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REGULAR ARTICLE

What is the significance of the arbuscular mycorrhizal colonisation of many economically important crop plants?

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Abstract Arbuscular mycorrhizal (AM) symbioses are widespread in land plants but the extent to which they are functionally important in agriculture remains unclear, despite much previous research. We ask focused questions designed to give new perspectives on AM function, some based on recent research that is overturning past beliefs. We address factors that determine growth responses (from positive to negative) in AM plants, the extent to which AM plants that lack positive responses benefit in terms of nutrient (particularly phosphate: P) uptake, whether or not AM and nonmycorrhizal (NM) plants acquire different forms of soil P, and the cause(s) of AM 'growth depressions'. We consider the relevance of laboratory work to the agricultural context, including effects of high (available) soil P on AM fungal colonisation and whether AM colonisation may be deleterious to crop production due to fungal 'parasitism'. We emphasise the imperative for research that is aimed at increasing benefits of AM symbioses in the field at a time of increasing prices of P-fertiliser, and increasing demands on agriculture to feed the world. In other words, AM symbioses have key roles in providing ecosystem services that are receiving increasing attention worldwide.

Keywords Arbuscular mycorrhizal symbiosis · Plant phosphorus nutrition · Soil phosphate · Mycorrhizal growth response · Crop growth and yield

Abbreviations

AM	arbuscular mycorrhizal
NM	non-mycorrhizal
MGR	mycorrhizal growth response
MGD	mycorrhizal growth dependency

Introduction

The present title is essentially the question asked by JL Harley (1959) as his last words in 'Biology of Mycorrhiza' at a time when the experimental phase of research into arbuscular (AM) symbioses was in its infancy, and their widespread distribution among plants (including nearly all crop plants) was not yet known. The issue has been raised many times subsequently, for example in reviews co-authored by Alan Robson (e.g., Abbott et al. 1995; Smith et al. 1992); indeed, whole volumes have been devoted to the subject of management of mycorrhizas in agriculture, forestry and horticulture (e.g., Robson et al. 1994). In this special issue of *Plant and Soil* it must be emphasised that Alan Robson's research outputs

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since the late 1970s have covered many facets of soil-plant relations (not only AM symbiosis), and have lain firmly in the context of agriculture. During this period much knowledge of outcomes of AM symbiosis was gained worldwide, and issues were raised that are still important. With significant input from Lynette Abbott and postgraduate students, and with many visitors (including SES, hence the review by Smith et al. 1992), the work on AM symbiosis at the University of Western Australia has ranged widely. It has covered diversity among responses to different AM fungi as associated with amount of colonisation and hyphal spread (including tracer P and Zn uptake and transfer to plants), important aspects of soil chemistry (availability of soil P, influence of pH, etc), and importance of root traits, including root-hair length. (We cite examples of this research later.) These are all ongoing fields of research worldwide but—despite the many publications that have considered the issues—the question posed as our title is not yet fully answered, whether in functional, agronomic or social contexts.

In fact it is rather ironic given the past history of the research, often with agricultural plants, that the roles of AM symbiosis are now highlighted strongly for natural ecosystems, but significance in agriculture has a very mixed reputation. This is often due to failure to recognise that AM fungi are integral components of root systems in agricultural plants, just as in 'wild plants'. Root-related traits are receiving considerable attention worldwide with respect to the need to increase crop yield by improving nutrient uptake (e.g., Gahoonia and Nielsen 2004), but AM symbiosis sometimes receives very little consideration (e.g., Cornish 2009; Lynch 2007; Richardson 2009). Practical problems have emerged from past agriculturally-oriented research, as follows. 1) there is realisation that there appear to be no universal 'elite' AM fungi to maximise growth of all plants that can form AM symbioses; 2) production of high-quality inoculum requires suitable host plants, because AM fungi are obligate symbionts, hence production is not cheap and ongoing quality testing for different hosts and soil-types is crucial; 3) large-scale inoculation in the field is not easy or likely to be cheap, and survival of AM fungal inoculants is problematic, as it is for other biological inoculants. Additional issues arise when supply of P fertiliser is plentiful and affordable for farmers because in general even where plants have large growth increases arising from AM symbioses in low-P soils they do not in high-P soils (Abbott and Robson 1977, 1978, 1982; Oliver et al. 1983; Schweiger et al. 1995; Smith et al. 1979). Not surprisingly therefore, a role for AM symbiosis in production agriculture has been questioned (Ryan and Graham 2002), and it has even been suggested that in the wheat belt of SE Australia there should be selection of wheat (*Triticum aestivum*) varieties for low AM colonisation to improve growth, because of perceived parasitism by AM fungi (Ryan et al. 2005). Nevertheless, as the non-renewable sources of P rock are increasingly depleted, P fertilisers will become more expensive and all plant strategies that increase P uptake and use efficiencies will be increasingly valuable.

In this review we attempt to be realistic as regards the role of AM symbiosis in influencing yield of agricultural plants. Accordingly, we ask a series of focused questions which, if not all "FAQ" ("frequently asked questions"), are relevant to the question in our title, to which we return later. A particular aim is to overturn some entrenched concepts that are now obsolete, including some of our own past beliefs, or at least to raise doubts based on recent research. We do not address issues relating to drought or pathogen tolerance of AM plants, nor changes in soil microflora. Finally, we ask an even bigger question, which is whether AM symbiosis can in fact be better harnessed to improve crop productivity, despite the present problems and doubts. As pointed out by Miller et al. (1994), ability to generalise from experiments to ascertain how effectiveness of AM symbiosis may be increased in managed ecosystems requires knowledge of mechanisms that may be involved. Accordingly, we start the questions at the laboratory scale before proceeding to field-scale issues.

What determines responsiveness of AM plants?

Here we define 'responsiveness' as a change in plant biomass that results from the symbiosis; hence we talk of 'mycorrhizal growth response': MGR. The conventional 'responsiveness' equation is:

$$\mathrm{MGR} = 100.(AM - NM)/NM \qquad (1)$$

from Hetrick et al. (1992), where *AM* and *NM* refer to biomass of AM and NM (non-mycorrhizal) plants.

Values can be positive or negative, i.e. there can be AM 'growth depressions'. MGR will be infinite if the NM plants fail to grow. AM plants are also said to have 'dependence' (or 'dependency') on the symbiosis, and there is also a conventional mycorrhizal growth 'dependence' (MGD) equation:

$$\mathrm{MGD} = 100.(AM - NM)/AM \qquad (2)$$

from Plenchette et al. (1983), giving MGD values of 100% if the NM plants fail to grow. The two equations are used as alternatives and sometimes 'MGD' is defined using Eq. 1. We prefer MGR because 'dependence' has stronger functional overtones than 'responsiveness' (Smith et al. 2009); we discuss this point below. The most studied cause of positive MGR is increased uptake of soil P via the AM fungal pathway, and equations similar to Eqs. 1 or 2 can be used to calculate 'mycorrhizal P responsiveness' in terms of plant P content, or indeed any other AM-related growth or plant nutrient parameter.

The short answer to the question posed here is that MGR of any plant taxon (even cultivar) depends on a whole range of factors that have received extensive coverage in the literature. These include 1) plant genomic traits, especially root architecture (length, branching, fineness and formation of root-hairs; 2) AM fungal genome, e.g. ability to take up soil P efficiently and to lose it to the plant (without growth detriment) in exchange for plant photosynthate (organic carbon: C); 3) soil chemistry, especially availability of immobile and potentially growth-limiting nutrients such as P and Zn, but also any other growth-limiting nutrient that AM fungi can transfer faster than non-mycorrhizal (NM) roots can acquire; 4) other factors that directly or indirectly influence plant and fungal growth, e.g. temperature, water, soil pH, ability of AM to minimise root disease and to stabilise soil, etc. All of these factors have been extensively reviewed (e.g., Bolan 1991; Marschner and Dell 1994; Marschner 1995; Gahoonia and Nielsen 2004; Smith and Read 2008; Smith et al. 2010). Responsiveness also has a time factor, i.e. it depends on speed of colonisation, and can change over a plant life-span (Li et al. 2005; Smith 1980). Most laboratory growth experiments do not continue beyond the vegetative stage, so knowledge of AM effects on growth to normal reproductive stages and harvest times is comparatively very limited. Some of these investigations show that growth depressions are transitory and are not apparent as reductions in seed production (i.e. yield) (e.g. Li et al. 2005).

