolutionary benefits of the very widespread facultative arbuscular mycorrhizal (AM) associations, in which the plants can grow and reproduce whether or not they are colonized by AM fungi. Here we address the significance of new research findings that are overturning conventional views that facultative AM associations can be likened to parasitic fungus–plant associations. Specifically, we address the occurrence and importance of phosphate uptake via AM fungi that does not result in increases in total phosphorus (P) uptake or in plant growth, and possible signalling between AM fungi and plants that can result in plant growth depressions even when fungal colonization remains very low. We conclude that, depending on the individual AM fungi that are present, the role of facultative AM associations in the field, especially in relation to plant competition, may be much more subtle than has been previously envisaged.

Introduction: reviewing the current paradigms

The importance of symbioses – both mutual and antagonistic – in the lives of organisms has been increasingly appreciated over the many years since the term was introduced by de Bary (1879). The range of scales involved is enormous, spanning evolution of eukaryotes from prokaryote ancestors, and transition of multicellular plants to life on land as a consequence of associating with arbuscular mycorrhizal (AM) fungi. Nevertheless, the significance of symbioses has often been

disregarded in broader contexts and as recently as 1991 David Smith wrote: ‘despite its all-embracing importance as a phenomenon, symbiosis usually receives no more than lip service from most mainstream biologists, whose ideas about it are usually naïve and half a century out of date’ (Smith, 1991). Part of the problem lies in the increased complexities that consideration of symbioses introduces into already complex research areas. Those symbioses that are obligate are accepted as ‘normal’, as with lichens, corals, etc., and so are symbioses between eukaryote plants and nitrogen (N)-fixing bacteria,

despite the fact that addition of N-fertilizer can make the last artificially facultative (i.e. the plants can then complete their life-cycle when nonsymbiotic). Other symbioses that are apparently facultative are more easily glossed over, as with some AM associations, despite the fact that arbuscular mycorrhizas are the normal nutrient-absorbing organs of most vascular plants. Nevertheless, progress is being made: at the field scale many plant ecologists are making serious efforts to understand how soil–root interactions, including mycorrhizal fungal symbionts, influence plant interactions in ecosystems. Despite the fact that AM associations in many important crop plants seem to be facultative, some agronomists are also turning their attention to mycorrhizas at this time of rapidly increasing phosphorus (P)-fertilizer costs and diminishing supplies of high-quality rock phosphate. The tasks of both groups are made difficult because knowledge of how mycorrhizas function is still imperfect. Experimental investigation of AM associations has largely been based on short-term experiments encompassing the vegetative stages of growth and nutrient uptake of single plants in pots. In consequence, concepts of the trade-off in exchange of resources, in this case photosynthetic carbon (C) for soil-derived nutrients, particularly P, and outcomes in terms of supposed benefit to the plants, have not extended to the complete life cycles of the plants. Thinking has also frequently been constrained by generalized concepts established relatively early in AM research, which are now being re-examined and found to require considerable revision. Understandably, it is the conventional concepts that are first applied by nonspecialists in broader contexts of plant ecology and agronomy.

One particular conundrum that has faced researchers interested in the forces influencing the evolutionary persistence of AM associations is the very considerable functional diversity in the outcomes at the whole-plant level (Johnson *et al.*, 1997; Jones & Smith, 2004). Some associations do result in increased plant nutrient uptake and growth, but others do not. In the latter cases (called ‘unresponsive’ or ‘nonresponsive’) it has been very difficult to envisage any evolutionary benefits of the plant–fungus associations that result from P acquisition by the fungi. Why then have such apparently nonbeneficial associations persisted? One possible explanation is that formation of AM symbiosis has benefits beyond increased growth and nutrient uptake, encompassing tolerance to pathogens or drought (Newsham *et al.*, 1995). These may, indeed, make contributions to improving ‘fitness’ of AM plants. However, new insights into the quantitative contributions of AM fungi to plant P uptake at the physiological and molecular levels, as well as into the functional basis of ‘growth depressions’ in AM plants, are improving understanding of the extent of mutualism in the associations. Here we bring together new knowledge to explore the ‘balance of trade’ in AM symbioses, and hence increase understanding of roles in ecosystems and evolutionary persistence of these very widespread symbioses. We focus particularly on plants that are AM-unresponsive and those that show growth depressions when AM.

