

# Nutrient transfer in plant–fungal symbioses

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**Almost all plant species form symbioses with soil fungi, and nutrient transfer to plants is largely mediated through this partnership. Studies of fungal nutrient transfer to plants have largely focused on the transfer of limiting soil nutrients, such as nitrogen and phosphorous, by mycorrhizal fungi. However, certain fungal endophytes, such as *Metarhizium* and *Beauveria*, are also able to transfer nitrogen to their plant hosts. Here, we review recent studies that have identified genes and their encoded transporters involved in the movement of nitrogen, phosphorous, and nonlimiting soil nutrients between symbionts. These recent advances in our understanding could lead to applications in agricultural and horticultural settings, and to the development of model fungal systems that could further elucidate the role of fungi in these symbioses.**

## Plant–fungal Interactions

Over 90% of all plant species form symbioses (see [Glossary](#)) with soil fungi. These partnerships were initiated over 450 million years ago, and this early origin suggests that beneficial plant–fungal interactions had an important role in the global colonization by land plants [1]. Benefits imparted to the plant as a result of such an interaction with a soil fungus include increased mineral nutrient absorption, water-use efficiency, and disease resistance, and the interaction may also facilitate plant-to-plant communication [2,3]. Of particular interest is the transfer of soil nutrients mediated by the fungal symbiont to the plant host. Colonization of plant roots by fungi increases the surface area from which plants can scavenge nutrients. Thus, root-colonizing fungi facilitate the absorption of crucial and often limiting soil nutrients, which results in increased photosynthetic ability and enhanced growth, productivity, and overall plant health [4].

Approximately 5000 fungal species are thought to be capable of colonizing roots and subsequently furnishing plants with soil nutrients [5]. The two major groups of beneficial plant-associating fungi are the mycorrhizal fungi and the endophytic fungi. One characteristic that distinguishes mycorrhizal fungi from endophytic fungi is that mycorrhizal fungi cannot survive in the absence of their plant hosts because most mycorrhizal fungi are obligate biotrophs [1]. However, even though endophytes are

defined as microorganisms that live asymptotically inside healthy plant tissues, many of these ‘endophytic’ fungi have multiple life stages and are known to be decomposers of organic detritus, plant pathogens, or pathogens of soil insects at other points in their life cycle [6–8]. Nevertheless, this definition of endophyte is being challenged as more information regarding the important ecological role of endophytes is being uncovered.

Seven different types of mycorrhizal fungus have been identified: ectomycorrhizal, endomycorrhizal (arbuscular mycorrhizal), ectendomycorrhizal, ericoid, arbutoid, monotropoid, and orchid [5]. Although little is known about the specific evolutionary origins of these symbioses, more than 2000 species of mycorrhizal fungi are able to colonize the roots of over 300 000 species of plants in ecosystems around the world [9]. Most mycorrhizal fungi can be classified as either ectomycorrhizal or arbuscular mycorrhizal [10]. Ectomycorrhizal fungi are typically members of the Ascomycota or Basidiomycota and occur, predominantly, in forested areas, where they colonize roots of trees, such as pine (Pinaceae) and beech (Fagaceae), as well as the roots of tropical species, such as members of the Dipterocarpaceae [11]. These fungi initially form symbioses with lateral roots and create a mycelial sheath that envelops the root tip. The fungus is then able to penetrate the plant root to create a complex network of mycelia surrounding the epidermal and outer cortical cells, known as a ‘Hartig net’ [11].

Arbuscular mycorrhizal fungi are obligate biotrophic symbionts and are all members of the phylum Glomeromycota [12]. Arbuscular mycorrhizal fungi differ from ectomycorrhizal fungi in that the hyphae penetrate the cells of the

## Glossary

**Arbuscular mycorrhizal fungus:** fungal symbiont of plant roots in which the fungus enters the cortical cells of the roots and forms specialized, branched structures called ‘arbuscules’.

**Ectomycorrhizal fungus:** fungal symbiont of plant roots where the fungus does not enter the cortical root cells but grows intercellularly in a complex network of mycelia called a ‘Hartig net’.

**Endosymbiotic:** a symbiotic relation in which one symbiotic partner lives inside the other.

**Fungal endophyte:** an asymptomatic plant-colonizing fungus that lives a portion of its life cycle inside the plant.

**Mutualism:** a specific form of symbiosis where both partners benefit from the relation, as typically seen in mycorrhizal relations.