Most attention has been paid to root traits and it is well established that plants with relatively small, "coarse" root systems and low root-hair length and density have high positive MGR when soil P availability is low (Baylis 1970; Schweiger et al. 1995). Because hyphae can extend further from roots than root-hairs they can potentially acquire more P; thus, the 'mycorrhizosphere' from which the AM plant acquires P can be much larger in volume than the rhizosphere, with less depletion of soil P that can greatly limit uptake directly into NM roots. In such plants, with increasing supply of available soil P, MGR progressively decreases to zero and can even become negative. However, such AM plants may have higher P concentrations (P per g biomass) and P content (P per plant) at high soil P than the equivalent NM plants, i.e. there can remain an AM 'P response' which indicates that AM fungal P uptake remains active (Oliver et al. 1983). Examples of plants with high positive MGR at low soil-P include many legumes, including pasture legumes such as clovers (Abbott and Robson 1977; Smith 1982; Tawaraya 2003). Plants with extensive root systems and high root-hair length and density often have low (positive) or no MGR even in low-P soils; examples include grasses, including prairie grasses (Wilson and Hartnett 1998) and cereals such as wheat (Hetrick et al. 1992, 1993; see also Tawaraya 2003). Direct comparisons of P uptake into pasture legumes and *Lolium rigidum* (annual rye grass), with emphasis on root characteristics, are given by Bolan et al. (1987a) and Schweiger et al. (1995). Tawaraya (2003) lists a very large number of plant species, and again emphasises the importance of root traits in determining AM growth responses, in this case based on Eq. 2. However, it has to be emphasised that there can be large differences in MGR between cultivars of the same species, including wheat, when grown under the same laboratory conditions (Hetrick et al. 1996).

Presence or absence of MGRs can only be shown rigorously in laboratory ('pot') experiments that include the same plant genotype in an NM state—which would be wholly artificial under nearly all field conditions. Under normal agricultural conditions it is virtually impossible for AM fungi to be eliminated from soil in the field, with no other effects on soil chemistry or microbial populations (especially pathogens) that might

influence apparent MGR. This is shown by comparison of crop plants that are AM with others that are constitutively NM, such as members of Brassicaceae (Thingstrup et al. 1998; Ryan and Angus 2003). Even in the laboratory potential artefacts arise from sterilisation. An alternative approach that has not been applied extensively in the field is to use a plant mutant in which AM colonisation is suppressed or eliminated but that has no growth phenotype (compared with the wild-type) when NM, thus avoiding soil treatment and associated confounding factors (Cavagnaro et al. 2006). It must be emphasised that the mutants and corresponding wild-types must be very thoroughly tested for equivalence in terms of growth, root architecture and P responses in the absence of AM fungi (Cavagnaro et al. 2004; Facelli et al. 2010; Rillig et al. 2008). An important conceptual issue is that in a laboratory experiment in which soil has been sterilised for growth of the NM plants and fungal inoculum added to this soil to produce AM plants, the former are controls and the latter a treatment. In the field with a pre-existing population of AM fungi, it would usually be NM plants that would be an experimental treatment.

Do non-responsive AM plants 'benefit' from the symbiosis in terms of increased P uptake?

'Benefit' is conventionally defined as increase in growth or plant nutrient content. It seemed self-evident until about 15 years ago that lack of positive MGR meant that there were no such nutritional benefits, i.e. that the AM fungi were possibly acting as parasites in the conventional sense of acquiring C but giving little or no P. In fact it is often explicitly stated that the cause of zero or negative MGR is fungal parasitism (Bethlenfalvay et al. 1982; Johnson et al. 1997; Smith 1980). However, it is wrong to assume that no positive MGR or, strictly, no change in plant P content, means that there is no P delivery via the AM fungus. Delivery is consistently shown with supply of radioactive P (^{32}P or ^{33}P) to hyphae in compartmented growth systems in which the roots cannot access the radioactive P. Non-responsive agricultural plants that have been studied in this way include barley (*Hordeum vulgare*), cucumber (*Cucumis sativus*), field pea (*Pisum sativum*), tomato (*Solanum lycopersicum*) and wheat (see Smith and Smith 2011 for references).

Figure 1 shows an example in which the hyphal compartment is kept small, so as not to bias the experiment by allowing hyphae to access a much larger soil volume than the NM plants, as is the case in other such systems that have been widely used. Quantification of amounts of P taken up via the AM fungal pathway from the pot as a whole is possible using the system shown in Fig. 1, and with underlying assumptions and calculations as in Smith et al. (2003, 2004). As shown in Table 1 even when plants have zero or negative MGR they can still take up large amounts of P via the AM fungal pathway, which means that uptake directly through the roots is suppressed by the fungus. This is an important finding, because it indicates a hitherto unexpected consequence of AM fungal colonisation, including possible 'manipulation' of plant function induced by fungal signals. Confidence in the original calculations was established by results obtained with positively responsive plants, i.e. by comparing non-responsive tomato with responsive flax and medic (Smith et al. 2003, 2004). Those results also show that amounts of P taken up via the AM fungal pathway depend on individual AM fungal taxa. There are two field studies with ^{32}P supplied in hyphal compartments that demonstrate P uptake via AM fungi into wheat and peas (Schweiger and Jakobsen 1999; Schweiger et al. 2001), though the amount could not be

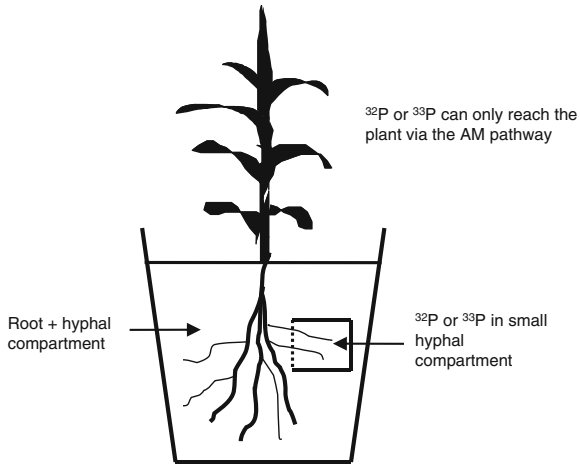

Fig. 1 Compartmented pot, with application of ^{32}P or ^{33}P in a small hyphal compartment that is accessible to AM fungal hyphae (but not roots) through a fine nylon mesh. The design ensures that the total soil volume is almost the same for both arbuscular mycorrhizal (AM) and nonmycorrhizal (NM) plants, and so does not bias growth in favour of AM plants in the same experiment

Table 1 Summary of effects of addition of phosphate (P) on arbuscular mycorrhizal (AM) wheat (*Triticum aestivum*) (a) and tomato (*Solanum lycopersicum*) (b), as obtained with compartmented pots (Fig. 1). Data are mean dry weight (DW) of AM plants, mycorrhizal growth responses (MGR), percent colonisation (% col AM), percent contribution of the AM pathway to P uptake via the mycorrhizal P uptake pathway (% P via MPU). Total P per plant via MPU and direct P uptake via the epidermis (DPU) into AM plants, and DPU into equivalent non-mycorrhizal (NM) plants are also shown. P0, P20 and P60 are levels of P added to the soil, all as mg kg^{-1}, with P added as $CaHPO_4$ (Ca), ammonium polyphosphate (APP), H_3PO_4 (H), Na_3PO_4 (Na), or KH_2PO_4 (K). a) from Li et al. (2006), b) from Nagy et al. (2009)

Soil P addition	DW AM plant (g per plant)	MGR	% col AM	% P via MPU	MPU AM (mg P per plant)	DPU AM (mg P per plant)	DPU NM (mg P per plant)
(a) wheat							
P0	3.2a	0	60c	75c	4.5	1.5	6.0
P20-Ca	6.4c	0	35a	60ab	7.5	5.5	13.3
P20-APP	5.2b	−17	40ab	50a	6.0	6.2	12.8
P20-H	6.1c	−19	45b	55a	6.6	8.4	15.0
P20-Na	6.1c	−20	35ab	65bc	8.4	6.6	15.0
(b) tomato							
P0-K	2.5a	−45	75c	75c	3.2	1.1	6.5
P20-K	6.0b	0	35b	30b	3.7	8.7	12.9
P60-K	6.5b	0	20a	10a	2.2	19.4	22.4

Percent P uptake via the AM pathway is as in the original publications, calculated using the equation of Smith et al. (2004), but here slightly rounded off, as is percent colonisation. Where present, statistical differences (a, b, c) in columns apply separately to each experiment at 0.05 probability levels, as given in the original publications. Values for total P uptake via the two pathways are our calculations, using the means for total P uptake per plant, as in the original publications.

quantified. Such findings have overturned the past belief that when there is no MGR the AM fungi are acting simply as parasites, which are generally considered to give no or negligible nutritional benefit to the plant in terms of P first acquired by the AM fungus. There is such a benefit, but it is 'hidden' because of the lack of positive MGR or increase in total plant P; in other words there is no *net* benefit. This is why the 'dependence' terminology can be misleading, because although the AM plants show no MGD in terms of Eq. 2 they do in fact depend functionally on the fungus to supply P.

The physiological demonstration of P uptake by the AM fungal pathway is supported by the expression of AM-specific P transporter genes in colonised cells in the cortex of the AM plants, both responsive and otherwise (Javot et al. 2007), though such expression does not help quantify how much P is acquired by the fungal pathway (Grace et al. 2009). There is also indirect evidence for the importance of AM fungal P uptake by non-responsive plants, including increased success of AM plants when they compete with NM (mutant) plants for soil P resources (Cavagnaro et al. 2004; Facelli et al. 2010), and decreased uptake of arsenate compared with the NM state (Christophersen et al. 2009). More research is needed to establish whether or not AM fungal colonisation often suppresses direct P uptake in responsive plants, but when there is a large MGR it is not an important issue, as suppression of direct uptake would have relatively little effect on total uptake.