Functional diversity in AM associations

It is now well recognized that change in plant growth resulting from formation of AM associations (i.e. the ‘mycorrhizal growth response’, MGR) varies widely among plant species and even varieties when soil P-availability limits growth. Some plants ‘typically’ show large positive MGR, while others ‘typically’ show no MGR or even negative MGR (Tawaray, 2003; Smith & Read, 2008). ‘Typically’ is in inverted commas because most results are obtained in pot experiments in which single plants are inoculated with single AM fungi, with growth under controlled or semicontrolled conditions. Wide ranges of MGR occur both in wild and agricultural plants, and there is no strong evidence that plant breeding has been a major influence in the latter. However, the extent to which results can be extrapolated to the real world of the field is often uncertain. The problem with field studies lies in obtaining realistic nonmycorrhizal (NM) controls – remembering that these are the artificial state – and discounting confounding factors such as suppression of plant root disease or release of nutrients or toxins that can arise from soil sterilization or fumigation and so influence MGR. These factors can be prevented or minimized in glasshouses and growth-rooms where, for example, many wild grasses and varieties of cereals such as barley and wheat – but by no means all – show low positive, zero or negative MGR (Azcón & Ocampo, 1981; Hetrick *et al.*, 1992, 1996; Ravnskov & Jakobsen, 1995; Graham & Abbott, 2000; Zhu *et al.*, 2003; Li *et al.*, 2008; and references within these articles). Their survival in low-P soil when they are NM obviously must depend on properties of the plant genome alone, such as ability to take up P efficiently owing to adaptations such as long, fine roots and long root-hairs and extrusion of solutes that increase P availability (Koide, 1991a; Jakobsen *et al.*, 2002; Janos, 2007). By contrast, other plants can be highly (positively) responsive in low-P soil: good examples are legumes such as species of *Trifolium* (clover) and *Medicago* (medic). In effect these are obligately AM because of very poor P uptake and growth when NM. This results mainly from properties of root systems that are the converse of those in unresponsive plants (i.e. low ratios of root–shoot weight and root length–root weight, and lack of lengthy root hairs) (Manjunath & Habte, 1991; Jakobsen *et al.*, 2002). In such plants the influence of individual AM fungi in promoting positive MGR in low-P soil is imposed via individual fungal–plant genomic interactions, in particular those involved in symbiotic ‘P trade’ (P uptake via the fungus) and ‘C trade’ (plant C supply to the fungus). However, different AM fungi can influence MGR even in relatively unresponsive plants, as shown by Burleigh *et al.* (2002) with 10 AM fungi in combination with *Medicago truncatula* (positively responsive) or a variety (76R) of *Solanum lycopersicum* (tomato) that shows relatively low MGR. Environmental factors (light intensity, temperature, available soil P, soil pH, etc.) will also influence outcomes in terms of MGR, whether positive, zero or negative. The outcomes can

arise from effects on the plant, and/or AM fungus, and on their interactions, as discussed by Smith & Read (2008: see their Chapter 4). They illustrate diagrammatically (their Fig. 4.1) the two potential pathways for P uptake by AM plants: (1) the AM pathway involving uptake by extensive external fungal hyphae, with translocation and release into the root, and (2) direct uptake through root hairs and epidermis, as in NM roots.

The choice of the AM fungus used to inoculate the plant is increasingly known to be important in relation to MGR. Smith *et al.* (2003, 2004) followed up Burleigh *et al.* (2002) by comparing three AM fungi and three plant hosts, again in low-P soil. Fungal colonization became well established in all combinations. In terms of weight, two AM fungi (*Glomus caledonium* and *Glomus intraradices*) caused large but different positive MGR in flax (*Linum usitatissimum*) and medic but none in tomato; the other (*Gigaspora rosea*) caused a relatively small positive MGR in *Linum usitatissimum* (flax) but no response in medic and a depression in tomato. Effects on P uptake per plant were very similar to those on growth. It can be argued that *G. rosea* (also used by Burleigh *et al.*, 2002) was a poor choice because it might not be well suited to the growth conditions used (e.g. the soil type, pH, etc.). Nevertheless, the results clearly demonstrated considerable AM functional diversity under the same experimental conditions. Graham & Abbott (2000) grew wheat with 10 different AM fungi, all isolated from wheat-belt soils and all caused growth depressions. Munkvold *et al.* (2004) showed that different effects of AM fungi occur even at intraspecific level. In more ecologically oriented work individual AM fungi isolated from natural plant ecosystems demonstrate functional diversity when partnered with individual plants with which they co-occur. Thus van der Heijden *et al.* (1998) and Klironomos (2003) showed that different AM fungi in partnership with the same plant species gave differences in MGR, which ranged from positive to negative. However, different plant species responded differently to the individual AM fungi. By contrast, Pringle & Bever (2008) found that differences in MGR caused by individual AM fungi occurred consistently across a range of plants, both in the laboratory and in the field. They used preinoculated plants in the field to minimize confounding factors arising from the AM fungi already present, thus minimizing the problem of having no NM 'controls'. These results all need explanation in functional terms.

Although explanations for lack of positive MGR seem relatively simple – if the question of what the balance of P and C trade in a facultative AM association actually involves is ignored – plant growth depressions in low-P soil are another matter. Over the years they have been explained in terms of competition for soil P between AM fungus and plant (Crush, 1973), C drain to the fungus that exceeds benefits in terms of its P supply to the plant (Tinker, 1975: still the favourite explanation) or both (Bethlenfalvay *et al.*, 1982a,b). Despite past suggestions (Koide & Schreiner, 1992), these growth

depressions cannot by any means now be ascribed solely to artefacts under controlled conditions (e.g. low light that limits photosynthetic C supply). This is not to say that low light cannot lower MGR or cause growth depressions: it can, as shown by many 'shading' experiments (Graham *et al.*, 1982; Son & Smith, 1988; Smith & Gianinazzi-Pearson, 1990; Facelli *et al.*, 1999). Even where growth depressions can arise because of choice of 'unsuitable' AM fungi or experimental conditions, their causes are unresolved.

Costs and benefits; causes and effects

As already indicated, a simple cost–benefit approach is generally used when analysing mycorrhizal growth responsiveness. Ideally, such an analysis should be based on a single resource, which Fitter (1991) suggested should be C, so that a growth depression arises if the C cost to the plant exceeds the benefit in C that arises from increased P uptake resulting in increased photosynthesis. There is no C cost to the fungus because it can only gain C from the plant. On this basis, P supply via the fungus is regarded as a causal intermediary in the plant's net C benefit and hence growth. More recently, Fitter (2006) has proposed that the plant will only give large amounts of C to an AM fungus if it receives P from it (i.e. the fungi cannot act as parasites ('cheaters')); again, this puts P trade first in the cause–effect relationship. He further proposed that the C trade and P trade are spatially separated between intercellular cortical hyphae and arbuscules respectively, as suggested by Gianinazzi-Pearson *et al.* (1991). Such separation does not seem fundamental to his cause–effect argument, and it could not occur in *Paris*-type AM, which have no such intercellular phase. Although Fitter's model does not allow cheating by individual AM fungal taxa in association with individual plants, the occurrence of plant growth depressions has led to the view that cheating does occur and must be taken into account in understanding the basis of functional diversity in AM associations (Johnson *et al.*, 1997).

