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«Οι σύγχρονες τεχνικές βιο-ανάλυσης στην υγεία, τη γεωργία, το περιβάλλον και τη διατροφή»

Roles of Arbuscular Mycorrhizas in Plant Nutrition and Growth: New Paradigms from Cellular to Ecosystem Scales

Sally E. Smith and F. Andrew Smith

Soils Group, School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Adelaide, South Australia 5005, Australia; email: sally.smith@adelaide.edu.au, andrew.smith@adelaide.edu.au

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plant nutrient uptake pathways, phosphorus uptake, nitrogen uptake, mycorrhizal growth responses

Abstract

Root systems of most land plants form arbuscular mycorrhizal (AM) symbioses in the field, and these contribute to nutrient uptake. AM roots have two pathways for nutrient absorption, directly through the root epidermis and root hairs and via AM fungal hyphae into root cortical cells, where arbuscules or hyphal coils provide symbiotic interfaces. New physiological and molecular evidence shows that for phosphorus the mycorrhizal pathway (MP) is operational regardless of plant growth responses (positive or negative). Amounts delivered cannot be determined from plant nutrient contents because when responses are negative the contribution of the direct pathway (DP) is reduced. Nitrogen (N) is also delivered to roots via an MP, but the contribution to total N requirement and the costs to the plant are not clear. The functional interplay between activities of the DP and MP has important implications for consideration of AM symbioses in ecological, agronomic, and evolutionary contexts.

Contents

Glomeromycota: the phylum of fungi to which the arbuscular mycorrhizal fungi belong

INTRODUCTION

Arbuscular mycorrhizas, which involve approximately 80% of terrestrial plant species and obligately symbiotic fungi in the phylum Glomeromycota, are the most common and widespread terrestrial plant symbioses. They are extremely ancient (>450 million years), representing a very long period of coevolution and indicating considerable selective advantage of the symbiosis for both partners (134). Arbuscular mycorrhizal (AM) symbioses are biotrophic and also (usually) mutualistic, based on bidirectional transfers of organic carbon (C) from the plant and soil-derived nutrients [particularly phosphorus (P) but also nitrogen (N) and zinc (Zn) from the fungi] (76, 134).

With the exception of plants that form other types of mycorrhiza (ecto-, ericoid, and orchid mycorrhiza) and the relatively few species that are never mycorrhizal (151), the AM condition is normal for plants growing in most field situations. The nonmycorrhizal (NM) condition is found naturally only under extreme soil conditions (e.g., highly disturbed or waterlogged soils) and is therefore not usually the control situation but rather the treatment (as with plants grown experimentally in sterilized soil). Recognition that the NM state is very unusual in nature should alter perspectives of the roles of AM in plant function and their evolutionary persistence.

The last review in this series specifically addressing physiology of AM symbioses was published over 20 years ago (132). Since then cellular and molecular research has led to enormous advances in knowledge of signaling and cellular interactions between the symbionts, control of development of AM symbioses, and the expression and function of genes involved (10, 12, 50, 106, 107). Ecologists have increasingly become aware of the likely significance of AM symbioses in nature but have (mainly) tended to ignore underground symbiosis-driven processes, even though effective prediction of plant responses to changed conditions (e.g., competition) requires an understanding of mechanisms (74). Together with agronomists, they have often relied on well-entrenched functional models to interpret potential roles of arbuscular mycorrhizas in plant interactions and productivity. Physiological experiments over the past 10–15 years, coupled with molecular biology

and advanced microscopy, have provided new information that has overturned many aspects of these established models. This new information includes the range of fungal structures formed between AM fungi and plant roots (22); the diversity of growth responses to AM colonization, from highly positive to negative (75, 76); and the significance and contribution of the mycorrhizal uptake pathway in delivering nutrients (particularly P) to plants, regardless of whether they respond positively or not (126). We now bring together this new research to provide a better picture of the integration of plant and AM fungal nutritional processes that contribute to plant growth and productivity. The outcomes have important implications for understanding AM symbiosis at scales from cellular through whole plant to ecological interactions.

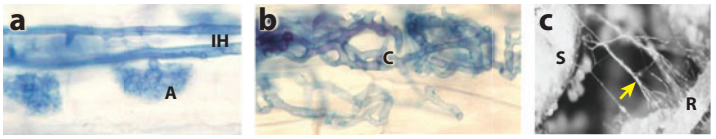

Figure 1

Photomicrographs of arbuscular mycorrhizal colonization of tomato roots (*a*,*b*) and extraradical mycelium (*c*). (*a*) Intercellular hyphae (IH) of *Glomus intraradices* leading to arbuscules (A) in cortical cells; (*b*) intracellular hyphal coils (C) of *Gigaspora rosea*; (*c*) extraradical mycelium of an AM fungus (*arrow*) growing between a root (R) and a soil particle (S). Panels (*a*,*b*) are from Smith et al. (138), reproduced with permission of *New Phytologist*. Panel (*c*) is from Olsson et al. (103), reproduced with permission of Springer-Verlag.

STRUCTURAL DIVERSITY IN ARBUSCULAR MYCORRHIZAL SYMBIOSES: FUNCTIONAL IMPLICATIONS

Intraradical Colonization

An AM fungus lives in two environments, the root from which it receives organic C and to which it delivers nutrients, and the soil from which it absorbs those nutrients. The intraradical mycelium (IRM) grows in an environment controlled by plant homeostasis, whereas the extraradical mycelium (ERM) encounters considerable environmental variations, such as soil pH, nutrient availability, and soil moisture.

Colonization of roots by AM fungi involves subtle signaling between the symbionts, leading to expression of key genes and tightly programmed cellular events (10, 50, 106, 107). The outcome is considerable fungal growth in the root cortex, where interfaces involved in nutrient exchange develop. A varied range of structures is formed by AM fungi in the roots of plants, as first highlighted by Gallaud (32). Use of a relatively small number of species of plants and AM fungi led to the belief that arbuscules, which are terminal, dichotomously branched, intracellular fungal structures (**Figure 1*a***), are the sole defining feature of an arbuscular mycorrhiza. Dependence on arbuscules for definitive identification of an AM root and failure to recognize the common occurrence and importance of intracellular coiled hyphae (**Figure 1*b***) as alternative AM structures has almost certainly led to underestimation of the number of plant species that form arbuscular mycorrhizas in nature (128). Demonstration that hyphal coils, arbuscules, and intermediate structures are involved in the nutrient transfers that underpin a functional symbiosis has been a major step forward (16, 21, 22, 33, 78, 128). Experiments show that identities (and hence genomes) of both plant and fungal partners determine the mycorrhizal type (14, 21, 22).