Do AM and NM plants acquire different forms of soil P?

There is no simple answer to this question, although it is generally believed that roots and AM fungi access inorganic soil P from the same sources. This belief comes from many studies showing that when carrier-free ^{32}P is added to soil (in non-compartmented pots) and allowed to equilibrate with soil P, the specific activities (^{32}P per total plant P) of AM and NM plants are the same (for references, see Bolan 1991; Bolan et al. 1984; Frossard et al. 2011). Bolan et al. (1984) tested the approach by using soil in which different levels of ferric hydroxide were allowed to react with different levels of P (KH_2PO_4), with subsequent addition of carrier-free ^{32}P and further equilibration.

They showed that P uptake by AM *Trifolium subterraneum* (subterranean clover) increased with increasing soil P but was unaffected by the ferric hydroxide. Uptake of P into NM plants was lower at all soil P levels and was further decreased by ferric hydroxide; i.e. ferric hydroxide decreased P availability to NM plants, but not to AM plants. However, ^{32}P specific activities were the same throughout. Differences in P uptake were mirrored by differences in amounts of P extracted by different methods. Again, however, ^{32}P specific activity of extracted soil P was the same, irrespective of the extractant and amount of P extracted. The results showed that the view that AM and NM plants obtain P from the same 'source' is over-simple, because forms of soil P that differ in availability to AM and NM plants can become uniformly labelled by ^{32}P. In a second study, Bolan et al. (1987b) examined effects of P application on growth of shoots supplied (in order of decreasing solubility) with KH_2PO_4, colloidal ferric P and crystalline ferric P (strengite). Subterranean clover had higher MGR than annual ryegrass for all P sources but for both species MGR was highest in the least soluble source, i.e. strengite. The results are again important in showing differences between AM and NM plants in ability to acquire P from a poorly available source, and also that MGR is affected by P supplies of different availability. Bolan et al. (1984b) and Bolan (1991) gave several possible explanations that all involve higher rates of desorption of soil P by AM plants. These are 1) exploitation by AM fungal hyphae of soil beyond the depletion zone in the rhizosphere, thus decreasing the distance that P ions must diffuse before uptake; 2) faster P uptake from solution by the AM pathway, possibly involving a lower K_m; 3) differences in pH between mycorrhizosphere and rhizosphere; and 4) production of exudates by AM plants. These possibilities are summarised in Fig. 2, which also shows outcomes in terms of ^{32}P specific activity in plants following its addition to soil as orthophosphate. The possibilities in Fig. 2 all need more attention. For example, Tawaraya et al. (2006) have shown that hyphal exudates of AM fungi solubilise $FePO_4$ *in vitro*. The possibilities in Fig. 2 are very relevant when considering growth of plants in agricultural soils with differences in P sources, and in particular evidence that MGRs are particularly strong in subtropical and tropical soils with poorly available P (Cardoso and Kuyper 2006).

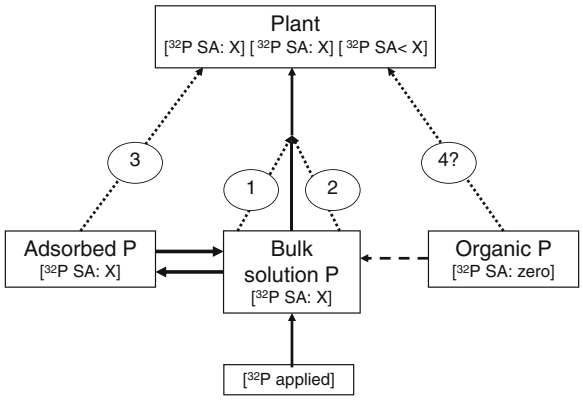

Fig. 2 Possible mechanisms for increased uptake of P by arbuscular mycorrhizal (AM) plants, modified from Bolan et al. (1984). Thick arrows indicate interconversions of forms of P during an experiment, with the thick dashed arrow indicating net hydrolysis of organic P. Thin dashed arrows indicate effects of AM fungi, as suggested by Bolan et al. 1) Extensive physical exploration of soil by fungal hyphae; 2) higher substrate affinity of P uptake into hyphae than directly into roots; 3) combined extensive physical exploration and chemical modification of adsorbed P that speeds up soluble P release; 4) possible fungal hydrolysis of organic P. The figure also shows specific activities (SA) of ^{32}P after addition as inorganic P (orthophosphate), where SA is ^{32}P/(available P) in soil phases and ^{32}P/(total P) in the plant, and a representative value (X or < X). Adsorbed P can be in several forms that become uniformly ^{32}P-labelled (see text)

Do AM plants hydrolyse significant amounts of organic P?

The question already seems to have been answered by the evidence from the soil ^{32}P-labelling just described, in that specific activities of AM plants should be lower than those of NM plants if the AM plants hydrolyse significantly more organic P than NM plants (see Fig. 2); this is not the case. However, there is other evidence that is conflicting. With the use of compartmented systems, phosphatase activity and/or breakdown of organic P have been found in association with AM fungal hyphae in some studies (e.g., Tarafdar and Marschner 1994a, b) but in others the role of AM fungi was very small (e.g., Joner and Jakobsen 1994, 1995; Joner et al. 1995). Joner et al. (2000) pointed out that difficulty in maintaining soil-based systems free of microbes other than the AM fungi hampered unambiguous conclusion that the latter were responsible for mineralisation, especially as the surface of hyphae may be enriched with bacteria relative to bulk soil. They showed with compartmented monoxenic root organ cultures that

some forms of organic P can be hydrolysed and P transferred to the plant by the AM fungal pathway (Joner et al. 2000). Again, the issue is how much of the total P in soil-grown plants can be obtained in this way. Returning to the issue of similar plant ^{32}P specific activities in NM and AM plants there is always the possibility that the AM fungi may replace phosphatase production and extrusion by NM roots with their own, which might result in similar specific activities overall. There is need to investigate soils that are rich in organic P compared with inorganic P, and in experiments that minimise mineralisation of organic P caused by soil sterilisation that might leave only forms that are unavailable to AM fungi.

Is there significant AM fungal delivery of nutrients other than P?

It has been known for some time that soil nutrients other than P are taken up via the AM fungal pathway, again as shown with experimental systems with compartments accessible only to AM fungal hyphae. A good example is Zn (Bürkert and Robson 1994; Jansa et al. 2003), with others including Ca, Cu, K, S as SO_4^{2-} and N as NH_4^+ and NO_3^- (Marschner and Dell 1994; Rhodes and Gerdemann 1980). The question is again whether such uptake provides a substantial proportion of plant needs, and this is still unresolved in all these cases. The amount of NH_4^+ and NO_3^- taken up by the AM fungal pathway under natural conditions is particularly uncertain. The uncertainty is largely because these ions are much more mobile in soil than orthophosphate ($H_2PO_4^-$). In agricultural soils NO_3^- is the predominant form of combined N and is not likely to be depleted significantly in the rhizosphere by direct uptake into roots. The argument is not so convincing for NH_4^+, but this ion is still very mobile compared with phosphate. Roots and hyphae are accordingly expected to have similar uptake efficiencies for soil N, and scavenging for N at a distance from roots by hyphae does not seem likely to be advantageous compared with that for P. Evidence for positive MGR or increased tissue N concentrations in soil-grown AM plants due to N uptake via the AM fungal pathway has only been obtained in a few investigations; in others AM symbioses did not improve N nutrition (for references, see George et al. 1995; Smith and Smith 2011). Because plant tissues have N:P ratios of approximately 22:1 on a molar basis, large direct effects of AM fungi on N uptake should be easily detectable, but this has mostly not been the case in laboratory experiments with plants grown in pots. There are several examples of uptake of ^{15}N-labelled N from hyphal compartments into non-responsive plants such as celery (*Apium graveolens*) and cucumber (Ames et al. 1983; Johansen et al. 1992, 1994) and maize (*Zea mays*) (Frey and Schüepp 1993). If such uptake were a significant component of total N uptake where non-responsive AM and NM plants have access to the same amounts of soil N it would mean that direct N uptake through roots must be suppressed, analogous to effects on P uptake, i.e. there would be 'hidden' N uptake via the AM fungal pathway. However, the necessary quantitative experiments have not been carried out.