The alternative basis for the cost–benefit analysis is P itself, as analysed by Koide (1991a) in terms of P supply and demand. On this basis, growth depressions would arise if for any reason P supply to an AM plant per unit time via an AM fungus (or via the AM roots and fungus combined) was less than P supplied directly via the roots of the equivalent NM plant. In terms of P, there is certainly a cost to the fungus (i.e. P lost to the plant). Again on this basis, AM fungi that, contrary to Fitter's (2006) model, obtain C from the plant but trade little or no P in return could be at an evolutionary advantage over 'beneficial' AM fungi, given that the P uptake and transfer along hyphae (probably as polyphosphate) are not energetically cheap, even if P loss to the plant is. Accordingly, from a mycogenic viewpoint, cheating would be expected to be relatively common, as also argued by others on more general grounds (Egger & Hibbett, 2004; Kiers & van der Heijden, 2006).

Table 1 Phosphorus (P) uptake via the arbuscular mycorrhizal (AM) pathway into plants that in the same experiments showed little or no mycorrhizal growth response (MGR) in terms of dry weight

Plant	AM fungus	MGR (%)	% Total P via AM pathway	Reference
<i>Cucumis sativus</i> (cucumber)	<i>Glomus caledonium</i>	9	c. 100	Pearson & Jakobsen (1993)
		9	c. 100	Ravnkov & Jakobsen (1995)
	<i>Glomus invermaium</i>	15	c. 20	Pearson & Jakobsen (1993)
		-7	c. 0	Ravnkov & Jakobsen (1995)
	<i>Scutellospora calospora</i>	10	c. 7	Pearson & Jakobsen (1993)
<i>Bromus inermis</i> (bromegrass)	<i>Glomus intraradices</i>	-12	Not quantified	Ravnkov <i>et al.</i> (1999)
	<i>Glomus etunicatum</i>	-10	Not quantified	Hetrick <i>et al.</i> (1994)
<i>Triticum aestivum</i> (wheat)	<i>Glomus caledonium</i>	-8	c. 100	Ravnkov & Jakobsen (1995)
	<i>Glomus invermaium</i>	0	c. 0	Ravnkov & Jakobsen (1995)
	Two mixed spp. ^b		Not quantified	Hetrick <i>et al.</i> (1996)
	Unknown (field)	?	Not quantified	Schweiger & Jakobsen (1999)
	<i>Glomus intraradices</i>	0 to -1 ^a	c. 50–60 ^a	Li <i>et al.</i> (2006)
<i>Pisum sativum</i> (pea)	Three mixed spp. ^c	0	Not quantified	Gavito <i>et al.</i> (2002)
<i>Solanum lycopersicum</i> (tomato)	<i>Glomus caledonium</i>	0	c. 20–30	Smith <i>et al.</i> (2004)
	<i>Glomus intraradices</i>	-10	c. 90–100	
	<i>Gigaspora rosea</i>	-30	0? (see text)	
<i>Hordeum vulgare</i> (barley)	<i>Glomus intraradices</i>	-28	Not quantified	Zhu <i>et al.</i> (2003)
		-45 ^d	c. 41 and c. 55 ^d	Grace <i>et al.</i> (2009)

MGR has been calculated from the original data as $100 (AM - NM) / NM$, where AM and NM are mean DWs of AM and NM plants, respectively. Values for P uptake via the AM pathway were obtained (where possible) by the original or present authors, by extrapolating ^{32}P or ^{33}P uptake from hyphal compartments to the whole pot.

^aFour P treatments; ^btwo mixed spp.: *G. etunicatum* and *G. mosseae*; ^cthree mixed spp.: *G. intraradices*, *G. caledonium* and *G. claroideum*;

^dtwo P treatments.

Whatever basis is used for the cost–benefit analysis, it has to be remembered that the real cost to an AM plant may be trivial if photosynthesis can cope with the fungal C demand. Further, root–shoot weight ratios can decrease because the fungus is supplying the limiting nutrient (P); here a decrease in root biomass compared with an NM plant represents a saving in C that is transferred to the fungus. Also, it is well recognized that costs and benefits can change during plant growth (e.g. depressions often occur at early growth stages, and the plants recover later) (Bethlenfalvay *et al.*, 1982a,b; Pearson & Jakobsen, 1993; Li *et al.*, 2005). These points aside, it follows from the conventional cost–benefit approach that growth depressions are inevitably considered to be deleterious to the plant – they seem to reduce ‘fitness’ (e.g. in competing with plants that show high or no MGR), even if only temporarily. They are at the far end of the positive-to-negative MGR spectrum (Francis & Read, 1995; Johnson *et al.*, 1997; Egger & Hibbett, 2004) and the same ‘poor fitness’ argument arises if a plant that can be very positively AM-responsive is colonized by an AM fungus that produces little or no positive MGR.