Intracellular fungal growth involves development of specialized cytoplasmic assemblies that ultimately lead to formation of symbiotic interfaces, including marked invagination, increase in surface area of contact, and modification of the plant plasma membrane to form a perifungal membrane [or periarbuscular membrane (PAM) when associated with arbuscules] (33, 34). The fungus remains outside the plant cytoplasm such that the symbiotic interfaces involve plasma membranes of both fungus and plant, separated by an apoplastic compartment (**Figure 2**) that has an acidic pH and contains some modified plant wall material (7, 136). The plant membranes are strongly modified, particularly in association with arbuscule formation. Variations in location of specialized membrane domains surrounding AM structures of

Symbiosis: the living together of two dissimilar organisms; includes a spectrum of interactions from beneficial to detrimental

Biotrophic: a symbiotic organism that obtains nutrients from the living cells of its partner

Mutualistic: a symbiosis that is beneficial to both partners

Mycorrhiza: literally "fungus-root," a symbiosis between specialized soil fungi and roots or other underground organs of land plants

Mycelium: network of branching hyphae

Arbuscule: a highly branched structure formed inside root cells by arbuscular mycorrhizal fungi; creates a large interface between fungus and plant

Hyphae: long, branching, tubular structures formed by fungi

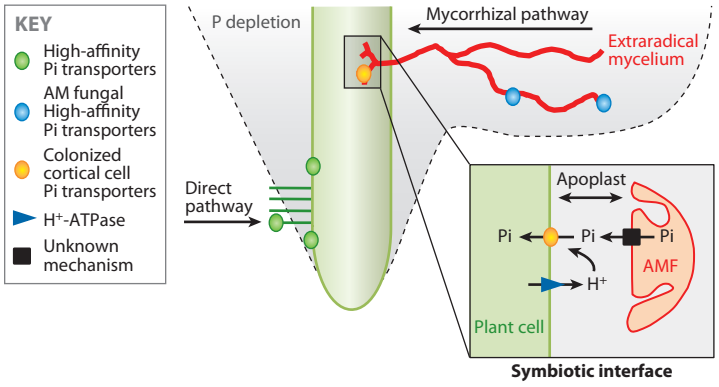

Figure 2

Diagrammatic representation of the direct and mycorrhizal orthophosphate (Pi) uptake pathways in an arbuscular mycorrhizal (AM) root. The direct pathway (DP) involves high-affinity Pi transporters located in root hairs and epidermal cells near the root apex. DP activity results in progressive depletion of Pi concentration close to roots (*dashed black line*) because uptake is faster than replacement by diffusion or mass flow. The mycorrhizal pathway (MP) develops behind the root hair zone. It involves uptake of Pi by AM fungal high-affinity Pi transporters in the extraradical mycelium (ERM), followed by translocation of phosphorus (P) along the hyphae to intracellular structures in the root cortex and transfer to the root. Inset shows transfer across the symbiotic interface, which involves efflux of Pi from the arbuscular mycorrhizal fungus (or AMF, by unknown mechanism; *black square*) into the apoplast and uptake into the plant cells by Pi transporter(s) that are preferentially or specifically expressed in colonized cortical cells. H^+-ATPases are involved at all Pi-uptake steps (shown only in the symbiotic interface). Activity of the MP results in extension of the phosphate depletion zone as far as the ERM extends. The MP may also operate in nitrogen (N) uptake (see also **Figure 4**). Based on a diagram by E.J. Grace.

different types (arbuscules, coils, and intercellular hyphae when present) probably occur (78, 110). The consequences of variation remain to be fully explored, but a key conclusion is that arbuscules are not the only AM fungal structures having significant functional interfaces with plant cortical cells. Physiological, molecular, and field studies must include awareness of this diversity to gain a better picture of the occurrence and function of different types of AM colonization of wild and cultivated plants.

Development of Arbuscular Mycorrhizal Fungal Mycelium in Soil

The ERM (**Figure 1*c***) plays critical roles in uptake and rapid translocation of nutrients to the intraradical structures and in foraging to locate new roots on the same or different plants, which are new sources of organic C (103). Mycelia produced by different fungi have quite varied characteristics, in terms of hyphal diameters (usually in the range of 2–20 μm), extent of growth away from the root, and ability to absorb nutrients at a distance [up to 25 cm (65)] and translocate them to the root (23, 63, 99, 127). Many AM fungi produce runner hyphae of relatively large diameter that can subtend tufts of finely branched hyphae; the latter turn over rapidly and are probably involved in nutrient uptake (4). Hyphal length densities in soil associated with plants in pot experiments are variable and usually in the range of 1–40 m g^{-1} depending at least in part on the identity of the AM fungus (61, 99, 138). They are very much higher than the root length densities of associated plants [e.g., 2.6 versus 0.04 m g^{-1} for AM fungal hyphae and wheat roots, respectively (89)], emphasizing how effectively the fungi can explore soil. Implications of variability in structure and function of the ERM are becoming recognized, and it appears that where several fungi colonize a root (as is normal in the field), their nutrient acquisition activities are complementary (66, 81).

The ERM may be associated with several plants of the same or different species, forming an interconnected network (62, 134). Hyphae from the same fungal mycelium, and sometimes from different isolates of the same species, can anastomose (fuse) frequently. This process allows for exchange of nuclei, network repair, and fusion of two or more separate mycelia into larger units facilitating transfer of phosphorus (P) (2, 62, 97). The extent of sharing of costs and benefits of a common mycelial network among the symbionts requires further research.

Root and Fungus Provide Two Pathways for Nutrient Uptake

An AM root superficially retains many of the structural features of an NM root. Root apex, epidermis, root hairs, and lateral root branches remain recognizable. Root hairs still occur on AM roots, although their length and density may be lower than in equivalent NM plants

(82, 104). In the context of nutrient uptake, the soil-root interface provides the direct pathway (DP), in contrast to the mycorrhizal pathway (MP) (**Figure 2**). The latter involves uptake by the ERM and rapid translocation, sometimes for many centimeters, to the IRM. Delivery is followed by nutrient export from the fungus across the interfacial apoplast to the plant. The perifungal membrane contains orthophosphate (Pi) and NH_4^+ transporters that are preferentially or specifically expressed in AM roots (12, 47, 68, 110). Likewise, H^+-ATPases energize perifungal membranes that surround both arbuscules and intracellular coils (37, 38, 83, 116, 136). Overall, the MP is a highly regulated, rapid transit system delivering nutrients that were absorbed considerable distances away from roots by the ERM directly into cortical cells. This contrasts with the DP, which absorbs nutrients from the immediate vicinity of the root (rhizosphere) into epidermal and root hair cells.

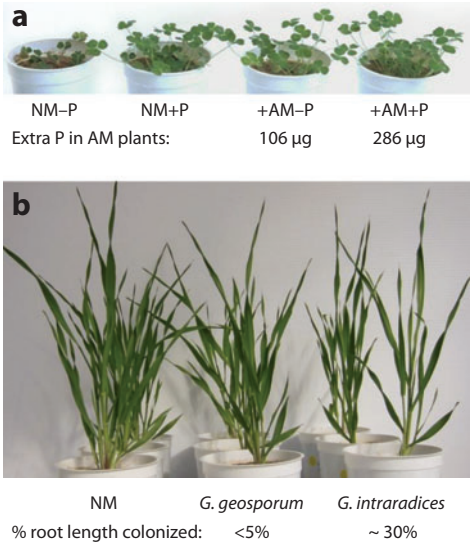

Figure 3

Positive (*a*) and negative (*b*) mycorrhizal growth responses (MGRs) influenced by P supply and identity of AM fungal symbionts. (*a*) *Trifolium subterraneum* grown in low P soil without inoculation (NM-P) or with added P (NM+P) showed a positive MGR when inoculated with *Glomus mosseae* in low P soil (+AM-P). The MGR was less marked when P was added (+AM+P), but AM plants absorbed more P than equivalent NM plants (Extra P), even when growth was similar. (*b*) *Hordeum vulgare* inoculated with *Glomus geosporum* or *G. intraradices* showed similar negative MGR, despite large differences in AM colonization. Low colonization by *G. geosporum* indicates that high fungal biomass is not necessarily correlated with negative MGR. Original photos by (*a*) S.E. Smith and (*b*) E.J. Grace.