**Obligate biotroph:** a parasitic or symbiotic organism that cannot survive outside of its host (i.e., mycorrhizal fungi).

**Symbiosis:** a relation that exists when two species live in direct contact with each other. This can be mutualistic, antagonistic, or commensal. Mycorrhizal–plant interactions are typically mutualistic. The relation that exists between EIPF and their plant host has yet to be defined in terms of a specific symbiotic relation.

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inner root cortex to form specialized branched structures called 'arbuscules' [13]. Arbuscules create a specific nutrient transfer interface that is embedded with several plant and fungal transporters, facilitating nutrient transfer between the symbionts [14,15]. However, arbuscular mycorrhizal fungi do not enter the protoplast of the plant cell; instead, the plant cell wall accommodates the entering arbuscule by forming a new and distinct periarbuscular membrane (PAM) continuous with the plasma membrane of the cortical cell [15].

Endophytes can be defined as endosymbiotic microorganisms that live in plant tissues without causing symptoms of disease. Endophytes have been found to associate with almost all plant species, and some are capable of internal migration within plant tissue to inhabit foliage, stems, and bark, as well as roots [16,17]. Root-associating endophytic fungi share numerous similarities with their mycorrhizal counterparts; however, most endophytes do not have an obligate biotrophic life stage and live at least part of their life cycle away from the plant [18]. However, the mechanism of plant colonization, and the nature of the symbiosis is poorly understood when compared with mycorrhizal fungi [10]. The ability of fungal endophytes to transfer nutrients to the host is a relatively recent discovery [19,20], and the mechanisms of this transfer are unknown.

Nutrient transfer is common among most plant–fungal symbioses [21]; however, for many of these fungi, the specific mechanisms and gene products involved in nutrient transfer remain to be elucidated. Despite the importance of nutrient transfer to plants by mycorrhizal fungi, there is a paucity of information with respect to the fungal genes involved, and most of the genetic information obtained has been inferred from a plant perspective [21]. Mycorrhizal fungi are difficult to culture *in vitro* or manipulate using traditional transformation technology. Therefore, research has focused on plant molecular biology and the use of model plants, such as barrel clover (*Medicago truncatula*), that can be genetically modified [14,22,23]. However, the discovery that several endophytic fungi have the ability to colonize plants has provided an opportunity to analyze genetically the fungal role in this symbiosis because these fungi can be stably transformed [24–26]. Fungal symbionts can transfer limiting soil nutrients, including phosphorus, nitrogen, and sulfur, to their plant hosts, and the plant typically reciprocates by transferring plant-derived carbohydrates to the fungus [27]. Here, we review recent advances in understanding regarding the transfer of nitrogen, phosphorus, and nonlimiting soil nutrients by mycorrhizal and endophytic fungi to plants, and discuss the elucidation of novel symbiotic pathways that relate to widespread plant–fungal nutrient exchange.