Rethinking the balance of trade in AM-unresponsive plants: ‘hidden’ P uptake

Equating lack of positive MGR and development of growth depressions (negative MGR) with substantial cheating assumes

by definition that the AM fungus supplies little or no P to its host. This assumption inevitably arises from a general belief that direct P uptake by the roots and uptake via the fungus are additive. Conversely, a positive MGR is thought to be a straightforward P trade ‘bonus’ produced by the fungus. However, there are now many experiments showing transfer of radioactive P (^{32}P or ^{33}P), supplied as orthophosphate, into unresponsive plants from compartments accessible only to AM fungal hyphae (HCs). Using three different AM fungi, Pearson & Jakobsen (1993) compared uptake into cucumber of ^{32}P from HCs with uptake of ^{33}P from compartments accessible to both roots and hyphae (RHCs). They calculated that *G. caledonium* provided all of the plant’s P, showing that root P uptake was completely inactivated. By contrast, *Glomus invermaium* (*G. sp.* WUM10’) provided c. 20% and *Scutellospora calospora* < 10% of total plant P. The MGR was very small with all fungi and occurred only in shoot biomass. Table 1 shows this and other examples where the plants showed low positive, no or negative MGR. Fungal colonization was well established in all examples shown, and in all cases except one (Zhu *et al.*, 2003) P concentrations in NM and AM plants were very similar, so that values of MGR are very similar to those for responsiveness in terms of total P uptake. In the case of Zhu *et al.* (2003) the AM plants had higher P concentrations than the NM plants, but taking into account the growth depressions the total P contents in AM and NM plants were the same. Hetrick *et al.* (1996) compared the

unresponsive wheat cultivar Newton with the highly AM-responsive cultivar Turkey (the latter is not in Table 1). Cultivar Newton took up much more ^{32}P via the AM pathway than cv. Turkey. This difference showed that 'mycorrhizal function is not impaired even in cultivars that do not display a biomass increase in response to mycorrhizal symbiosis' (Hetrick *et al.*, 1996). An extreme case in Table 1 where AM fungal hyphae took up no detectable tracer P from HCs was an experiment with cucumber and wheat colonized by *G. invermaium* (Ravnkov & Jakobsen, 1995): this fungus was then apparently acting as an 'absolute' cheater rather than a very inefficient AM fungus, as had been the case in the experiment by Pearson & Jakobsen (1993). However, it transferred ^{32}P to flax (AM-responsive; not shown in Table 1), and *G. caledonium* transferred P to all three hosts. Other examples where there was extensive tracer P transfer from HCs into plants that showed relatively high positive MGR (not shown in Table 1) include medic and (again) flax (Smith *et al.*, 2003, 2004), cucumber (Munkvold *et al.*, 2004) and tomato (Poulsen *et al.*, 2005). The cultivars of cucumber and tomato were the same as those that showed little or no MGR in the experiments in Table 1.

It has to be stressed that there are dangers in extrapolating transfer of tracer P by AM fungi from HCs to fungal P uptake in the main compartment (pot). Obviously, if the hyphae are dense around the roots in the main compartment but do not enter the HC at all, lack of tracer P in the plant does not mean that there is no hyphal P uptake in the main compartment. Similarly, if AM colonization is slow and the hyphae reach the HC slowly, tracer P transfer may underestimate P uptake via the AM fungus in the main pot. Tracer P uptake and hyphal length densities need to be measured at different times to cope with this problem, as was done in most of the experiments in Table 1 where P uptake by the AM pathway was quantified. Very few hyphae of *G. rosea* entered the HCs in the experiment carried out by Smith *et al.* (2003, 2004), so that low ^{33}P transfer by *G. rosea* did not itself mean that this fungus was acting as a cheater. Much more compelling was the fact that, unlike the other two AM fungi, *G. rosea* produced no positive MGR in medic (also shown by Burleigh *et al.*, 2002) and only a small positive MGR in flax; *G. rosea* is included in Table 1 for this reason. Alternatively, if the HC is a more favourable environment for hyphal P uptake than the main compartment (e.g. owing to lack of competition by roots), tracer P transfer from HCs may overestimate the contribution of the AM pathway in the pot as a whole. These issues were addressed by Pearson & Jakobsen (1993), Ravnkov & Jakobsen (1995) and Joner *et al.* (1995). It was overall similarity between the ^{33}P -specific activities in the AM-responsive medic and flax and the unresponsive tomato variety that persuaded Smith *et al.* (2003, 2004) that P uptake into tomato via the hyphal pathway could be substantial, and that similar previous findings (Table 1) deserved more recognition.

Even allowing for variations in different experiments, it now has to be recognized that the unresponsive AM plants and the corresponding NM plants can be functionally very different in terms of mechanisms of P uptake, in that direct uptake by the AM roots can be reduced to an extent that depends on the individual AM fungus. We call the P influx via the AM pathway 'hidden' because it is not evident as a contribution to total P uptake. Extrapolating from the findings summarized in Table 1, we believe that the default situation for unresponsive plants that grow in association with populations of AM fungi both in natural and agricultural ecosystems is that there will be some hidden P uptake – the question is how much?

Aside from the tracer P work, expression of AM-inducible transporters for P (as orthophosphate) is impressive evidence for operation of the AM uptake pathway irrespective of responsiveness (Rausch *et al.*, 2001; Glassop *et al.*, 2007). In some cases there is apparently no complementary downregulation of epidermal P transporters (Karandashov *et al.*, 2004; Nagy *et al.*, 2005; Poulsen *et al.*, 2005; Grace *et al.*, 2009), but in others there is (H. Liu *et al.*, 1998; Burleigh *et al.*, 2002; Harrison *et al.*, 2002; Paszkowski *et al.*, 2002; Glassop *et al.*, 2005; H. Christophersen, unpublished). This difference is not necessarily a problem, because a young plant exposed to an AM fungus is likely to take up some P before it becomes colonized, with the rate of colonization depending on inoculum potential. This is likely to be an ongoing situation in any developing root system where P-absorbing regions close to root tips may be always NM (Smith *et al.*, 1986). Also, it has to be remembered that the degree of P transporter gene expression cannot necessarily be correlated with the P flux (i.e. amount of P transferred per unit time per unit plant biomass). Expression of epidermal P transporter genes is likely to be very high when the plant is scavenging for P at very low concentrations externally, even though the P flux is likely to be low, as found by C. Liu *et al.* (1998) with NM tomato. All that can be safely concluded is that if there is no P transporter gene expression at a given location there is no P uptake there via that transporter.