FUNCTIONAL DIVERSITY OF AM SYMBIOSES

When growth of AM and NM plants is compared, as in simple pot experiments, the mycorrhizal growth response (MGR) can be highly positive, neutral, or negative and influenced by the identities (genotypes) and developmental stages of the partners, the environmental conditions (e.g., nutrient availability and light intensity), and community interactions (17, 27, 75, 76, 130). Positive MGRs arise largely from increased P uptake via the MP, alleviating P deficiency (**Figure 3*a***), but can also come from increased uptake of other growth-limiting nutrients (134).

Plant and fungal factors that may, separately or together, influence MGR include fungal growth, development of interfaces within the root, and root characteristics such as growth rate, branching, and root hair development. **Table 1** [modified from table 11.3 of Smith et al. (129)] shows a range of such factors; for simplicity, possible nutrient or organic C transfer between plants via common mycelial networks, interactions with other soil microbes that might increase nutrient availability, and suppressive effects on soil pathogens are ignored. The aim is to emphasize that MGR depends on many factors at scales from molecular (e.g., transporter gene expression) to ecological (e.g., plant and fungal composition, density, and competition). The influence of the factors in **Table 1** appears mostly self-evident. For example, AM fungi that rapidly develop extensive interfaces with plants are much more likely to give positive MGR than those that do not. The same applies to plants that have relatively poor root systems. However, as we shall show,

Rhizosphere: the zone of soil very close to a root and under the immediate influence of it

Table 1 Factors that may influence mycorrhizal growth responses of plants to colonization by arbuscular mycorrhizal fungi[a]

Fungal hyphae	Interface(s)	Root	Growth environment
Extraradical:	Rate of development	Root:shoot (weight) ratio	*Soil nutrient availability*
Root colonization rate	Contact area	Length and diameter	*Light intensity*
Growth rate	Longevity	Branching	Other stressful soil conditions[c]
Extension in soil	*Nutrient transfer to roots*	Root hair length and density	Plant density and competition[d]
Nutrient uptake capacity	*Organic carbon transfer from roots*	*Rhizosphere modifications*[b]	
Intraradical:		*Nutrient uptake capacity*	
Growth rate		*Organic carbon delivery to interface(s)*	
Nutrient delivery to interface(s)			

[a]The table is to be read vertically; factors in italics are physiological, relating to resource acquisition and transfer.
[b]Modification via production of organic acids, phosphatases, etc.
[c]Stressful conditions: high soil salinity, compaction, waterlogging, contamination, etc., under which arbuscular mycorrhizal (AM) fungal populations are expected to be low, and possibly also high-input agriculture if this too lowers AM fungal populations.
[d]Plant competition might be interspecific or intraspecific.

prediction of MGR from structural features is very unsafe due to the diversity behind the physiological features given in **Table 1**. Conventionally, MGR is considered in simple terms of P benefits and C costs. When MGR is positive, cost of the fungus in terms of organic C is presumed to be offset by increased photosynthesis as a result of increased P nutrition or increased sink strength. However, the cost is only "real" when C supply limits plant growth (75). **Table 1** does not show possible causes of negative MGR (discussed below). Importantly, the magnitude of MGR is strongly influenced by how well the NM plants grow under given experimental conditions [as exemplified by the high biomass of NM barley (*Hordeum vulgare*) in **Figure 3*b***]. Change of soil type, for example, can modify MGR by increasing or decreasing growth of NM plants, without necessarily altering growth of AM plants (64). Hence, uncritical use of MGR as an indicator of plant dependency on AM fungi for nutritional benefits without evaluating overall growth is very risky.

It has been stated that "the extent of AM colonization is strictly controlled by the plant" (105). It is certainly true that both plant and AM fungal genes facilitate different colonization steps, as shown (mainly) with plant mutants (106). What is much less clear is how far a plant manipulates the extent of colonization and hence fungal cost, especially with high soil P supply when the fungus is supposedly not needed to increase P uptake (e.g., 74, 85). Under these conditions, colonization per unit root length (percent colonization, commonly used as a measure of fungal biomass or abundance) is frequently lower, with the magnitude depending on growth of both plant and AM fungus. In fact, percent colonization is not a valid measure of fungal biomass per plant, and decreases with increasing soil P can be due to increases in root length, with constant AM biomass per plant (135). True suppression of fungal biomass per plant and decreased frequency of arbuscules may occur only at very high soil P. It cannot be concluded that a plant (or indeed a fungus) is in control of the symbiosis simply on the basis of changes in percent colonization.

Plants showing zero and negative MGR have received much less attention than those with positive MGR. It is unclear why some plants (including major crops, especially cereals) typically show such responses, which may be much more complex than previously thought. Despite some early doubts, zero and negative MGR are not artifacts, as they occur in the field as well as in pot experiments (15, 48, 80, 98). Until recently it was assumed that poor response arises from efficient P uptake by roots alone (DP), with small uptake via the

MP. Low or zero AM benefit is conventionally set against large C costs of maintaining the fungus (9). The range of MGR, interpreted in this way, has led to the widely accepted concept of the mutualism-parasitism continuum, as defined by Johnson et al. (75). The question that then arises is why the plant does not eliminate AM fungi that apparently behave as parasites, especially if the plant is capable of controlling the symbiosis. This conundrum—both in ecological and evolutionary terms—has been addressed by suggesting that AM fungi can deliver benefits to disease or drought tolerance, which are unrelated to nutrient supply via MP (75, 76, 102). However, use of radioactive ^{32}P or ^{33}P in compartmented pots has shown that the MP can make a major contribution to P uptake, regardless of the size or direction of MGR (137, 138). As discussed in more detail in the next section, this finding means that in the absence of a positive MGR, AM fungi cannot be regarded simply as parasites (76, 126). This is a new functional paradigm if parasitism implies one-way resource transfer to the fungus rather than imbalanced C–P trade.

Another new paradigm is that negative MGR is not always associated with high fungal C use, which is usually derived from percent colonization and, where measured, hyphal length density of ERM. Positive MGR certainly decreases when percent colonization is high and plants are shaded and hence C-limited (45, 133, 144). However, large growth depressions can also occur at high light when colonization is low, indicating that high fungal C use is not the only determinant of negative MGR (41, 44, 88). Differences in growth depressions caused by different fungi may arise from differences in the balance between P uptake via MP and DP rather than C demand (126). Thus, there will be negative MGR if colonization reduces P uptake via DP, but MP provides inadequate compensatory P (27, 42, 88, 126). This alternative explanation based on P rather than C limitation requires more investigation as to why P uptake by NM plants can be more efficient than uptake by well-colonized AM plants. It may be due to favorable changes in root architecture, including better root hair development or organic acid extrusion. If negative MGR is not caused by excessive C cost, this will drive a conceptual change both in understanding the interacting controls of uptake by MP and DP and in the way the fungus rather than the plant may manipulate the symbiosis to its own advantage (see **Supplemental Text** section Negative Mycorrhizal Responses; follow the **Supplemental Materials link** from the Annual Reviews home page at **http://www.annualreviews.org**).