A very different picture has emerged from experiments with monoxenic root organ culture in compartmented systems which show hyphal transfer of very large amounts of ^{15}N as arginine (Jin et al. 2005). Arginine is a monovalent cation at physiological pH, and if its movement through hyphae is balanced by P, either as orthophosphate ($H_2PO_4^-$) or polyP$^-$, with complete breakdown of arginine at the fungus-plant interface to release 4 molecules of N per molecule of arginine, this would amount to a contribution of almost 20% of plant N (based on N:P ratios). The problem is that the levels of organic C and nutrients for root organ cultures are wholly artificial compared with soil-grown plants. In the latter, analysis of intraradical hyphae and arbuscules suggests that most of the ionised P is balanced by inorganic cations such as K^+ and Mg^{2+} (Ryan et al. 2003; Ryan et al. 2007). Molar ratios of Mg, K and P were 1: 2: 4, which, taking into account presence of other cations (Ca and Na) and differences in valency of cations, suggests little scope for univalent arginine to balance P in the intracellular fungal structures. However, arginine could not be measured by the techniques used. Issues of ionic charge-balance are discussed in detail by Smith and Smith (2011). Consideration of these issues at all stages of nutrient uptake into plants may seem specialised in terms of plants and agriculture but charge-balance is fundamentally important in relation to plant nutrition because, with very few exceptions, soil nutrients are taken up as ions. An intriguing possibility is that plants take up more N via the AM

fungal pathway when soils are dominated by NH_4^+ rather than by NO_3^-, the latter being the case in agricultural soils.

In relation to this last point, comparison of natural ^{15}N composition of plants in natural ecosystems with different growth strategies has shown that those that form AM symbioses have distinctly different $\delta^{15}N$ values than those that do not (Pate et al. 1993). This strongly suggests differences in pathways of acquisition of soil N by AM compared with NM plants in natural ecosystems at least.

What causes AM 'growth depressions'?

After some debate (Mosse 1973), it has become generally accepted that the cause of negative MGRs (growth depressions) is C cost of AM symbiosis in the absence of no net benefits from P uptake: in other words, an extreme C-P 'trade imbalance'. Growth depressions in the laboratory occur in agricultural plants that grow well when NM, though they can be temporary (e.g., Bethlenfalvay et al. 1982; Li et al. 2005). They also occur in wild plants inoculated with co-occurring AM fungi (Klironomos 2003). In that study—intriguingly—individual plant taxa showed different MGR with different AM fungal taxa which, in turn, produced different MGR with different plant taxa; clearly the C-P trade balance is not a simple matter in terms of individual plant-AM fungal associations, which raises important questions of recognition and signalling between the symbiotic partners.

The widespread occurrence of growth depressions was discussed in detail by Johnson et al. (1997) in terms of the general ecological concept of a mutualism-parasitism continuum, as shown in Table 2. Although 'commensalism' seems compatible with absence of MGR, it would be problematic to use the term in AM symbioses, and Johnson et al. (1997) did not use it in their broad analysis of mutualism and parasitism, because the term indicates that benefits are gained by one partner with no effect on the other. The problem in AM symbioses is that benefits to the fungus (organic C) are gained at the expense of the plant, which is why the use of 'parasitism' is conventionally used both where there is both zero and negative MGR (growth depression). 'Parasitism' is also misleading in implying no P transfer to the plant via the AM fungal pathway, as emphasised above. There is also a large problem in equating growth depression with C-cost in that the latter is only deleterious when the plant cannot compensate for development of an AM symbiosis, as it might with 1) increased C-delivery to roots if photosynthesis is limited by 'sink strength', 2) decrease in relative root biomass (higher shoot:root ratio—which is particularly common), or even 3) decreased extrusion of organic C to soil when plants are AM. To quote an AM fungal respiratory C-cost of 5–20% as the likely cause of a growth depression is misleading if there is simply a transfer of cost from growth of roots to growth of the AM fungus. As well as higher shoot: root ratios, changes in root architecture when plants become colonised, including decrease in root-hair formation (Kothari et al. 1990; Maillet et al. 2011; Orfanoudakis et al. 2010) might be deleterious if they produce limitation in uptake of nutrients that the AM fungi do not take up. However, that should not be the case if the experiment has adequate nutrient supply.

We have recently suggested that AM growth depressions at low soil P are not necessarily caused by the C-cost of the symbiosis, because they can occur when colonisation and hence C-cost are very low (Grace et al. 2009; Johnson et al. 1997; Li et al.

Table 2 Outcomes of arbuscular mycorrhizal (AM) symbiosis, as mycorrhizal growth response (MGR), with responses of AM fungus and plant. Also shown are equivalent stages in the AM 'mutualism-parasitism' continuum (Johnson et al. 1997). Net resource benefit to fungus or plant: (+); no net resource benefit to plant: (0); net resource cost to plant: (−). "Commensalism" is in inverted commas because it is difficult to apply the term to AM symbioses; "Parasitism" is in inverted commas because we query the original assumptions; see text

	Positive MGR	Zero MGR	Negative MGR
AM fungal response	(+)	(+)	(+)
Plant response	(+)	(0)	(−)
Symbiotic outcome	Mutualism	"Commensalism"	"Parasitism"

2008; Smith et al. 2009). Again, our suggestion relates to suppression of direct P uptake, but in this case there is little or no P uptake via the fungal pathway because of the very low percent root length colonised. Why colonisation is so low is not clear, and must involve issues of plant-fungus recognition and signalling that are beyond the scope of this review. We have also suggested (Smith et al. 2011) that AM growth depressions should perhaps considered more realistically as a result of stress-related growth increase in NM plants, caused by absence of their natural AM symbionts. The NM plants may need to expend more C in order to acquire nutrients that may be essential at later growth stages.

Growth depressions at high soil P are also conventionally ascribed to symbiotic C-cost, but there is conceptual contradiction in that at high soil P percent colonisation is often relatively low compared with that at low P and may include lower arbuscule density in colonised regions, and accordingly C-cost per plant of maintaining the AM fungal symbiont ought to be relatively low. It also seems less likely that AM fungal colonisation significantly decreases direct uptake of P through the roots, though this cannot be ruled out.

Johnson (2010) has pursued the 'mutualism-parasitism' theme in comparing growth and MGR of AM and NM plants in field soils with different amounts of inorganic N and P, and has proposed an interesting expanded trade balance model. The model features 'parasitism' (negative MGR: growth depression) particularly when soil contains both high P and high inorganic N. This is equivalent to the condition in some experiments where P is increased to a high level and other nutrients are maintained at non-limiting levels, but in fact such negative MGR is not always found (e.g., Schweiger et al. 1995). As presented, the new trade balance model does not specify whether or not N is taken up by the AM fungal pathway, though this would affect C costs. There is a good physiological basis for negative MGR ('parasitism', as stated by Johnson 2010) if with high soil N there is substantial fungal N uptake and transfer as arginine, with breakdown to NH_4^+ for transfer across the interface, but release of CO_2. We have pointed out that this would involve loss of a substantial amount of C derived originally from the plant and might indeed be a substantial C-cost (Smith and Smith 2011). This cost does not directly represent fungal parasitism of its host in the conventional sense, because it is loss of C from the fungus before N is transferred to the host. Nevertheless, it is equivalent in terms of trade imbalance: in this case C-N trade rather than C-P trade. To test issues raised above, there is a clear need here for some rigorous experiments to check relative amounts of N and P delivered by the AM fungal pathway, and C lost from the symbiosis. However, we believe it sensible to replace the 'mutualism-parasitism continuum' in Table 2 with 'positive-negative responsiveness continuum', and avoid the use of 'parasitism'. The word is certainly capable of misinterpretation unless defined carefully as net costs exceeding net benefits, rather than total lack of benefits. Such careful definition would include some transfer of P (and N) via the AM fungal pathway.

How relevant is laboratory work as summarised above in the agricultural context?

The answer here has to be equivocal: it depends both on the aim of the study, and the mind-set of a researcher who focuses on field applications of AM symbiosis in relation to crop yield. Artificial substrates or soil-sand mixtures are often used because they have great advantages in extracting roots and AM fungal hyphae for measurement of biomass (root and hyphal), P concentrations, DNA etc. Also, growth of a responsive plant in a low P soil is the simplest way of demonstrating that a particular AM symbiosis is functional in transferring P via the AM fungal pathway. Such work with low P soil appears more relevant to natural ecosystems, where AM now receive a lot of attention (e.g. Klironomos 2003). However, some crops worldwide are grown in soils with low P-availability.