In this context the effects of large P depletion zones that develop around NM roots growing in low-P soil certainly have to be considered. As these zones develop the roots would be expected to increase expression of epidermal P transporter genes to maximize P uptake. An analogous high expression in culture solution with low P was shown with NM tomato (C. Liu *et al.*, 1998). However, when the AM uptake pathway comes to dominate P uptake from soil, the epidermal uptake pathway may become so ineffectual in scavenging P-depleted rhizospheres that downregulation of expression (or operation) of epidermal P transporters occurs as a consequence of fungus-plant signalling. The difficulty in untangling these possibilities in AM plants is that P transporter gene expression is most often measured on a 'whole-root system' basis. Unlike those in NM roots, differences in P uptake pathways in different parts of AM roots have received no attention, (Daram *et al.*, 1998; Gordon-Weeks *et al.*, 2003).

Rethinking growth depressions – not only caused by the ‘C trade’?

It seems intuitively obvious that high C costs in relation to net P benefits are the main cause of growth depressions when highly colonized roots are growing in low-P soil. Depressions can also occur at high soil P, and it is usually argued that the AM uptake pathway gives P benefits that are trivial compared with those from direct uptake, so that the depressions might again reflect the underlying C demand of the AM fungi (Johnson *et al.*, 1997). However, Mosse (1973) suggested that the cause of such depressions is P toxicity in the plant caused by high P uptake through the roots. Others have associated them with changes in root carbohydrates that are caused by increases in plant P status (Thomson *et al.*, 1986; Peng *et al.*, 1993). It is not clear how much the depressions at high soil P help with understanding those that occur at low soil P, especially taking into account that in the former case different (low-affinity) epidermal P transporters would be expected to operate; their molecular biology is a mystery as far as we are aware. It has to be borne in mind that decreasing fungal biomass per plant at high soil P (where this occurs) would be expected to offset growth depressions if these are caused by C drain to the fungus.

The simple intuitive explanation that growth depressions at low soil P are caused only by excessive C drain to the AM fungi becomes very unlikely when very large depressions occur with very low per cent colonization or (more convincingly) with very low total fungal biomass per plant. Such cases are scattered through the literature, of which the most famous – or infamous – is the tobacco stunt disease caused by *Glomus macrocarpum* (Modjo & Hendrix, 1986). Graham & Abbott (2000) showed that the range of growth depressions in wheat was similar whether the AM fungi were ‘aggressive’, resulting in rapid extensive colonization or ‘nonaggressive’, resulting in slow and less extensive colonization. They concluded that the negative MGRs at low P could not be linked to C drain to the fungi. Interestingly, they found that growth depressions were also caused by both groups of fungi with high soil P – again indicating that high C drain to the fungi is not an adequate explanation. Li *et al.* (2008) found very large growth depressions in wheat by *Gigaspora margarita* at very low biomass, but smaller or no depressions by *G. intraradices* at much higher biomass. The suggested explanation was inhibition of the direct P uptake pathway, as can occur when colonization is extensive (see above), but now with delivery of only small amounts of P via *G. margarita* and a consequent reduction in growth. Grace *et al.* (2009) similarly showed that large growth depressions in barley caused by *Glomus geosporum* were similar to those caused by *G. intraradices*, even though colonization by *G. geosporum* was very low (2% and 20% at 4 wk and 6 wk, respectively) compared with colonization by *G. intraradices* (61% and 72% at 4 wk and 6 wk, respectively).

Inhibition of P uptake via the epidermis may also explain the surprisingly large growth depression with negligible

colonization by *Glomus versiforme* in a mutant of tomato 76R that in general shows reduced mycorrhizal colonization (*rmc*) by AM fungi (Poulsen *et al.*, 2005). There was no growth depression in *rmc* with *G. intraradices* WFVAM 23, the only AM fungus known to colonize *rmc* extensively (Gao *et al.*, 2001), and which transferred ^{32}P to *rmc* from HCs, or with *G. intraradices* BEG 87, which showed negligible colonization and no transfer of ^{32}P (Poulsen *et al.*, 2005). There were no growth depressions at all in the wild-type, which was well colonized by all three AM fungi, with substantial (though different) amounts of ^{32}P uptake from HCs. Only when there was ^{32}P transport via the AM pathway in either the wild-type or *rmc* were AM-inducible P transporter genes expressed. Inactivation of epidermal P uptake was suggested by Neumann & George (2005) as one possible explanation for very large growth depressions in *rmc* that arose from surface colonization by a mixed inoculum of *G. mosseae* and *G. intraradices* when the inoculum was AM propagules in the presence or absence of living roots of a wild-type tomato (not 76R). The other suggested explanation was activation of defence responses leading to cellular dysfunction. This would be a case of AM ‘antagonism’ (Francis & Read, 1995).