Supplemental Material

FOCUS ON PHOSPHORUS NUTRITION

Forms and Availability of Phosphorus in Soil

Globally, soil P availability is generally low, with many soils deficient and unable to support productive crops unless fertilized. Pi is the main form absorbed by plants and AM fungi, being released from organic forms by soil microorganisms. Pi anions are strongly adsorbed to the cations iron (Fe) and aluminum (Al) at low pH and calcium (Ca) at high pH, so that Pi concentrations in soil solutions are usually less than 10 μM (124). Low solubility results in very low mobility, so that when Pi is absorbed by roots, replacement from bulk soil is extremely slow, and depletion zones develop that reduce uptake by the epidermis and root hairs via the DP. Depletion is lower around small-diameter AM fungal hyphae (125, 146). Factors influencing plant Pi uptake are therefore more closely related to the ability of the root system to access Pi from undepleted soil than to the kinetics of uptake processes and hence the characteristics of Pi transporters (124, 125).

Uptake and Translocation of Phosphate by Arbuscular Mycorrhizal Fungal Hyphae and Delivery to Intraradical Interfaces

Operation of the MP starts with active Pi uptake into the ERM against a large electrochemical potential gradient, via high-affinity Pi

Monoxenic culture: the rearing or growing of an organism with only one known species of associated organism

transporters and energized by H^+-ATPases (12, 29, 52, 68, 134). Following Pi uptake, polyphosphate (polyP: linear chains of Pi residues linked by phosphoanhydride bonds) accumulates in hyphae, where it buffers cytoplasmic Pi concentration, provides temporary P storage, and translocates P along hyphae (26, 56). Reported amounts are quite variable, probably because polyP dynamics are strongly influenced by P availability and because of difficulties of measuring total polyP over the full range of chain lengths from small and soluble to large and insoluble. Nevertheless, polyP is consistently implicated in rapid, long distance P translocation from sites of uptake in the ERM to sites of transfer to the plant (150). Both Pi and polyP carry negative charge, which must be balanced by cations. In soil-grown plants, K^+ and Mg^{2+} may play this role (120, 121), but experiments with monoxenic cultures (with high sugar and N supplies) suggest that arginine (Arg^+) is translocated with P [as $polyP^-$ (69)] (**Figure 4**).

Molecular mechanisms promoting Pi efflux from the IRM are unknown. PolyP chain lengths in ERM are longer than in IRM, suggesting that localized hydrolysis leads to high internal Pi concentrations, facilitating efflux,

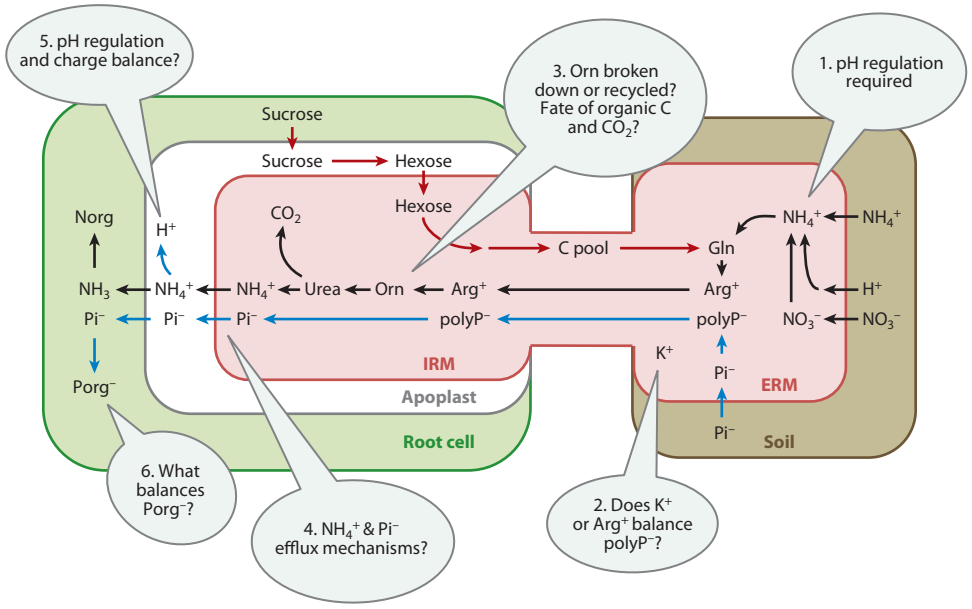

Figure 4

Diagrammatic representation of hypothetical arbuscular mycorrhizal (AM) fungal N transfer (*black arrows*) and associated sugar (*red arrows*) and phosphate (*blue arrows*) transfer between soil and root cortical cells, based on a diagram presented in Jin et al. (69). Uncertainties are indicated in boxes 1–6. Ammonium (NH_4^+) and/or nitrate (NO_3^-) are depicted as absorbed by the AM extraradical mycelium (ERM) from soil and assimilated into glutamine (Gln), then arginine (Arg^+). Assimilation will generate excess H^+ or OH^- with nitrate and ammonium, respectively, so that pH regulation is required (box 1). Ionic charge balance during Arg^+ translocation to the intraradical mycelium (IRM) is envisaged as being maintained by negative charge on polyphosphate ($polyP^-$), as in the original scheme (69), but K^+ or Mg^{2+} are possible alternative counter-ions (box 2). Arg^+ is envisaged as being broken down either partially or completely (box 3), generating CO_2, NH_3 (not shown), and NH_4^+. Efflux mechanisms for Pi^- and NH_4^+ from the intra-radical mycelium (IRM) to the interfacial apoplast are unknown (box 4). Transfer of NH_4^+ to the plant cells and subsequent assimilation will again generate H^+ ions, and pH regulation will be required (box 5). Which ions balance negative charges on organic P ($Porg^-$) in the plant cells are unknown (box 6). Abbreviation: Orn, ornithine.

which can be slightly (10%) increased by C supply (142, 143, 150). AM fungal Pi transporters similar to those involved in plant Pi remobilization may be involved in efflux (109). Additional uncertainties include the extent to which P delivery is linked to C supply, the pH of the apoplast that will influence Pi speciation, and the Pi concentration that will influence which kinetic characteristics of AM-inducible plant Pi transporters will be most efficient with respect to uptake into root cortical cells. Whatever the mechanism, transfer of Pi^- will require ionic charge balance.

Hidden Phosphate Uptake: Contributions of Mycorrhizal and Direct Pathways for Uptake

Most early research on effects of AM colonization on plant P nutrition centered on plants grown in low-P soil with consistently large positive MGR, and with higher total P than NM counterparts [i.e., also a positive mycorrhizal P response (MPR); **Figure 3*a***]. It was assumed that the MP simply contributed extra P to AM plants and that the DP contribution was not changed by colonization; i.e., the two contributions are additive. Based on this premise, inflow of P (uptake per unit root length per unit time) was calculated as a measure of the efficiency of AM and NM roots and, by difference, the MP contribution (123, 139). The latter was very large in positively responsive plants like onion, leek, and clover and was assumed to be zero in plants with zero or negative MPR.