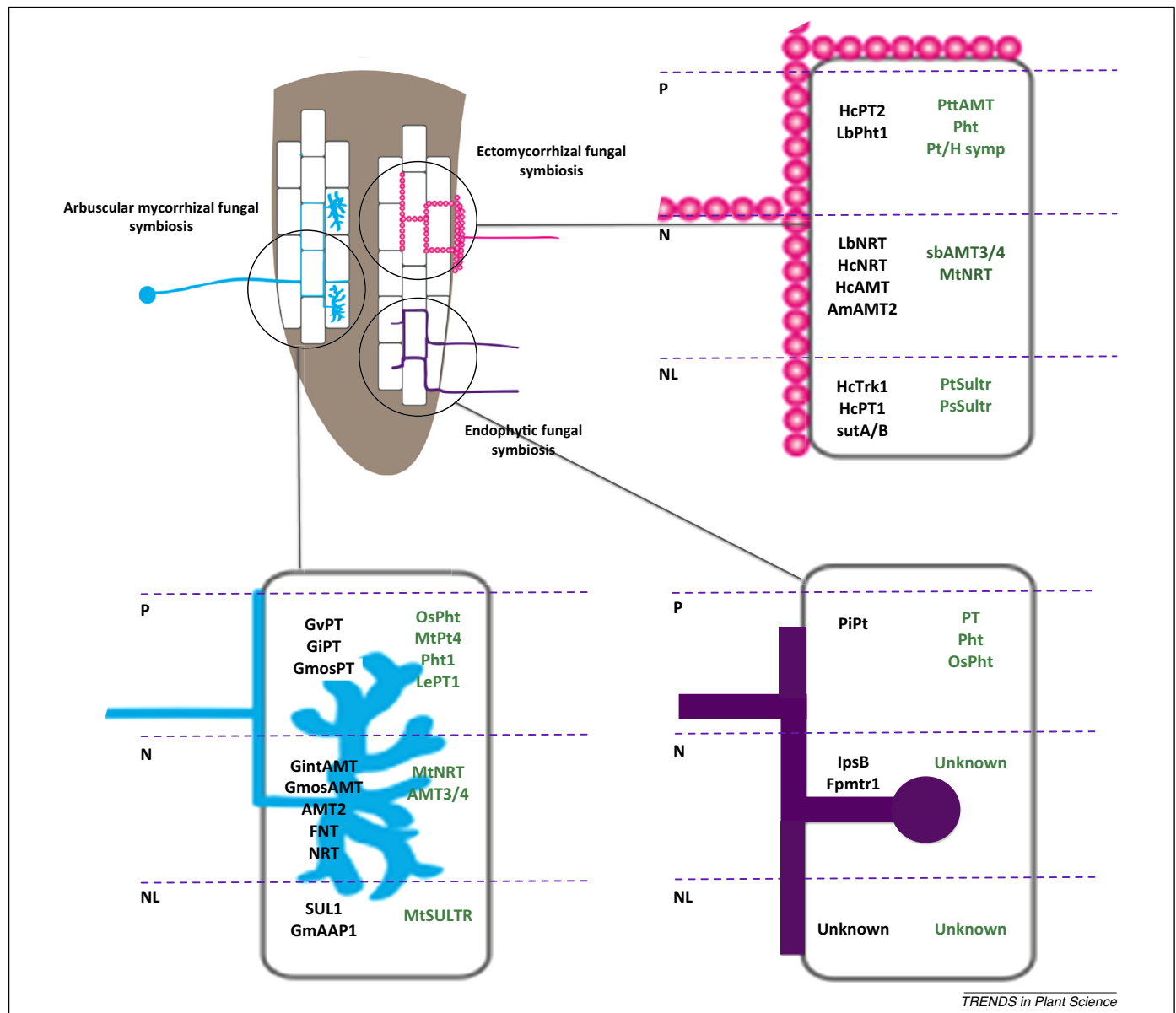
### Nitrogen transfer from fungal symbiont to plant hosts

In almost all ecosystems, including agricultural conditions, nitrogen availability limits primary productivity, and nitrogen cycling and storage is highly variable [28]. The rate of photosynthesis can be directly linked to nitrogen availability; plants require relatively large quantities of nitrogen compared with phosphorus [29]. Over 50% of leaf nitrogen is devoted to photosynthesis [30]; hence, nitrogen availability is integral for carbon fixation.

There are two routes by which atmospheric nitrogen is deposited into terrestrial ecosystems, atmospheric deposition and nitrogen fixation [28]. In the atmosphere, several forms of nitrogen exist, such as  $N_2$  (the most abundant, comprising 78% of the atmosphere), NO,  $NO_3^-$ , and  $NH_3$  gas [28]. Although some of these forms can be readily incorporated into terrestrial ecosystems,  $N_2$  requires fixation by soil and/or plant symbiotic bacteria [31]. Once fixed in terrestrial systems, nitrogen may be bound in organic matter and must subsequently be mineralized to release inorganic nitrogenous compounds that can be used by plants [32]. Nitrogen conversion is largely mediated by fungal decomposers in the soil [33]: several soil fungi are able to exploit the energy stored in the redox potential of organic nitrogen [34]. For example, mycorrhizal fungi are able to excrete exoenzymes (i.e., proteases) that break down organic matter and subsequently capture nitrogen-containing compounds, thus providing a direct path from organically bound nitrogen in the soil to the plant [35]. That is, mycorrhizal fungi provide the host plant with access to nitrogenous reserves that would otherwise remain inaccessible in the soil.