It can be argued that where AM colonization is low the cause of the reduced direct P uptake is that the initial colonization (or attempt to colonize) includes a signal (from AM fungus or plant) telling the plant to ‘expect’ P transfer via the AM pathway. The plant then switches off or decreases the direct uptake pathway but, because colonization becomes very limited, little or no P is then delivered by the fungus, so plant growth suffers. Given that AM fungal propagules will continue to interact with the plant as it grows, whether or not the plant would ‘learn’ that it is being invaded by a cheater and so attempt to prevent further colonization would depend on (1) whether relevant plant signalling was systemic or not, and (2) whether the plant was able to prevent colonization by the potential cheater. Why the latter is possible in some cases but not in others (see Table 1) is not at all obvious. Where ongoing colonization is prevented, Fitter’s (2006) model might come into play (i.e. colonization becomes restricted because the plant delivers very little C to the AM fungus). However, this possibility would not negate the involvement of P-related signalling as the immediate cause of inhibition of direct P uptake. Apart from tobacco stunt disease (Modjo & Hendrix, 1986), it is possible that large growth depressions associated with very low AM colonization arise from combinations of plants and AM fungi that, along with the growth conditions, would never occur naturally. Such effects are hardly beneficial to the ecological success of the fungi that cause them. However, this is not an issue that negates the need to understand the cause(s) of the large growth depressions associated with low colonization where these occur in the laboratory. It also seems necessary to consider the possibility that growth depressions when colonization is high may involve an effect on P uptake by the plant as well as the C drain to the fungus.

What makes an AM plant unresponsive in low-P soil?

The traditional answer to this question as far as constitutively unresponsive (i.e. facultatively) AM plants are concerned is that efficient P uptake by the roots renders any additional benefit in terms of P uptake by the AM pathway unnecessary to maintain a high growth rate. However, this answer is no longer adequate given that growth of such plants can involve varying and sometimes very high hidden P uptake via the AM pathway. The simplest answer in functional terms is that the direct and AM pathways are not additive but are complementary. If this is also the case in highly (positively) responsive plants, cessation of direct P uptake through the inefficient root system will have only a small effect on the MGR that would be achieved when both pathways are operating. In both unresponsive and positively responsive plants there will be environmental restraints on operation of the two pathways, as already indicated (e.g. light intensity, temperature, soil pH, etc.). Fungal inoculum potential and inherent plant growth rate immediately after seed germination may also play a role in determining which of the two P uptake pathways 'gets ahead'. For positively responsive plants the plant density per unit soil volume is an important factor in relation to MGR, in that at high plant density AM fungi can provide little or no increase in total P uptake per plant because competition for soil P is high in both the AM and NM plant populations (Koide, 1991b; Facelli *et al.*, 1999). This raises the possibility that in laboratory experiments, even with single plants in pots, MGR may be reduced if both external hyphae and roots are competing for the same soil P in overlapping and P-depleted rhizospheres. There is no doubt that this might be a factor if pot sizes are small and growth periods are long, but such competition would be expected to develop only gradually as plants grow, so that positive MGR decreases with time, which is not usually the case. Some studies have had built-in treatments to test this factor. For example, Zhu *et al.* (2003) showed that two barley varieties were equally unresponsive to *G. intraradices* in pots of two different sizes (950 g and 2850 g).

Classic studies that have shown large differences in MGR among different plant species in the field, where roots are expected to be less confined, include that of Plenchette *et al.* (1983a), using a range of species that included oats and wheat, both of which had zero MGR although other plants had high MGR. Constitutively NM plants (cabbage and beet) provided controls showing that soil fumigation with methyl bromide had not produced artefacts that altered the growth of NM controls. The results were confirmed in glasshouse experiments that showed that when fumigated soil was reinoculated MGRs were restored in some AM plants but others remained unresponsive – the latter again including wheat (Plenchette *et al.*, 1983b). Pringle & Bever (2008) have shown that effects of five AM fungi were similar in the laboratory and in the field.

The best explanation as to why the direct and AM pathways are not additive but are usually complementary has to be some form of signalling between the symbionts. As already noted, simple downregulation of expression of plant genes for P transport across the epidermis when plant genes are expressed at the AM interfaces does not seem to be the general answer, which must be sought in more sophisticated signalling, possibly via P loading to the xylem and delivery to the shoot. As to the question why such signalling should occur when provision of extra soil P can increase growth of NM plants, there is again no obvious answer. However, it has to be borne in mind that, owing to the relatively efficient P uptake by the NM plants, P-induced increases in growth of AM-unresponsive plants grown in low-P soil are usually small compared with those for highly AM-responsive plants, as shown by Baon *et al.* (1993) with eight barley genotypes, Bryla & Koide (1998) with two tomato genotypes and Kaeppler *et al.* (2000) with 28 maize genotypes. Where AM plants that are unresponsive in terms of weight have higher P concentrations than in NM plants there must be another level of regulation, or limiting factor, that prevents the improved P nutrition from being immediately converted to additional vegetative biomass. To help sort out these uncertainties (in the laboratory at least) requires measurement of AM-induced changes in expression of genes involved in P uptake and P signalling along growing roots, in tandem with measurement of tracer P uptake and (ideally) P depletion in the rhizosphere: technically this is not an easy requirement.

Putting a positive spin on growth depressions?

Most experimental work that has demonstrated growth depressions in low-P soil has focused only on vegetative stages of growth, during which depressions can become smaller and can reverse (Bethlenfalvay *et al.*, 1982a,b). However, Li *et al.* (2005) showed that, depending on the amount of P fertilizer supplied, growth depressions in wheat caused by *G. intraradices* at early growth stages were overcome at grain development. In this case, the AM plants probably would have had lower uptake of soil resources other than P, including – importantly in South Australia at least – lower water use over the whole growth cycle. Putting it another way, the AM plants reached the reproductive stage with lower investment in resources than in the 'unnatural' larger NM state in which the plant had to grow faster to achieve its reproductive goals. It is not yet clear how far this apparent advantage of early growth depressions can be extrapolated but it should not be forgotten with respect to both natural and managed (agricultural) plant ecosystems. In particular, more research seems needed to verify the conclusion by Ryan *et al.* (2005) that, owing to 'parasitic' AM fungi, wheat in south-eastern Australia may benefit from reduced AM colonization, which could be achieved through selected crop rotations or possibly through targeted wheat breeding programs.