The question is most important in attempting to extrapolate laboratory work to crop production in developed agriculture where there is high fertiliser P application. These studies have generally shown that MGR decreases with increasing soil P, as already noted, though generalisation is unwise in that such decreases vary greatly between plant taxa, even at cultivar level. Hetrick et al. (1996) showed considerable functional diversity in responses to AM colonisation among wheat cultivars. They grew ten cultivars for 14 weeks in prairie soil with no added P or with P added at 10 or 50 mg per kg soil, and with five individual AM fungal isolates representing three species, and also NM plants. Hetrick et al. (1996) showed very different MGRs

among cultivars (from slightly negative to highly positive) with no soil P addition, and large differences in decreases of MGR caused by increasing P amendment. Cultivars with high MGR showed high correlation between MGR and percent colonisation, while (unsurprisingly) MGR of cultivars with no or negative MGR was independent of percent colonisation. In a separate experiment, use of a compartmented pot system with ^{32}P showed that P was taken up via the AM fungal pathway into both a positively responsive and a non-responsive cultivar. As well as concluding that the mechanisms whereby AM fungi increase growth of some cultivars but decrease growth of others were unclear, Hetrick et al. (1996) emphasised—importantly—that percent AM colonisation itself is not an appropriate indicator of AM responsiveness for a particular cultivar. This last message has been delivered repeatedly elsewhere (e.g., Marschner 1995, Ch. 15; Smith et al. 2011; Smith and Smith 2011), and its relevance to field-based studies should be obvious. Nevertheless, percent colonisation is the most convenient and common measure in field-based studies as an indicator of AM fungal activity and hence supposed 'benefits'. For example, Lekberg and Koide (2005) published a detailed meta-analysis of 290 field and glasshouse studies, aimed at ascertaining if plant performance under a variety of agricultural management practices is limited by 'abundance' (their word) of AM fungi. As the measure of abundance they used change in percent colonisation between 'control' treatments and '+AM fungal treatments' where the latter included shortened fallow, reduced soil disturbance, avoidance of constitutively NM crops in rotations and inoculation of AM fungi into non-sterile soils. All these are known from previous studies to give relatively high AM fungal biomass in soil, in contrast to the 'controls, which were the converse treatments. (Studies with sterilised soils were omitted.) It seems to us possible that a difference in percent colonisation (say 20%) over a low range of values in treatments will not have the same effect on growth parameters as the same (e.g. 20%) difference over a high range of values. However, we recognise the unavoidable limitations in such a meta-analysis. Data selection and analysis by Lekberg and Koide (2005) showed many cases of association between higher percent colonisation and improved plant performance. In such cases it is sometimes possible to propose with confidence causal relationships in the field. Work in subtropical NE Australia showed that periods of long fallow and pre-cropping with NM crops decreased AM fungal inoculum as well as percent colonisation and yield of a range of crops Owen et al. 2010; Thompson 1987). These included wheat, though yield decreases were small. A contrary example is extensive work that used high percent colonisation and lack of improved performance as a possible indicator of parasitism in wheat in south-eastern Australia (Ryan et al. 2005). It is not known if the wheat variety used can show positive MGR, i.e. based on comparison with NM plants. If not, no correlation between percent colonisation and growth in the field would be expected, and parasitism cannot be assumed. Possible functional reasons for lack of correlation in field-pea (Ryan and Angus 2003) are more problematical, given that AM-forming legumes generally have high MGR in laboratory experiments (Tawaraya 2003). Jakobsen (1986) showed in a field experiment with pea that fumigation almost completely prevented AM colonisation but did not decrease growth. However, fumigation decreased shoot P content, showing that the AM pathway for P uptake was operating in the plants from untreated plots. In an experiment with pots that were larger than those mostly used, Gavito et al. (2002) found no increase in shoot P content, but there was considerable uptake of ^{33}P from hyphal compartments, again showing AM-mediated P uptake. As with wheat, the extent to which the different results obtained with pea reflect the use of different plant cultivars, AM fungi, or growth conditions remains very unclear. We agree strongly with Ryan and Angus (2003) that further investigation under field conditions is required. However, returning to the question raised for this section, we believe that there are dangers in avoiding laboratory-based research that is needed especially to establish constitutive differences among plant cultivars in their ability to establish positive MGR. Such an approach might provide information that is very relevant to functional roles of AM symbiosis in the field and potential for increased yield.

Relating to agriculture, does high (available) soil P really suppress colonisation?

This is another issue that is not as straightforward as is sometimes assumed. As already noted, much laboratory work has shown that percent of root length colonised decreases with increasing soil P (Table 1),

but at intermediate soil P levels this will reflect relatively fast root growth, compared with rate of colonisation. Hence there is an increase in the divisor, i.e. total root length per plant, without necessarily a decrease in the colonised root length per plant. As with most AM-related plant properties, decreases in percent colonisation differ greatly between plant genotypes (e.g., Schweiger et al. 1995). At high P levels, both colonised root length and 'intensity' of AM fungal biomass per colonised root length do decrease (e.g., Thomson et al. 1986); only when this latter effect occurs is there true suppression. A recent study that focused on decreased colonisation in tomato (Nagy et al. 2009) included an experiment with intermediate P supply, and showed that formation of arbuscules and amount of P taken up via the AM fungal pathway remained constant per plant, even though percent colonisation and percent of total P uptake via the AM pathway went down (Table 1b). Even at the high P level there is some uncertainty about true suppression of uptake because of movement of ^{32}P from the hyphal compartment to the main compartment in the absence of hyphae, resulting in ^{32}P uptake by the NM plants as well as that from the hyphal compartment into the AM plants. Uptake of ^{32}P, again from hyphal compartments into (non-responsive) wheat, increased when fertiliser was added (Li et al. 2006) (Table 1a). The actual cause of the suppression of colonisation, when it does occur, is still unresolved, but will involve shoot-to-root signalling, as shown by supply of P only to foliage or in split pots (Sanders 1975; Balzergue et al. 2011). Put simply, a decrease in percent colonisation at high P will exaggerate true suppression of colonisation of the whole root system. Relatively low percent colonisation (say within a range 20–40%, especially without information on arbuscule density) should not be used loosely as showing that AM symbiosis is unimportant in P uptake under agricultural conditions.

Might AM colonisation be deleterious in the field?

If most crops are AM and the soil in which any crop is growing contains AM fungal propagules the issue has to be that the AM state might be 'deleterious' compared with an unnatural NM condition, i.e. that a population of AM fungi is for some reason dominated by truly parasitic fungi. As emphasised above, it is not valid to hypothesise parasitism just because there are no perceived AM benefits, as was done by Ryan et al. (2005). We cannot see a causal association between relatively high percent colonisation and wheat growth in that study, especially taking into account factors that could have differed in the experimental plots following the different crop rotations. For example as well as differences in P-fertiliser application, Ryan et al. (2005) mentioned that in one case deleterious organisms other than the known pathogens that were assessed might have contributed to relatively poor wheat growth. However, it was the belief in parasitism that led to the suggestion that crops should be bred to give lower AM colonisation (Ryan et al. 2005). While perhaps acceptable at the time in the context of the suggested functional explanation of the 'mutualism-parasitism continuum' (Johnson et al. 1997), the recommendation now looks hazardous, even for the soils that were studied in SE Australia.

We know of no convincing evidence for deleterious effects in the field that can confidently be ascribed to AM symbiosis, which is not to say that AM fungal populations in any location will comprise the taxa that can give the largest MGR. Indeed, there are suggestions that prolonged growth of crop monocultures can select less mutualistic AM fungi, in accord with ecological theory that predicts selection of 'cheaters', i.e. symbionts that maximise acquisition of resources (in this case C) but provide few in exchange (Johnson 1993; Johnson et al. 1997; Kiers et al. 2002).

We have suggested (Smith et al. 2010) that there may be a positive aspect to plant strategies that lower vegetative growth, as in AM growth depressions, if these have relevance to success in the field. 'Lower' vegetative growth would give savings in plant water use which might be beneficial in drought-prone conditions as long as reproductive capacity (yield, in agricultural terminology) is not lowered. Again, this is relevant to the perspective from which growth depressions in the laboratory are viewed, i.e. whether the NM or AM condition is regarded as the norm.

What is the significance of the arbuscular mycorrhizal colonisation of many economically important crop plants?

The short answer is that there is nothing unusual in terms of C-P trade in crop plants, and probably

resource trade involving other soil nutrients. Whether such resource trade leads to positive, zero or negative MGR is a complex issue in terms of plant and fungal traits, and environmental conditions, as outlined above. Colonisation by AM fungi should not be regarded as an 'add-on' response of plants to avoid P deficiency, as is sometimes done (Richardson 2009): it is a normal fact of life for most plants. The extent to which increasing soil P results in true suppression of colonisation and P transfer through the AM fungal pathway requires much more attention, using soil-P amendments within the range used on farms across the world and tracer techniques to quantify actual P transfer. Even under farm conditions, cases where AM fungal propagules become very sparse seem rare, as with one soil used by Baon et al. (1992). Beyond the relatively short history of plant agriculture, AM fungal uptake of P into non-responsive plants helps explain why AM symbioses persist in evolutionary time when there are (apparently) no net nutritional 'benefits': there are actual benefits, as discussed above. Suppression of direct P uptake through the roots shows that AM fungi have considerable control over the symbiosis, at least in soils with low or moderate amounts of plant-available P, showing that is over-simple to believe that the plant controls the symbiosis just because the AM fungus has obligate dependence on it for survival (e.g. Fitter 2006). However, there is no doubt that plants can, in part, control levels of colonisation, especially with high soil-P but also depending on other soil factors such as pH (e.g., Abbott and Robson 1985).

Can benefits of AM symbiosis in the field be increased?