Typically, nitrogen bound in organic matter is present as peptides, proteins, and free amino acids, which can be directly absorbed by fungi [35]. Both arbuscular mycorrhizal and ectomycorrhizal fungi release peptidases and proteases into the soil that cleave organically bound nitrogen and subsequently absorb the nitrogenous monomers [1,36]. Inorganic nitrogen in the soil is preferentially taken up by fungi as nitrates ( $NO_3^-$ ) or ammonium ( $NH_4^+$ ), and it has been suggested that, once this nitrogen has been absorbed by the extraradical mycelia, it is converted into the amino acid arginine for transport into the intraradical mycelia [37]. Once internal migration is complete, the arginine would be broken down through the urease cycle into ammonium for transport into the plant. This hypothetical pathway is supported by work showing that, during fungal association, arginine in host plant roots is increased threefold, and is the most abundant free amino acid owing to the fungal presence inside the root [38]. This pathway would indicate preferential absorption of  $NH_4^+$  by fungal hyphae; however, this is not always the case. For example, the root:shoot ratio of  $^{15}N$  in cowpea (*Vigna unguiculata*) plants was higher for plants supplied with  $NO_3^-$  than for cowpeas grown with  $NH_4^+$ , indicating that nitrate is transferred more rapidly within the plant compared with ammonium, and that transfer of  $NO_3^-$  may rely on independent pathways within the fungus [37]. Preference for the form of inorganic nitrogen absorbed by the hyphae may also relate directly to soil type. In most soils, nitrate is more mobile than ammonium; this effect is exacerbated in clay-based soils because clay minerals adsorb  $NH_4^+$  owing to its positive charge [39]. Thus, preference for a specific nitrogen type may be influenced by the soil in which the symbiosis occurs.

Although the transport of nitrogen in most plant–fungal symbioses seems to focus heavily on nitrates and ammonium, the specifics of the transfer vary between fungal species (Figure 1). For example, arbuscular mycorrhizal fungi construct a specific nutrient transfer interface with the host plant, the aforementioned PAM, and ammonium



**Figure 1.** Plant–fungal root symbioses. Plant genes (depicted in green) and fungal genes (depicted in black) involved in nitrogen (N), phosphorus (P), and other nonlimiting soil nutrient (NL) transport in arbuscular mycorrhizal fungal [40,41,56,57,66,80,81], endophytic fungal [61,82], and ectomycorrhizal fungal [59,67,83,84] symbioses.

transporters in *Glomus intraradices* have been characterized within this membrane [40]. Specifically, the ammonium transporters GintAMT1 and GintAMT2 are expressed by mycorrhizal fungi colonizing cortical cells [40,41]. The nitrogen transfer ability of other *Glomus* species has also been investigated, which led to the identification of a *Glomus mosseae* ammonium transporter (GmAMT4.1) during arbuscule development inside soybean (*Glycine max*) roots [42]. In several of the endomycorrhizal symbioses, a distinct upregulation of the AMT family of ammonium transporters has been observed and fungal nitrate transporters have been found in ectomycorrhizal fungal-colonized roots [43]. In both *Laccaria bicolor* and *Hebeloma cylindrosporum*, high-affinity nitrate transporters in the NRT family are expressed during plant colonization [44,45].

Endophytic fungi are also capable of transferring nitrogen to their plant hosts. The dark septate endophyte

*Heteroconium chaetospira* has been shown to transfer nitrogen obtained from decaying organic matter in the soil to the roots of Chinese cabbage (*Brassica campestris*) [46]. Other endophytes have been found to increase the nitrogen uptake efficiency of young plants in nitrogen-depleted soils [47]. Nitrogen can also be cycled into plants through endophytic insect-pathogenic fungi (EIPF). This unique group of root-colonizing fungi is able to transfer nitrogen harvested from belowground insect hosts into different plant species [19,20]. Species of the insect pathogens *Metarhizium* and *Beauveria* are able to infect soil insects and subsequently transfer insect-derived nitrogen to plants [19,20]. *Metarhizium*, *Beauveria*, and *H. chaetospira* all have similar habitat, host specificity, and nutrient transfer ability, similarities that may be of future use in accurately classifying nutrient cycling endophytes. The specific form of nitrogen transfer is unknown, although nitrogen is potentially converted to ammonium within the

intraradical mycelia and transferred into plant roots in this form, much like the pathways that have been postulated for the transfer of nutrients in the arbuscular mycorrhizal and ectomycorrhizal–plant symbioses [48]. There are costs as well as benefits associated with endophytism, for both the plant and the fungus [18]. However, these costs have yet to be specifically elucidated in symbioses involving *Metarhizium* or *Beauveria*.