Are 'dependence', 'mycotrophy' and 'benefits' useful terms?

We have deliberately used 'responsiveness' terminology rather than 'dependence' or 'dependency' for reasons that should now be clear. As conventionally defined, AM 'responsiveness' or 'dependence' are based on differences in weight of AM and NM plants, with a divisor that is usually (but by no means always) the NM weight when 'responsiveness' (and hence MGR) is used (Hetrick *et al.*, 1992; Baon *et al.*, 1993; Table 1), or the AM plant when 'dependence' or 'dependency' are used (Plenchette *et al.*, 1983a; Hetrick *et al.*, 1992). Variations are listed by Janos (2007). Both terms can be defined with respect to other growth-related parameters such as plant P content or even P concentration (Zhu *et al.*, 2003). Given the occurrence of hidden P transfer, the 'dependence' terminology is potentially very misleading, because where AM plant weights or P contents are no larger than those of NM plants (i.e. zero 'dependence'), there may in fact be a very large dependence on the AM fungus for P uptake. In other words, the conventional 'dependence' terminology only relates to outcomes of trade-offs between the two P uptake pathways under the given experimental conditions. Where there is a growth depression, to say that the plant growth response is negative is meaningful in functional terms, but to talk of negative 'dependence' is not functionally helpful without further clarification – the plant does not give the fungus P, though it does give it C. Accordingly we think that use of 'dependence' or 'dependency' as value-loaded terms based on plant growth-related responses should be avoided.

Janos (2007) has defined 'mycorrhiza dependence' in a very different way, as the inability of the NM plants to grow or survive without an increase in soil fertility (i.e. P). Thus, there is low 'mycorrhiza dependence' in plants such as cereals that take up P efficiently when NM; there is high 'mycorrhiza dependence' when NM plants take up P inefficiently. Plants with the conventional negative MGR would have negative 'mycorrhiza dependence' in that the NM plants would need less P to achieve the same size as the AM plant. Sawers *et al.* (2008) pointed out that MGR as defined conventionally involves both a 'dependence' component as defined by Janos (2007) and a 'nondependence' component, and that it is the latter that involves the AM fungus–plant interactions. They considered performance differences in cereals (mainly maize) and possibilities for breeding in these terms. The measure suggested by Janos (2007) is certainly useful in focusing on P supply from soil, but a term that completely prevents confusion with conventional 'dependence' terminology would be better (e.g. 'mycorrhizal soil P index'). Even this term is unhelpful functionally in that it still avoids the issue of whether a plant with a zero or negative 'P index' value actually depends on an AM fungus for at least part of its P uptake when it is AM. To use 'mycotrophy' ('feeding on the fungus') – again conventionally based on responsiveness – is even worse when a plant

showing no or negative MGR may actually be 'feeding on the fungus' for some of its P.

'Benefit' is often used as a synonym for positive response, especially in ecological studies of AM associations in the context of 'fitness'. For example, West *et al.* (1993) studied the response of the grass *Vulpia ciliata* to removal of AM fungal colonization (using the fungicide benomyl) and to P application under natural conditions. They showed that plant response to P was slight in that P inflow (rate of P uptake per unit root length) was unrelated to per cent AM colonization and concluded that colonization was not giving the plants any benefit in terms of improved P supply and plant performance at their field sites. They then addressed, at length, the issue of why the plants continued to support the AM fungi – a question that we posed earlier – and could find no simple answer except that 'benefits ... may be dependent on local conditions and may be due to other effects than increased P uptake'. Unsurprisingly, given the timing of the study, hidden P uptake was not considered to be a factor.

Some extrapolations to ecology and evolution

Despite lack of positive MGRs in pot experiments with single plants and single AM fungi, important ecological consequences might be expected if the AM pathway can dominate P uptake into unresponsive AM plants in the field. Here we address them only briefly. In particular, acquisition of P into unresponsive plants via extensive external hyphae would be expected to allow better competition with highly responsive plants than if the AM fungal pathway in the former was inefficient in supplying P. This point was demonstrated in a simple way by Cavagnaro *et al.* (2004a), who showed that although under NM conditions the tomato *rmc* mutant grew at the same rate as its wild-type parent (76R), it competed poorly when the wild type was AM. When *rmc* was inoculated with the only AM fungus known to form a functional symbiosis (the isolate now classified as *G. intraradices* WFVAM 23), it grew better in competition with AM leek than when only the latter was colonized (Cavagnaro *et al.*, 2004b). These results were explained as efficient capture of P via extensive external hyphae of AM plants when in competition with NM plants. However, it is possible that hyphae encountering roots of the (NM) mutant 'switched off' its epidermal P uptake. In any case, the end result was MGRs induced by competition either directly or indirectly – positive in the wild-type and negative in the *rmc* mutant, except when the latter was colonized. Another example of competition-induced MGR that almost certainly involved hidden P uptake was the outcompetition of *Lolium perenne* by *Holcus lanatus* when both were AM, although they both grew at approximately the same rate when NM (Fitter, 1977). More generally, when considering the competitive interactions in the field between positively responsive and unresponsive AM plants it can no longer be assumed that the latter can be lumped together with

constitutively NM plants as being 'nonmycotrophic or weakly mycotrophic' (Urcelay & Diaz, 2003).