This is the question on which many researchers have focused since the beginning of the experimental phase of research into AM symbiosis. There have been ongoing changes in emphasis in different parts of the world, reflecting on needs on the one hand to develop 'sustainable' management systems that will reduce environmental impacts of excessive inputs of fertiliser and pesticides and on the other to maximise yields of food while minimising production costs. Underlying many initiatives is awareness that P fertiliser is a non-renewable resource, with increasing price and (eventually) decreasing quality (Cordell et al. 2009).

Research has addressed two interacting aspects: 1) managing AM populations in the field, and 2) identifying and utilising plants in which well developed AM symbiosis provides strong benefits in terms of growth and yield, compared with plants where AM symbiosis is poorly developed. There is little doubt that AM populations in the field can be enhanced by managing soils and crops. Expected AM benefits will vary considerably, depending on the farming systems in question, and encompass not only yield, but also economic or environmental benefits. Plenchette et al. (2005) provided four case-studies of the roles, management and possible benefits of arbuscular mycorrhizas in situations ranging from extremely low fertiliser-input, subsistence farming where the target outcomes should be increased food production, to high input cereal and livestock production where the significant issues were offsite pollution. The most recent European Union (EU) COST Action (Food and Agriculture Action (FA) 870) had the major objective of 'tak(ing) a multidisciplinary approach to increase the knowledge needed for implementation of arbuscular mycorrhizal fungi in agricultural systems, *in order to reduce agricultural inputs and reduce losses to the environment.*' Here (our) italics reflect changing attitudes toward sustainable systems that minimise waste and maximise economic benefits, rather than increasing efficiency of food production. The changed viewpoint may require some modification with the recognition that food security remains a significant issue worldwide. Secondary objectives of the EU COST Action program are to identify plant genes which control the responsiveness of crop plants to AM fungi' and to 'facilitate the development of AM fungal inoculum with specificity for specific crops under different soil conditions and fertilisation regimes'. These objectives recognise that 'inherent' responsiveness of a crop to both AM symbiosis and P fertilisation is a very important consideration. As we have emphasised above, determination of MGR in the field is very difficult. However, there is sufficient evidence from pot experiments that for crops that characteristically have positive MGRs (e.g. maize, soybean (*Glycine max*), faba bean (*Vicia faba*), cowpea (*Vigna unguiculata*) and pasture legumes such as subterranean clover, to name but a few) soil and crop management may lead to positive benefits. These may include lower P fertiliser application to achieve good yields, as suggested by Abbott and

Robson (1991). Management approaches may therefore provide economic benefits (Miller et al. 1994), as well as minimising contamination by nutrient losses.

High populations of AM inoculum in soil are important to achieve rapid colonisation of plant roots and growth and yield benefits, and can be encouraged by introduction of minimum or zero tillage or inclusion of pasture leys or break crops that are AM, avoidance of long periods of bare fallow, burning of crop residues or frequent cropping with non-host (NM) species such as members of the Brassicaceae or Chenopodiaceae (e.g. canola or beet) (Abbott and Robson 1991). As shown in Queensland, pre-cropping with canola effectively reduced populations of AM fungi and also of parasitic nematode populations (Owen et al. 2010). The outcome of the pre-cropping was decreased wheat yield, because the variety used (cv Batavia) was responsive to AM colonisation with the fertilisers applied, despite being nematode-susceptible and intolerant. Owen et al. (2010) emphasised that outcomes might be even more deleterious when AM-responsive plants, such as cotton (*Gossypium hirsutum*), sorghum, maize, chickpea (*Cicer arietinum*) and faba bean followed the canola. As was pointed out, decreased yield will depend on 1) the degree of reduction of AM fungal inoculum caused by fallow or pre-cropping with a NM crop, 2) the level of P (and Zn) in the soil, and 3) the underlying AM responsiveness of the crop species. Clearly, where crop varieties have little underlying responsiveness decreased inoculum intensity and colonisation will not be a factor in growth and yield of a selected crop, but may be in a later rotation. However, generalization is dangerous, as shown by the detailed studies by Ryan and associates in SE Australia with crop varieties currently in use.

Where previous management has resulted in very low inoculum densities or where soils are fumigated to remove pathogens, then inoculation with AM fungi may be warranted. This will also almost certainly be the case in using newly-formed soils for agriculture, horticulture and forestry. However, identification of particularly 'beneficial' strains or combinations of strains of AM fungi has proved difficult, and as with many (if not all) microbial soil inoculants a major issue is ensuring survival of inoculated strains in the complex and competitive rhizosphere (Richardson 2009). Identification of AM fungal strains with specificity for particular crops and soil conditions poses a challenge, not least because those AM fungi that are easily propagated and suitable for inoculum production appear to have little specificity with respect to the plant taxa that they are able to colonise. An additional concern is production of high quality inoculum of AM fungi, which as obligate symbionts must be grown on host species. Quality control in terms of infectivity, absence of pathogens and growth promoting effects in relation to dosage is essential and well recognised, but effective guidelines for the expanding industry have not yet been adopted. Relatively high costs of production will probably limit inoculum application mainly to nursery-scale inoculation before transplanting or to field-scale inoculation only of high-value crops for the foreseeable future. There are still few sources that have been used with benefit in terms of increased yield in broad-scale cropping systems. This conclusion remains unchanged over several decades, although inoculation is being tested across the world. For example, Mäder et al. (2010) obtained significant increases in yield of wheat over 2 years at several sites (5×5 m plots) in India after applying AM fungal inoculum combined with plant growth-promoting rhizobacteria. Progress and prospects of inoculum production and use have recently been critically assessed (IJdo et al. 2011); we strongly recommend this review.

For crops that characteristically show little MGR in particular regions or cropping systems, genetic approaches to increasing contributions of AM symbiosis to yield may be warranted. The aims would be to increase yield, while optimising uptake of plant-available P stored in soil and minimising the need for high fertiliser applications, with both economic and environmental benefits. Approaches could include identification and adoption of plant varieties that do show positive MGR, as well as breeding for increased MGR. However, real progress will not be made unless there is a thorough understanding of the underlying mechanisms, and hence genes, including those involved in AM growth depressions. There is no doubt that AM fungi do contribute to nutrition (particularly P uptake) in poorly responsive crops and that there is concurrent loss of uptake by the direct pathway. We have challenged, as above, the established hypothesis that growth depressions are caused by excessive C use and proposed that they may be based on P deficiency, because the AM fungal uptake pathway fails to compensate for direct P uptake via

the roots. Revealing aspects of fungus-plant interaction, including signalling, that underpin lower direct uptake should be a research priority. The aim would be to make AM and direct uptake additive, rather than alternative.

Genetic approaches to maximising MGR need to be evaluated alongside other approaches to increase P uptake, some of which are genetic (such as root architecture, root-hair development and increased rhizosphere production of organic acids etc), and others which are based on microbial inoculants that we have not considered here.

Conclusions

Here we have tried to show that there is a strong need for researchers, whether laboratory- or field-oriented, to appreciate that there should be a 'research symbiosis' that includes a continuum between laboratory-scale research and field-scale research. Unfortunately the comment by David Read that relevance of mycorrhizal research is correlated with the scale of research (Read 2002) was hardly an encouragement to field-oriented researchers, whether focusing on natural or managed ecosystems, to pay attention to laboratory studies of AM function. At the very least, new knowledge from laboratory-scale research should not be ignored in attempts to explain field-scale research in functional terms, including reasons for associations between crop yield and AM symbiosis, whether positive or negative. In relation to this Special Volume of Plant and Soil, the research involving AM symbioses to which Alan Robson has contributed has always been set within the context of relevance to agriculture, as shown by the examples that we have cited. His name is hidden in the text in many examples of 'et al.'

Finally, it must not be forgotten that AM symbiosis is the default situation for most crop plants in the field. In consequence, the P fertiliser recommendations based on field trials inevitably incorporate any effects of AM symbioses, and identification of plant traits for P uptake or use efficiency will be potentially obscured by any AM fungal contribution and by alterations in that contribution as a result of fertiliser applications. Furthermore, AM symbioses may provide benefits unrelated to yield or P fertiliser use, and include tolerance to disease and drought, improved soil structure and C sequestration in soil. In other words they are fundamentally involved in the wide range of resources in both natural and managed ecosystems, nowadays called ecosystem services (Gianinazzi et al. 2010).