From the early 1990s, increasingly sophisticated compartmented pots were used to track radioactive P supplied to ERM, but not to roots, of plants growing in soil (55, 63, 113, 153). As more species were investigated, it became clear that the MP can make large contributions to P uptake even when MPR is zero or negative (**Figure 3*b***). Smith et al. (137, 138) introduced innovations to allow quantification of percent contribution of MP. ^{33}P was supplied in small hyphal compartments (HCs; approximately 10% of the total soil volume), minimizing overestimation of MP contribution when HC is large. In previous work, AM plants had access to much larger soil volumes, and hence nutrient supply, precluding valid comparison with NM plants growing in smaller volumes. Three plant species in symbiosis with three AM fungi showed a full range of MGR and MPR, from positive to negative, and the MP was active in all. Even in tomato, with consistently negative responses, one of the fungi delivered 100% of the P via the MP; the DP appeared completely inactive (137, 138). Clearly, contributions of DP and MP are not additive, and variation in percentage of total P delivered by the two pathways illustrates strong functional diversity in AM symbioses and different fungal efficiencies in absorption and delivery of P (27, 41, 99, 108). The method can also show differences in the extent to which DP is suppressed. Problems can arise when a fungus with poor ability to grow away from roots fails to access radioactive P in the HC, despite apparently absorbing considerable P close to the roots in the root hyphal compartment (RHC); in such situations, the MP is underestimated (99, 126, 127, 138). The key findings from plants with zero or negative MGR (or MPR) are that MP contribution cannot be determined from plant P contents. It remains hidden unless quantified using tracers, and DP makes a lower contribution to P uptake in AM than NM plants. Both hidden P uptake via MP and reduced contribution of DP have been slow to be recognized as physiologically significant, despite the insightful review by Jakobsen (61). More attention needs to be paid to MP and DP contributions in crop plants that show positive MGR and also in wild plants irrespective of whether MGR is positive or negative.

Previous emphasis on relative (percent) contributions of DP and MP to total P uptake has obscured comparisons of actual amounts taken up by the two pathways. Facelli et al. (27) showed that *Glomus intraradices* delivered a larger percentage of P to tomato via MP than *Gigaspora margarita*, but the former plants were smaller (more negative MGR), and hence amounts delivered per plant were similar. DP contributions to total P were much lower with

G. intraradices than *Gi. margarita*. In both cases, a major effect of colonization was suppression of the DP, compared with NM plants, which was not compensated for by the MP.

The interplay between colonization, P supply, and contributions of MP and DP to total P uptake is important in designing experiments to unravel underlying mechanisms. For example, Nagy et al. (101) showed that percent MP contribution (again in tomato) declined as P supply was increased, in line with effects on percent colonization. Further analysis of their data (see **Supplemental Text** section Effects of P Supply on Contributions of MP and DP to P Uptake by Tomato, including **Supplemental Table 1**) indicates that total (mg P plant^{-1}) and specific (mg P g^{-1} root) uptake via MP were similar at low and moderate P and markedly reduced only at the highest level. Accordingly, the conclusion that the MP is P repressible appears valid only at very high P, when expression of AM-inducible Pi transporters was barely detectable. Importantly, specific DP uptake was considerably lower in AM than NM plants at all P levels (101), as well as in negatively responsive wheat, barley, and (again) tomato (41, 87, 88, 108, 138). Reduced DP contribution is certainly implicated as a cause of negative MGR, where MP fails to compensate for the decrease (27, 42, 126). In plants with positive MGR, the effects are less clear, but some evidence points to lower DP contributions (E. Facelli, unpublished results; data recalculated from Reference 138). In such plants, the high MP contribution more than makes up for decreases in DP contribution. If molecular mechanisms underlying lower DP contributions in AM plants can be understood, it may be possible to eliminate them in nonresponsive crops, making MP and DP contributions additive to increase P uptake efficiency.

Changes in Orthophosphate Transporter Gene Expression in Arbuscular Mycorrhizal Roots

Operation of the two uptake pathways in AM roots is associated with changes in expression of transporter genes as compared with NM roots (reviewed in 12, 68). The DP involves Pi transporters of the PHT1 family, located in epidermal cells and root hairs, that transfer Pi ions ($H_2PO_4^-$) across the plant plasma membrane. Regulation of transcription is probably a major control mechanism (12, 84). Expression is preferentially localized near the root tip and in the root hair zone (20, 39); these regions encounter relatively high Pi concentrations in soil solutions (but still <10 μM) before there is any depletion consequent on uptake. Expression is lower in more mature regions of the root. How closely related the reductions are to normal death of root cells (93) has not been explored. Expression is also lower at high P supply (and hence high plant concentrations) and is often lower in AM than NM roots (68). In soil-grown plants, AM colonization first becomes established behind the root hair zone; root tips are rarely colonized (141). However, gene expression data have usually been obtained by sampling the whole root, and no developmental studies have compared noncolonized root tips with colonized older regions. These would clarify integration of DP and MP activities as roots develop. Not all reductions in expression of Pi transporter genes in the DP in AM roots are caused by increased plant P concentrations. Direct AM fungal effects may occur, including signaling from fungus to plant and unspecified antagonism, as also seen in nonhost plants (27, 31). Intriguingly, a complex pattern of expression of transporters in DP (downregulation) and MP (upregulation) has been observed in field-grown tomato in response to NH_4^+ application (118). The explanation was a shift to P delivery via MP to support N-induced growth increases. The extent to which DP uptake is quantitatively related to transporter expression or protein synthesis is still unclear (111).

In addition to effects on Pi transporter gene expression in the DP, cortical colonization results in localized, and sometimes exclusive, expression of AM-inducible plant Pi transporters in the membranes surrounding arbuscules or hyphal coils (8, 12, 51, 68, 110). This location strongly suggests a role in MP operation.

Similar localization of H^+-ATPases is consistent with active uptake of Pi (37, 38, 116). Variable numbers of AM-inducible Pi transporters have been reported both from plants that, in low P soil, commonly show a high positive MGR (such as *Medicago truncatula*, *Lotus japonicus*, and *Zea mays)* and from plants that often do not (such as *Triticum aestivum*, *Hordeum vulgare*, *Oryza sativa*, *Solanum lycopersicum*, and *S. tuberosum*) (summarized in 12, 68). Although expression of such genes indicates a potential for MP operation, it is very risky to assume that the level of gene expression can provide quantitative information on contributions to P uptake of the DP or MP. Such quantification can only be accomplished by tracking with radioactive P. High gene expression does not demonstrate high P flux but may instead be a starvation response (i.e., an attempt to increase P fluxes by maximizing transporter synthesis, as generally accepted in the DP). Absence of expression can, however, be a realistic predictor of lack of contribution (101), but a complication arises from the extent of overlap of function (redundancy) between multiple AM-inducible transporters in a single species. In tomato, knockout of LePT4 does not completely eliminate P transfer via the MP, so that LePT3 and LePT5 appear to be able to compensate for the loss (100, 152), but in *M. truncatula*, knockout of the single AM-inducible transporter gene (MtPT4) results in defective arbuscules and complete lack of external mycelium, and hence elimination of the MP (67). The suggestion that these effects are a consequence of failure of organic C transfer and hence starvation of the AM fungus requires experimental verification; suggested links between C and P fluxes in regulating symbiotic development remain largely speculative (30).