In the field, the roots of an AM plant will house a range of AM fungi that individually will be expected to show functional diversity in terms of P transfer, an issue that is increasingly important to mycorrhizally minded plant ecologists, especially with respect to potential cheaters. Indeed, it might be argued from a mycogenic viewpoint that competition for plant C is a major factor in determining the population of AM fungal taxa in roots. In this context, Fitter's (2006) model can apply if a cheater can 'slip in' behind the AM fungi that deliver P and (depending on the spatial aspects of colonization) capture some of the C released without being 'detected' by the plant. It can now be expected that effective AM fungi will strongly influence outcomes of plant competition in the field in terms of P transfer if they dominate root colonization in individual plant species over ineffective AM fungi. Accordingly, hidden P transfer should now be taken into account when considering the roles of AM in plant community structure and dynamics. This would especially be the case in grasslands, where it may provide a strong buffer against rapid replacement of unresponsive plants by highly responsive AM plants; in other words, it is a factor that should be added to those considered by Hartnett & Wilson (2002) in reviewing the role of AM associations in plant community structure and dynamics in grasslands.

It may also be necessary to reappraise some AM effects previously interpreted as nonnutritional. An example is the protection of AM fungi against the root disease caused by the fungal pathogen *Fusarium oxysporum* that resulted in a positive MGR in *Vulpia* (Newsham *et al.*, 1995). In the absence of positive MGR when the plant was not diseased (also shown by West *et al.*, 1993) it was concluded that the 'protection' had to be nonnutritional. If we now assume that there was hidden P uptake in the AM plants and that when they were infected by *F. oxysporum* the AM colonization maintained a P uptake that was faster than uptake through NM roots, the MGR may be P-related after all.

The fact that there is considerable functional diversity in AM associations helps explain why an AM plant that is repeatedly subject to cheating by individual AM fungi does not totally reject colonization, so that the species evolves towards the constitutively NM state. In this context we cannot emphasize too strongly that the AM condition is normal for most plants and that for such plants the NM condition is an artefact – except when the plant is colonizing 'new land' that is free of any AM fungal propagules (very rare), or the plant has evolved to form AM associations that are very specific for individual AM fungi that may not be present in all locations into which the plant can potentially spread. Therefore, the question is: How does a plant that is exposed to many AM fungi in the field 'know' that it will grow less well when inoculated in the laboratory with an AM fungus (i.e. that it will be cheated)? Only where a plant species has evolved to experience and

survive NM conditions naturally might a plant 'know' (depending on changes in gene expression and signalling in the NM and AM states). If the plant does not 'know' it is being cheated until colonization is well established it may then be too late to cast off the AM fungus – depending of course on whether or not there is systemic signalling against cheating and whether any mechanisms for rejection exist. (Decreased colonization that occurs in some plants in high-P soil is irrelevant in this context.) Again, paraphrasing Janos (1985; see also Smith & Smith, 1996), environmental fluctuations may change the relative efficiency of individual AM fungi so that none is consistently superior, and limitations in distribution of AM fungi may mean that neither fungus or host can afford to reject the association 'because encounter of an optimal partner cannot be anticipated' (Janos, 1985). Extrapolating from this point it seems likely that AM fungal preferences in some plants ('partial' or 'ecological' specificity) are likely to be a relatively recent stage of the evolution of AM symbioses. Further consideration of the ideas presented here is warranted in terms of plant ecology and evolution.

Conclusions: time for new paradigms?

Here we have attempted to demonstrate the relevance of hidden P uptake into unresponsive AM plants in low-P soils at scales that range from molecular to ecological. We have also expanded on the conclusion by Li *et al.* (2008) that growth depressions are not caused by C drain alone, but may involve large decreases in epidermal P uptake pathways in the absence of the AM-induced P uptake pathway. To what extent cause–effect relationships of the 'combined P trade' (via both uptake pathways) and 'C trade' can be safely distinguished in individual facultative AM associations is not clear.

Irrespective of the possible ecological consequences of hidden P transfer via AM fungi, its occurrence should be a considerable comfort to those who have worried about the evolutionary maintenance of facultative AM associations in the (apparent) absence of P 'benefits' (Fitter, 1985; West *et al.*, 1993). Hidden P transport means that it can no longer be assumed that growth depressions, or lack of MGR in highly responsive AM plants, can be equated with 'strong' cheating (i.e. where the AM pathway delivers little or no P); in other words, such cheating may be less frequent than previously considered. It may also be reassuring that, to our knowledge, there is no evidence that there are any AM fungi that are constitutively strong cheaters over their whole distribution range in soils, and among all potential plant hosts that they may encounter whether naturally or in pot experiments. However, strong cheating does occur (see Table 1) and this is only compatible with Fitter's (2006) model if negligible amounts of P supplied via an AM fungus can sometimes 'fool' the plant into releasing a lot of C. Hidden P transfer is also compatible with the opposing and mycogenic cause–effect argument. This argues that AM fungi have primarily evolved to gain as much C as

possible from their hosts, and when doing so lose some P, which the plant 'recognizes' and so does not seek to cast off the symbiosis completely – if it could, of course – irrespective of the size of the P supply via the AM pathway.

In conclusion, we reiterate our 'take-home' messages that: lack of a positive MGR does not mean that the AM fungus is not delivering P to its host; and hidden P uptake in AM plants is a factor to be reckoned with in extrapolating from the plant pot to the mesocosm or the field, especially when plants are competing for soil P. We hope that those who doubt the relevance of pot experiments to AM function in the field (Read, 2002) will keep this second point in mind and agree with Graham (2008) that consideration of functional diversity under natural conditions involving competition among both plants and AM fungi remains a formidable technical challenge. Finally, it seems likely that achieving the goal of utilizing AM to greatly improve productivity of unresponsive or poorly responsive crops such as cereals, while conserving soil P as much as possible (Sawers *et al.*, 2008), will necessitate making the two P uptake pathways wholly additive rather than at least partly complementary. It looks likely that this will only be achieved via subtle genetic engineering to modify whatever fungus–plant signalling regulates the P trade in AM symbioses.

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