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References

Abbott LK, Robson AD (1977) Growth stimulation of subterranean clover with vesicular arbuscular mycorrhizas. Aust J Agric Res 28:639–649

Abbott LK, Robson AD (1978) Growth of subterranean clover in relation to the formation of endomycorrhizas by introduced and indigenous fungi in a field soil. New Phytol 81:575–585

Abbott LK, Robson AD (1982) The role of vesicular arbuscular mycorrhizal fungi in agriculture and the selection of fungi for inoculation. Aust J Agric Res 33:389–408

Abbott LK, Robson AD (1985) The effect of soil pH on the formation of VA mycorrhizas by two species of *Glomus*. Aust J Soil Res 23:253–261

Abbott LK, Robson AD (1991) Field management of VA mycorrhizal fungi. In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth. Kluwer Academic Publishers, Dordrecht, pp 355–362

Abbott LK, Robson AD, Scheltema MA (1995) Managing soils to enhance mycorrhizal benefits in Mediterranean agriculture. Crit Rev Biotech 15:213–228

Ames RN, Reid CPP, Porter L, Cambardella C (1983) Hyphal uptake and transport of nitrogen from two ^{15}N-labelled sources by *Glomus mosseae*, a vesicular-arbuscular mycorrhizal fungus. New Phytol 95:381–396

Balzergue C, Peuch-Pagès V, Bécard G, Rochange SF (2011) The regulation of arbuscular mycorrhizal symbiosis by phosphate in pea involves early and systemic signalling events. J Exp Bot 62:1049–1060

Baon JB, Smith SE, Alston AM, Wheeler RD (1992) Phosphorus efficiency of three cereals as related to indigenous mycorrhizal infection. Aust J Agric Res 43:479–491

Baylis GTS (1970) Root hairs and phycomycetous mycorrhizas in phosphorus-deficient soil. Plant Soil 33:713–716

Bethlenfalvay GJ, Brown MS, Pacovsky RS (1982) Parasitic and mutualistic asssociations between a mycorrhizal

fungus and soybean: development of the host plant. Phytopathology 72:889–893
Bolan NS (1991) A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant Soil 134:189–207
Bolan NS, Robson A, Barrow NJ, Aylemore LAG (1984) Specific activity of phosphorus in mycorrhizal and non-mycorrhizal plants in relation to the availability of phosphorus to plants. Soil Biol Biochem 16:299–304
Bolan NS, Robson AD, Barrow NJ (1987a) Effects of phosphorus application and mycorrhizal inoculation on root characteristics of subterranean clover and ryegrass in relation to phosphorus uptake. Plant Soil 104:294–298
Bolan NS, Robson AD, Barrow NJ (1987b) Effects of vesicular-arbuscular mycorrhiza on the availability of iron phosphates to plants. Plant Soil 99:401–410
Bürkert B, Robson A (1994) ^{65}Zn uptake in subterranean clover (*Trifolium subterraneum* L.) by three vesicular-arbuscular mycorrhizal fungi in a root-free sandy soil. Soil Biol Biochem 26:1117–1124
Cardoso I, Kuyper TW (2006) Mycorrhizas and tropical soil fertility. Agr Ecosyst Environ 116:72–84
Cavagnaro TR, Smith FA, Hay G, Carne-Cavagnaro VL, Smith SE (2004) Inoculum type does not affect overall resistance of an arbuscular mycorrhiza-defective tomato mutant to colonisation but inoculation does change competitive interactions with wild-type tomato. New Phytol 161:485–494
Cavagnaro TR, Jackson LE, Six J, Ferris H, Goyal S, Asami D, Scow KM (2006) Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. Plant Soil 282:209–225
Christophersen HM, Smith FA, Smith SE (2009) Arbuscular mycorrhizal colonization reduces arsenate uptake in barley via downregulation of transporters in the direct epidermal phosphate uptake pathway. New Phytol 184:962–974
Cordell D, Drangerta J-O, White S (2009) The story of phosphorus: global food security and food for thought. Global Environ Chang 19:292–305
Cornish PS (2009) Research directions: improving plant uptake of soil phosphorus, and reducing dependency on input of phosphorus fertiliser. Crop Past Sci 60:190–196
Facelli E, Smith SE, Facelli JM, Christophersen HM, Smith FA (2010) Underground friends or enemies: model plants help to unravel direct and indirect effects of arbuscular mycorrhizal fungi on plant competition. New Phytol 185:1050–1061
Fitter AH (2006) What is the link between carbon and phosphorus fluxes in arbuscular mycorrhizas? A null hypothesis for symbiotic function. New Phytol 172:3–6
Frey B, Schüepp H (1993) Acquisition of nitrogen by external hyphae of arbuscular mycorrhizal fungi associated with *Zea mays* L. New Phytol 124:221–230
Frossard E, Achat DL, Bernasconi SM, Bünemann EK, Fardeau J-C, Jansa J, Morel C, Rabeharisoa L, Randrimanantsoa L, Sinaj S, Tamburini F, Oberson A (2011) The use of tracers to invesigate phosphate cycling in soil-plant systems. In: Bünemann EK, Obserson A, Frossard E (eds) Phosphorus in action: Biological processes in soil phosphorus cycling. Springer, Heidelberg, pp 59–91
Gahoonia TS, Nielsen NE (2004) Root traits as tools for creating phosphorus efficient crop varieties. Plant Soil 260:47–57
Gavito M, Bruhn D, Jakobsen I (2002) Phosphorus uptake by arbuscular mycorrhizal hyphae does not increase when the host plant grows under atmospheric CO_2 enrichment. New Phytol 154:751–760
George E, Marschner H, Jakobsen I (1995) Role of arbuscular mycorrizal fungi in uptake of phosphorus and nitrogen from soil. Crit Rev Biotech 15:257–270
Gianinazzi S, Gollotte A, Binet M-N, van Tuinen D, Redecker D, Wipf D (2010) Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20:519–530
Grace EJ, Cotsaftis O, Tester M, Smith FA, Smith SE (2009) Arbuscular mycorrhizal inhibition of growth in barley cannot be attributed to extent of colonization, fungal phosphorus uptake or effects on expression of plant phosphate transporter genes. New Phytol 181:938–949
Harley JL (1959) The biology of mycorrhiza. Leonard Hill, London
Hetrick BAD, Wilson GWT, Cox TS (1992) Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors. Can J Bot 70:2032–2040
Hetrick BAD, Wilson GWT, Cox TS (1993) Mycorrhizal dependance of modern wheat cultivars and ancestors: a synthesis. Can J Bot 71:512–518
Hetrick BAD, Wilson GWT, Todd TC (1996) Mycorrhizal response in wheat cultivars: relationship to phosphorus. Can J Bot 74:19–25
IJdo M, Cranenbrouck S, Declerck S (2011) Methods for large scale production of AM fungi: past, present and future. Mycorrhiza 21:1–16
Jakobsen I (1986) Vesicular-arbuscular mycorrhiza in field-grown crops. III. Mycorrhizal infection and rates of phosphorus inflow in pea plants. New Phytol 104:573–581
Jansa J, Mozafar A, Frossard E (2003) Long-distance transport of P and Zn through the hyphae of an arbuscular mycorrhizal fungus in symbiosis with maize. Agronomie 23:481–488
Javot H, Pumplin N, Harrison MJ (2007) Phosphate in the arbuscular mycorrhizal symbiosis: transport properties and regulatory roles. Plant Cell Environ 30:310–322
Jin H, Pfeffer P, Douds D, Piotrowski E, Lammers P, Shachar Hill Y (2005) The uptake, metabolism, transport and transfer of nitrogen in an arbuscular mycorrhizal symbiosis. New Phytol 168:687–696
Johansen A, Jakobsen I, Jensen ES (1992) Hyphal transport of ^{15}N-labelled nitrogen by a vesicular-arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. New Phytol 122:281–288
Johansen A, Jakobsen I, Jensen ES (1994) Hyphal N transport by a vesicular-arbuscular mycorrhizal fungus associated with cucumber grown at three nitrogen levels. Plant Soil 160:1–9
Johnson NC (1993) Can fertilization of soil select less mutualistic mycorrhizae? Ecol Appl 3:749–747
Johnson NC (2010) Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas accross scales. New Phytol 185:631–647
Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol 135:575–586
Joner EJ, Jakobsen I (1994) Contribution by two arbuscular mycorrhizal fungi to P uptake by cucumber (*Cucumis*

sativus L.) from ^{32}P-labelled organic matter during mineralization in soil. Plant Soil 163:203–209

Joner EJ, Jakobsen I (1995) Uptake of P-32 from labelled organic matter by mycorrhizal and non-mycorrhizal subterranean clover (*Trifolium subterraneum* L). Plant Soil 172:221–227

Joner EJ, Magid J, Gahoonia TS, Jakobsen I (1995) P depletion and activity of phosphatases in the rhizosphere of mycorrhizal and non-mycorrhizal cucumber (*Cucumis sativus* L.). Soil Biol Biochem 27:1145–1151

Joner EJ, Ravnskov S, Jakobsen I (2000) Arbuscular mycorrhizal phosphate transport under monoxenic conditions using radio-labelled inorganic and organic phosphate. Biotechnol Lett 22:1705–1708

Kiers ET, West SA, Denison RF (2002) Mediating mutualisms: farm management practices and evolutionary changes in symbiont co-operation. J Appl Ecol 39:745–754

Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301

Kothari SK, Marschner H, George E (1990) Effect of VA mycorrhizal fungi and rhizosphere microorganisms on root and shoot morphology, growth and water relations in maize. New Phytol 116:303–311

Lekberg Y, Koide R (2005) Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. New Phytol 168:189–204