ARBUSCULAR MYCORRHIZAL SYMBIOSES AND NITROGEN NUTRITION

Inorganic Nitrogen

It has been a long-standing view that the MP is quantitatively unimportant in uptake and transfer of N from soil to plants, based on the premises that (*a*) inorganic N (NO_3^- or NH_4^+) in soil is much more mobile than inorganic P, and (*b*) organic N is unavailable to AM fungi. Concentrations of NO_3^- and NH_4^+ in unfertilized soils are low [approximately 20–50 μM (96)] but are not depleted in the rhizosphere because of high mobility. Roots and hyphae are thus expected to have similar uptake efficiencies, and scavenging for N at a distance from roots by the ERM is not likely to be advantageous compared with that for P (35, 95). Furthermore, evidence for positive MGR or increased tissue N concentrations in soil-grown AM plants due to N uptake via the MP has only been obtained in a few investigations (15, 36). In others AM symbioses had no effect on N nutrition (1, 54, 115). Because plant tissues have N:P ratios of approximately 10:1 (mass basis, or 22:1 molar basis), major direct effects of AM fungi on N uptake should be easy to detect, but this has mostly not been the case. Increased total N per plant (content) and N concentrations (mg g^{-1} DW) are often observed in nodulated AM legumes compared with NM counterparts, but these findings have been attributed to positive effects of AM-mediated P uptake on nodulation and N_2 fixation (134). Nevertheless, statements are now frequently made that AM symbiosis can play a major role in N uptake (e.g., 28, 60, 74, 79). Here, we briefly assess experiments that have led to this changed view.

Experiments with compartmented pots using $^{15}NH_4^+$ or $^{15}NO_3^-$ supplied to ERM consistently show higher ^{15}N transfer to AM than to NM plants, although transfer in soil from HCs to RHCs by mass flow and diffusion has never been completely eliminated (1, 35, 53, 54, 71–73, 94, 145, 147). There are indications that transfer from soil NH_4^+ may be greater than from NO_3^-, but amounts vary with soil moisture content and hence mobility of inorganic N species (145, 147). In some experiments, but by no means all, AM fungal access to N in the relatively large HCs resulted in increased plant N content (35, 53, 54, 72, 94, 147). Hyphal uptake and transfer of N resulted in depletion in HCs (35, 71). Clearly, there is an MP for N transfer

from soil to plants, but estimates of amounts of N transferred vary considerably. To our knowledge, no experiments with soil-grown plants in pots with HCs containing ^{15}N allow confident calculation of the amount of N reaching the plants via the MP, although some estimates have been made. In two experiments, Johansen et al. (71) showed that AM fungi transferred 0.6 and 10% of total N to cucumber, a very small proportion considering the bias induced by relatively large HCs. At the other extreme, Mäder et al. (94) calculated that the MP contribution to total N in tomato was as high as 42%. Their analysis did not account for bias induced by a large HC and presumed that ^{15}N in the AM plants that could not be accounted for was delivered via the MP, both of which would overestimate MP; they also assumed there was no hidden N transfer, which would underestimate it. These uncertainties highlight the need for new experiments (using small HCs incorporating an air gap to eliminate mass flow or diffusion of inorganic N) to track ^{15}N delivery via AM fungal hyphae and calculate contributions of MP and DP, as has successfully been done for P.

Despite all the uncertainties about the quantitative contribution of the MP in soil-grown plants, use of monoxenic cultures of *G. intraradices* on Ri-T-DNA–transformed carrot roots (and in a few cases, soil-grown plants) is revealing details of inorganic N uptake and metabolism involved in N transfer. An NH_4^+ transporter (GintAMT1) has been cloned from *G. intraradices*. It has high sequence similarity to other fungal NH_4^+ transporters, complements defects in NH_4^+ uptake in yeast mutants, and has a high substrate affinity (92). The authors conclude that GintAMT1 is involved in uptake by the ERM when NH_4^+ is present at micromolar concentrations. Following uptake, enzyme activities and labeling patterns in ERM are consistent with assimilation of NH_4^+ via the glutamine synthase/glutamine oxoglutarate aminotransferase (GS/GOGAT) pathway (69, 140, 148) and of NO_3^- via nitrate and nitrite reductases (69, 77). NO_3^- uptake results in alkalinization of the medium, presumably due to efflux of OH^- generated during NO_3^- assimilation, or corresponding net H^+ influx along with NO_3^- (3, 6, 112). Acidification consequent to H^+ export following NH_4^+ assimilation has also been demonstrated (3, 91).

The ERM takes up N very rapidly in monoxenic cultures and incorporates it into amino acids, chiefly arginine (Arg) which accumulates to high concentrations. Labeling patterns following $^{15}NO_3^-$ or $^{15}NH_4^+$ assimilation indicate that Arg is the main form of N transported from ERM to IRM (5, 40, 69, 70). Concurrent ^{15}N and ^{13}C labeling suggests that synchronization of spatially separated reactions in the anabolic and catabolic components of the urea cycle are critical for effective N translocation along the ERM, and that N is released from Arg as NH_4^+ before transfer across the interface to root cells (19, 40, 69). The pathway as presently envisaged is shown in **Figure 4**; this builds on the original diagram by Jin et al. (69), who ignored the need to maintain ionic charge balance. Nevertheless, this need raises many crucial issues relevant to solute uptake and transfer from soil to plant that are highlighted in **Figure 4**. Uptake of inorganic N into the ERM poses no problems either for charge balance or pH regulation. However, once synthesized, Arg is actually positively charged (Arg^+), and movement to the IRM and breakdown require concomitant charge balance to be maintained at all stages. Ignoring the complex C chemistry of the urea cycle, complete breakdown of one Arg^+ would produce three NH_3 and one NH_4^+, with the charge of the latter balanced by whatever anion(s) balanced the original Arg^+ throughout its synthesis and delivery. Ionized P, either in $polyP^-$ or as Pi^-, is an obvious candidate (69) and, theoretically, would allow transfer of four N per P (molar basis), thus allowing contribution of approximately 18% of the total plant N, assuming a plant N:P mass ratio of approximately 10:1 and taking molecular weights of N and P into account. If only one NH_4^+ is released from Arg^+ at the interface, this would allow only one N per P transferred (again assuming that transfer with ionized P), or approximately 4.5% of the total plant N.

Intriguingly, an investigation of AM influences on transport in *Agropyron repens* (35) shows transfer of N and P from HCs in the ratio of 6:1 (molar basis), which means that more N was transferred than can be accounted for by Arg^+–$polyP^-$ coupling; perhaps glutamine, or glutamate balanced by K^+ or another cation, makes up the difference (136).