Li HY, Zhu YG, Marschner P, Smith FA, Smith SE (2005) Wheat responses to arbuscular mycorrhizal fungi in a highly calcareous soil differ from those of clover, and change with plant development and P supply. Plant Soil 277:221–232

Li HY, Smith SE, Holloway RE, Zhu YG, Smith FA (2006) Arbuscular mycorrhizal fungi contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the absence of positive growth responses. New Phytol 172:536–543

Li HY, Smith FA, Dickson S, Holloway RE, Smith SE (2008) Plant growth depressions in arbuscular mycorrhizal symbiosis: not just caused by carbon drain? New Phytol 178:852–862

Lynch JP (2007) Roots of the second green revolution. Aust J Bot 55:493–512

Mäder P, Kaiser F, Adholeya A, Singh R, Uppal HS, Sharma SR, Sahai V, Aragno M, Wiemken A, Johri BN, Fried PM (2010) Inoculation of root microorganisms for sustainable wheat-rice and wheat-black gram rotations in India. Soil Biol Biochem 43:609–619

Maillet F, Poinsot O, Pouch-Pagès AV, Haouy A, Gueunier M, Cromer L, Giraudet D, Formey D, Niebel A, Martinez EA, Driguez H, Bécard G, Dénarié J (2011) Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature 469:58–63

Marschner H (1995) Mineral nutrition of higher plants. Academic, London, p 889

Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159:89–102

Miller M, McGonigle T, Addy H (1994) An economic approach to evaluate the role of mycorrhizas in managed ecosystems. Plant Soil 159:27–35

Mosse B (1973) Plant growth responses to vesicular-arbuscular mycorrhiza. IV. In soil given additional phosphate. New Phytol 72:127–136

Nagy R, Drissner D, Amrhein N, Jakobsen I, Bucher M (2009) Mycorrhizal phosphate uptake pathway in tomato is phosphorus-repressible and transcriptionally regulated. New Phytol 181:950–959

Oliver AJ, Smith SE, Nicholas DJD, Wallace W, Smith FA (1983) Activity of nitrate reductase in *Trifolium subterraneum*: effects of mycorrhizal infection and phosphate nutrition. New Phytol 94:63–79

Orfanoudakis M, Wheeler CT, Hooker JE (2010) Both the arbuscular mycorrhizal fungus *Gigaspora rosea* and *Frankia* increase root system branching and reduce root hair frequency in *Alnus glutinosa*. Mycorrhiza 20:117–126

Owen KJ, Clewett TG, Thompson JP (2010) Pre-cropping with canola decreased *Pratylenchus thornei* populations, arbuscular mycorrhizal fungi, and yield of wheat. Crop Past Sci 61:399–410

Pate JS, Stewart GR, Unkovich M (1993) ^{15}N natural abundance of plant and soil components of a *Banksia* woodland ecosystem in relation to nitrate utilization, life form, mycorrhizal status and N_2-fixing abilities of component species. Plant Cell Environ 16:365–373

Plenchette C, Fortin JA, Furlan V (1983) Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. II. Soil fumigation induced stunting of plants corrected by reinoculation of the wild endomycorrhiza flora. Plant Soil 70:211–217

Plenchette C, Clermont-Dauphin C, Meynard JM, Fortin JA (2005) Managing arbuscular mycorrhizal fungi in cropping systems. Can J Plant Sci 85:31–40

Read DJ (2002) Towards ecological relevance-progress and pitfalls in the path towards and understanding of mycorrhizal functions in nature. In: van der Heijden MGA, Sanders IR (eds) Mycorrhizal ecology. Springer-Verlag, Berlin, pp 3–29

Rhodes LH, Gerdemann JW (1980) Nutrient translocation in vesicular-arbuscular mycorrhizae. In: Cook CB, Pappas PW, Rudolph ED (eds) Cellular interactions in symbiosis and parasitism. Ohio State University Press, Columbus, pp 173–195

Richardson AE (2009) Regulating the phosphorus nutrition of plants: molecular biology meeting agronomic needs. Plant Soil 322:17–24

Rillig MC, Ramsey PW, Gannon JE, Mummey DL, Gadkar V, Kapulnik Y (2008) Suitability of mycorrhiza-defective mutant/wildtype plant pairs (*Solanum lycopersicum* L. cv Micro-Tom) to address questions in mycorrhizal soil ecology. Plant Soil 308:267–275

Robson AD, Abbott LK, Malajczuk N (eds) (1994) Management of mycorrhizas in agriculture, horticulture and forestry. Kluwer, Dordrecht, p 238

Ryan MH, Graham JH (2002) Is there a role for arbuscular mycorrhizal fungi in production agriculture? Plant Soil 244:263–271

Ryan MH, Angus JF (2003) Arbuscular mycorrhizae in wheat and field pea crops on a low P soil: increased Zn-uptake but no increase in P-uptake or yield. Plant Soil 250:225–239

Ryan MH, McCully ME, Huang CX (2003) Location and quantification of phosphorus and other elements in fully hydrated, soil-grown arbuscular mycorrhizas: a cryo-analytical scanning electron microscopy study. New Phytol 160:429–441

Ryan MH, van Herwaarden AF, Angus JF, Kirkegaard JA (2005) Reduced growth of autumn-sown wheat in a low-P soil is associated with high colonisation by arbuscular mycorrhizal fungi. Plant Soil 270:275–286

Ryan MH, McCully ME, Huang CX (2007) Relative amounts of soluble and insoluble forms of phosphorus and other elements in intraradical hyphae and arbuscules of arbuscular mycorrhizas. Funct Plant Biol 34:457–464

Sanders FE (1975) The effect of foliar-applied phosphate on the mycorrhizal infections of onion roots. In: Sanders FE, Mosse B, Tinker PB (eds) Endomycorrhizas. Academic, London, pp 261–276

Schweiger PF, Jakobsen I (1999) Direct measurement of arbuscular mycorrhizal phosphorus uptake into field-grown winter wheat. Agron J 91:998–1002

Schweiger P, Robson A, Barrow N (1995) Root hair length determines beneficial effect of a *Glomus* species on shoot growth of some pasture species. New Phytol 131:247–254

Schweiger PF, Spliid NH, Jakobsen I (2001) Fungicide application and phosphorus uptake by hyphae of arbuscular mycorrhizal fungi into field-grown peas. Soil Biol Biochem 33:1231–1237

Smith SSE (1980) Mycorrhizas of autotrophic higher plants. Biol Rev 55:475–510

Smith SE (1982) Inflow of phosphate into mycorrhizal and non-mycorrhizal plants of *Trifolium subterraneum* at different levels of soil phosphate. New Phytol 90:293–303

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic, New York, p 787

Smith SE, Smith FA (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosytems scales. Annu Rev Plant Biol 62:227–250

Smith SE, Nicholas DJD, Smith FA (1979) Effect of early mycorrhizal infection on nodulation and nitrogen fixation in *Trifolium subterraneum* L. Aust J Plant Physiol 6:305–316

Smith SE, Robson AD, Abbott LK (1992) The involvement of mycorrhizas in assessment of genetically dependent efficiency of nutrient uptake and use. Plant Soil 146:169–179

Smith SE, Smith FA, Jakobsen I (2003) Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiol 133:16–20

Smith SE, Smith FA, Jakobsen I (2004) Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. New Phytol 162:511–524

Smith FA, Grace EJ, Smith SE (2009) More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses. New Phytol 182:347–358

Smith SE, Facelli E, Pope S, Smith FA (2010) Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. Plant Soil 326:3–20

Smith SE, Jakobsen I, Grønlund M, Smith FA (2011) Roles of arbuscular mycorrhizas in plant phosphorus (P) nutrition: interactions between pathways of P uptake in arbuscular mycorrhizal (AM) roots have important implications for understanding and manipulating plant P acquisition. Plant Physiol doi:10.1104/pp.111.174581

Tarafdar JC, Marschner H (1994a) Efficiency of VAM hyphae in utilisation of organic phosphorus by wheat plants. Soil Sci Plant Nutr 40:593–600

Tarafdar JC, Marschner H (1994b) Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. Soil Biol Biochem 26:387–395

Tawaraya K (2003) Arbuscular mycorrhizal dependency of different plant species and cultivars. Soil Sci Plant Nutr 49:655–668

Tawaraya K, Naito M, Wagastuma T (2006) Solubilization of insoluble inorganic phosphate by hyphal exudates of arbuscular mycorrhizal fungi. J Plant Nutr 29:657–665

Thingstrup I, Rubaek G, Sibbesen E, Jakobsen I (1998) Flax (*Linum usitatissimum* L.) depends on arbuscular mycorrhizal fungi for growth and P uptake at intermediate but not high soil P levels in the field. Plant Soil 203:37–46

Thompson JP (1987) Decline of vesicular-arbuscular mycorrhizae in Long Fallow Disorder of field crops and its expression in phosphorus deficiency of sunflower. Aust J Agric Res 38:847–867

Thomson BD, Robson AD, Abbott LK (1986) Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. New Phytol 103:751–765

Wilson GWT, Hartnett DC (1998) Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. Am J Bot 85:1732–1738