Transfer processes across the fungus-plant interface are also unresolved; these extend beyond charge balance. The ^{13}C labeling patterns suggest that CO_2 or HCO_3^- released during Arg breakdown is not transferred to the plant and refixed (40, 69). This seems wasteful (i.e., a C cost) unless refixation occurs rapidly in the fungus to give organic C transferred back along the ERM and used for more Arg synthesis, but even this would be energy-requiring. The C:N ratio in Arg is 1.5:1, so if 1.5 C (originally from the plant) is lost as CO_2 for every N transferred to the plant, there is a large C cost, that could decrease potential plant growth benefits. Taking a plant tissue C:N ratio of approximately 20:1 (molar basis), the C loss would be approximately 8% of total plant C if all N was acquired by complete breakdown of Arg. The identity of anions transferred with Arg^+ greatly complicates this issue. If Arg^+ transfer occurs only with P^-, the C loss would be approximately 1.4% of total plant C (from earlier calculations). Unfortunately, the fate of ornithine (Orn) is not known in detail with respect to the C arising from Arg breakdown. There are other issues at the plant-fungus interface. A plant NH_4^+ transporter (LjAMT2.2) is induced specifically in AM roots of *Lotus japonicus* (47). It has the interesting feature (demonstrated in *Xenopus* oocytes) that it binds NH_4^+ externally, but transport inwards does not result in flow of current. In the AM root, the relevant outside phase is the interfacial apoplast surrounding arbuscules or hyphal coils, where the low pH (49) ensures that concentrations of NH_3 (pKa of protonation 9.25) will be negligible. The conclusion was that LjAMT2.2 transports uncharged NH_3 into the plant from the interfacial apoplast, contrary to previous belief that NH_4^+ is transported. It was also concluded that the H^+ retained in the apoplast would contribute further to the low pH of that compartment (47). This is biophysically impossible unless other ionic membrane transport processes occur to balance charge. If the H^+ is taken into the plant, the overall transfer along with NH_3 would be equivalent to NH_4^+ transfer and, like the latter, would require charge-balance and pH regulation as the NH_4^+ was assimilated. Further analysis is well beyond the scope of this review but certainly needs to be sorted out, as was done for NM plants by Raven & Smith (112). Previous attempts to extend the analysis to AM plants were based on the assumption that transfer from AM fungus to plant was as electroneutral glutamine (136); this now appears unlikely if evidence from monoxenic cultures can be extrapolated to soil-grown plants.

A further complication in extrapolating N transfer in monoxenic cultures to soil-grown plants is that analysis of ERM of the latter suggests that ionized P in polyP is balanced by inorganic cations such as K^+ and Mg^{2+} (120), with no need for Arg^+ to perform this role, and again raising the issue of charge-balance during movement of Arg^+. The high concentrations of organic C supplied to the roots in monoxenic cultures, the high concentrations of inorganic N supplied to the ERM (>3 mM; 40), and lack of a shoot (preventing shoot-associated metabolic signaling and control of uptake) still leave the possibility that high Arg levels arise from the experimental conditions. A good way to resolve the issues would be to use sterile plantlets in the monoxenic systems, rather than transformed roots, together with realistic concentrations of N (e.g., 25). Concurrent measurements of potential balancing ions (Pi^-, K^+, and Mg^{2+}) are also needed to provide information relevant to whole plants grown in soil.

Organic Nitrogen

Organic N represents a large proportion of total soil N, and it has been assumed that AM fungi, having no saprotrophic ability, are unable to access this resource. Nevertheless, recent

studies have examined this issue using patches of organic matter of varying complexity labeled with ^{13}C and ^{15}N (e.g., 57–59, 86, 145). In several experiments, plants obtained ^{15}N from the patches via their AM fungal symbionts (57, 86). There was no transfer of ^{13}C, indicating that organic N was not absorbed and transferred to the plant intact. The amounts of N captured could be up to 72% of the N in the patches (provided as glycine in this case), but this was only approximately 7% of the total plant N (58). The conclusion must be that, unlike ecto- and ericoid mycorrhizas (134), arbuscular mycorrhizas are not involved in N release from organic matter. They may, however, increase the transfer of mineralized inorganic N to plants, possibly as a result of effective spatial exploitation of the patches and competition with the soil microflora.

As most fungi can take up amino acids, it is surprising that this ability has not been demonstrated in intact soil-grown AM plants. However, a cDNA sequence coding for an amino acid transporter (GmosAA1) has been obtained from *Glomus mosseae* grown on cucumber and has been functionally characterized in a yeast mutant (13). This transporter appears to be quite unspecific, as shown by the range of uncharged amino acids that competed with ^{14}C–proline uptake. However, negatively and positively charged amino acids (including Arg) competed poorly. A partial cDNA with close sequence similarity was also obtained from *G. intraradices* grown in monoxenic culture (13). Jin et al. (69) showed that the ERM of *G. intraradices* in monoxenic culture took up exogenous Arg, supplied at 2 mM. Clearly, the ability of AM fungi to take up exogenous amino acids from soil deserves further attention.

In conclusion, there is N transfer from soil to plants via the MP, but significant doubts remain both about amounts in proportion to plant requirements and about details of mechanisms. Realistic experiments are needed to determine amounts of N transferred to plants growing in soil to show whether AM fungi make a physiologically significant contribution to total N uptake and whether root function is changed by AM colonization, i.e., whether there is substantial hidden N uptake and reduction of DP contribution. Once these points are resolved, it will be worth investigating other influences on N transfer, such as relative transfer of NO_3^-, NH_4^+, and organic sources, identity of plant and fungal partners, and N supply and plant N demand. AM effects on plant N:P stoichiometry and costs of N transfer may also become clearer.

IMPLICATIONS OF NEW PARADIGMS AT WHOLE PLANT LEVEL

In this review, we have outlined new functional paradigms in AM symbiosis revealed at the level of whole-plant physiology and supported by information on transporter gene expression, particularly with respect to plant P acquisition but also for N.

Firstly, we have shown that the MP operates and AM-inducible P transporter genes are expressed not only in plants that respond positively to AM symbiosis but also in those that do not. This has several important consequences: The MP contribution to P uptake may be hidden unless tracers are used to demonstrate its activity; AM fungi must be regarded as mutualistic symbionts that transfer P to the plants (they are not parasites, because they deliver P as well as receive C even when MGRs are negative); and the cheating of plants by AM fungi to obtain C, but delivering no P (or N), must be rare. Hidden P transfer via the MP helps to explain the evolutionary persistence of AM symbioses that do not necessarily result in marked positive MGR.

Secondly, the contribution of the DP is reduced with respect to P uptake if the MP operates in plants that do not respond positively to AM colonization. It may also be reduced in plants that are positively responsive, but that will have less impact on total P uptake of the plants. From a practical standpoint, this means that contributions of AM fungi to plant nutrition cannot be determined from growth or nutrient contents of AM plants and their NM

counterparts. More significantly, mechanisms by which the DP is reduced in AM plants deserve increased attention. From ecological perspectives, increases in DP contribution to plant P and in root hair production in NM plants may be P starvation responses in species that are normally AM under field conditions.

Thirdly, negative MGRs and decreased DP contributions for P are not necessarily caused by high fungal biomass, i.e., by the C cost of the fungus. This argues further against fungal parasitism as a universal mechanism underlying negative MGR and suggests a hitherto unsuspected level of subtle fungus–plant cross-talk. Potential for AM involvement in the regulatory pathways implicated in plant responses to P deficiency is enormous. In plants not colonized by AM fungi (whether potential hosts or not) the pathways are highly complex and not fully understood. Phytohormones, sugar supply, and molecular regulators such as transcription factors, miRNAs, and genes induced by P starvation (IPS genes) play significant and interconnecting roles, resulting in changes in the expression of Pi transporters and in root architecture (117). Many of these factors influence, or are influenced by, AM colonization and are beginning to be incorporated into mainstream research on the P response signaling pathways (11, 24, 46). There is still a gap in research approaches that needs serious attention because the vast majority of plants are AM in field situations.

There is evidence for the involvement of an MP for N uptake by AM plants but considerable doubt about its quantitative contribution to total plant N, as well as its costs, and hence to its physiological and ecological significance. Likewise, processes involved in N uptake and transfer are poorly understood for plants grown in soil. More information is required on expression of genes involved in N uptake and soil-plant transfer, linked to measurements of amounts of N transferred. Important questions are whether there is hidden N transfer via the MP and whether AM colonization reduces the contribution of the DP. Attention must also be directed to mechanisms that maintain ionic charge balance during N transfer. Previous focus on imbalance in C–P trade as the main cause of negative MGR needs to be extended to C–N trade.

TOWARD MORE REALISTIC SCALING UP?

The new experimental findings mean that past perspectives about the functioning of AM associations and their effects on plant growth need considerable revision in relation to ongoing attempts to scale up from pot experiments to ecosystems. Such scaling up is generally accepted as very challenging but necessary if ecological functions of AM symbiosis are to be resolved (43, 74, 114). First and foremost, the wide range of MGR from positive to negative by itself gives no evidence for the conventional mutualism–parasitism continuum of AM fungal functioning, based entirely on C–P trade (75, 134). The default situation is that in the field there will be (unless proved otherwise) mutualism in terms of C trade and nutrient trade across AM interfaces, irrespective of MGR shown in pot experiments; this mutualism is especially likely because, in the field, individual roots will be colonized by many AM fungal taxa. Furthermore, statements in the literature that at high soil-nutrient levels the plant controls or eliminates the fungus need to be treated with great caution when it is only percent colonization that is lowered. Trade balance with regard to N uptake both in pots and in the field may be similar to that of P, taking into account the higher demand by plants for N, and despite the lack of measurements that might quantify the relative amounts of N uptake by MP and DP.

Operation of two interacting pathways for nutrient uptake, even when MGRs are zero or negative, clearly complicates interpretation of N:P uptake stoichiometry in AM versus NM plants, considered so far only on the basis of the conventional (and now obsolete) simple paradigms relating to MGR, parasitism, etc. (e.g., 74). Possible differences in N, P, and C trade balance between symbionts when soil N and P are high or low cannot be determined

simply from MGR, but must be determined from the actual contributions of the pathways, admittedly a daunting task. On a more positive note, nutrient uptake by the MP in the absence of positive MGR adds another dimension in competitive interactions between plants in the field. It has been shown (in pots) that an AM plant showing zero or negative MGR (when grown alone) can outcompete a constitutively NM plant due to the hidden contribution of its MP (17, 27). This finding adds to the evidence that AM symbiosis can be advantageous for individuals growing in competition, even though plants grown singly show negative MGR. In other words, an AM plant showing zero or negative responses cannot be assumed to be functionally equivalent (e.g., in terms of nutrient uptake or responses to competition) to a plant constitutively unable to form AM symbioses, as has—unsurprisingly—been done previously (149). Predictions about plant fitness based on such an assumption are likely to be incorrect. Even more generally, functional diversity among individual AM symbioses, with little or no consistency between individual plant species and AM fungal taxa in terms of MGR (80), now has a functional basis in terms of differences in operation of MP and DP. Contributions of the two pathways need to be explored more extensively in wild plant species (such as those used in Reference 80), extending findings from investigations using mainly crops. These new findings also necessitate reinterpretation of attempts to correlate yield of agricultural plants positively or negatively with (percent) colonization. For example, we see no valid functional grounds to the hypothesis that there is AM parasitism in wheat when (percent) colonization is relatively high and there are no perceived benefits in terms of growth (119, 122). What is needed is increased emphasis on how functions of MP and DP are integrated, with the aim of making the pathways additive and increasing P uptake efficiency in crops. However, caution is required because lower DP contribution may have other benefits, such as decreased uptake of arsenate, which enters via epidermal Pi transporters in the DP (18, 131).

Finally, earlier categorization of negative MGR as transitory (occurring only in young plants) or persistent (throughout the plant life cycle) (130) needs revisiting. Experiments should be extended beyond vegetative plant growth and should examine outcomes in terms of seed production and (in an agronomic context) yield and quality. It may turn out that early growth depressions are not always deleterious in terms of fecundity or final yield (9, 90). Further discussion of ecological and agronomic aspects is beyond the scope of this review, but we hope that the new paradigms introduced here will be considered in future by those who research AM symbioses at higher scales, and subjected to experimental testing in as close to field conditions as possible.

SUMMARY POINTS

1. The great majority of land plants naturally form arbuscular mycorrhizas. Therefore, knowing how the activities of arbuscular mycorrhizal (AM) fungal and plant symbionts are integrated is critical to understanding nutrient acquisition in ecological and agronomic contexts.
2. AM fungi live in two environments: in soil, where they form an extensive extraradical mycelium (ERM) that scavenges nutrients, and within the root, where they grow between and within cortical cells forming symbiotic interfaces (arbuscules or intracellular coils) involved in nutrient transfers.

3. Although plant growth responses to AM colonization are usually positive when soil phosphorus (P) limits growth, some AM plants grow less than their nonmycorrhizal (NM) counterparts. Such growth depressions [or negative mycorrhizal growth responses (MGRs)] occur not only when colonization by AM fungi is high but also when it is low and therefore unlikely to result in high organic C use. In these circumstances, the conventional explanation that growth depressions are caused by excessive C use by the fungi is unrealistic. Studies of integration of plant and AM fungal nutrient uptake are beginning to provide alternative explanations.
4. An AM root has two pathways for nutrient uptake. The direct pathway (DP) involves uptake from the rhizosphere by root epidermis and root hairs. The mycorrhizal pathway (MP) involves uptake by the ERM, rapid translocation over many centimeters, delivery to the symbiotic interfaces, and transfer to the plants. The two pathways involve different cell types and also different nutrient transporters, providing capacity for independent and coordinated regulation.
5. Experiments tracking activity of MP with ^{32}P or ^{33}P show that it makes an important contribution to P uptake, whether or not the plant grows more and takes up more P when AM than when NM. The amount of P delivered by the MP is not necessarily closely related to percent root length colonized by the AM fungi. Contrary to conventional ideas, this means that P delivery via DP can be lower in AM than NM plants and that MP contribution cannot be determined from plant nutrient content. Lower DP activity in AM plants, not compensated for by P delivery via MP, can lead to P deficiency and hence to negative MGR.
6. An MP for nitrogen (N) uptake has been demonstrated in soil-grown plants using $^{15}NH_4$ and $^{15}NO_3$, but it is not known what proportion of total plant N requirement is delivered via this route, nor whether there is higher DP activity in NM than AM plants, as there is for P.
7. Mechanisms underlying MP activity for N have been explored in monoxenic root organ cultures, where N is translocated as arginine but converted to NH_4^+ before transfer to the plant across the symbiotic interface. These investigations have not yet been extended to soil-grown plants, nor do they consider costs of N delivery or ionic charge balance, which must operate at all stages of N uptake and transfer.
8. Our new paradigms help resolve some ecological and evolutionary conundrums based on the conventional idea that negative MGR means that AM fungi are parasites (in the conventional sense), and yet plants have not evolved mechanisms to eliminate them. If AM fungi deliver P in exchange for C, they are not parasites but mutualists, regardless of plant growth response. Furthermore, MP activity increases competitive success even in plants that show negative MGR when grown alone. In an agronomic context, it may be possible to engineer plants to avoid reductions in DP activity and hence optimize P-uptake efficiency in crops like wheat and barley that often show negative MGR.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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138. Quantification of mycorrhizal pathway and direct pathway to plant P uptake in compartmented pots using ^{33}P.

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