A reciprocal upregulation of nitrogen transporters has also been observed in plants that accommodate fungal translocation of nitrogen. Plant ammonium transporters are upregulated in arbuscule-containing cells. For example, in sorghum (*Sorghum bicolor*), the expression of plant ammonium transporters SbAMT3;1 and SbAMT4 was induced only in arbuscule-containing cells [49]. Similarly, nitrate transporters were expressed in arbusculated cells of *Medicago truncatula*: the low-affinity nitrate transporters medtr7g116510.1, medtr4g136330.1, mtr.27765.1.s1, and medtr4g159100.1 all showed increased expression in cortical cells colonized by arbuscular mycorrhizal fungi [50]. This coordinated and specific expression of both plant and fungal ammonium and nitrate transporters in mycorrhizal-colonized cortical cells suggests the crucial importance of fungal nitrogen transfer in plants.

### Transfer of phosphate from fungus to plant

Phosphorous may also be limiting in some ecosystems; however, unlike nitrogen, atmospheric levels of phosphate do not have an important role in the cycling of phosphate to plants [51]. Most phosphorous is bound in rocks, minerals, and large oceanic deposits [51]. Geological processes, such as weathering, release phosphorous as phosphate ions into the soil, where it can be absorbed by plant roots [51].

The most abundant form of phosphate in soils is orthophosphate ( $\text{PO}_4^{3-}$ ) and, in this form, phosphate can be absorbed directly by plant roots or by roots colonized by mycorrhizal fungi [52].  $\text{PO}_4^{3-}$  is then rapidly translocated to fungal structures inside the root, such as intercellular hyphae or highly branched arbuscules, for transport directly into root cortical cells [53]. The transfer of  $\text{PO}_4^{3-}$  is a stepwise process that involves the conversion of  $\text{PO}_4^{3-}$  to a polyphosphate molecule for movement through the mycelia; the polyphosphate is then converted back into an inorganic phosphate molecule for transport into the plant [53]. Mycorrhizal fungi secrete enzymes, such as phosphomonoesterases and phosphatases, that are able to hydrolyze organic P compounds in soil, such as phenolphthalein diphosphate, providing plant hosts with phosphorous typically unavailable to non-mycorrhizal roots [54].

Initial studies of fungal nutrient transfer focused on the ability of *Glomus* spp. to transfer phosphate to their plant partners. It is now apparent that several mycorrhizal fungi have a key role in the cycling of soil phosphate [55], and numerous transporters involved in the process have been elucidated. For example, microarray analyses of *G. intraradices* and an associated plant (*M. truncatula*) showed a coordinated upregulation of fungal and plant phosphate transporters, specifically GmosPT and GvPT in the fungus and MtPt4 in the plant [56–58]. These transporters are specific to arbuscular mycorrhizal symbiosis and are localized in the PAM [58]. In addition, various transporters

crucial to the cycling of phosphate have been reported in ectomycorrhizal fungi. In the model organisms *L. bicolor* and *H. cylindrosporum*, there is increased expression of the phosphate transporters HcPT2 and LbPT during plant root association [59]. Expression is further increased when soil phosphorous levels are low, indicating the importance of these transporters in plant phosphate nutrition [59].

Significant upregulation of high-affinity phosphate transporters has also been reported in several mycorrhizal plants, including legumes and woody plant species [60]. For example, proteins in the Pht1 family are highly expressed during ectomycorrhizal symbiosis, and knock-down experiments have shown the importance of these transporters in phosphate transfer between the ectomycorrhizal *L. bicolor* and poplar (*Populus trichocarpa*) trees [60].

Endophytes are also capable of phosphorous transfer to plants; however, there is a paucity of information on this process. *Piriformospora indica*, a basidiomycetous root endophyte, was shown to increase the phosphate uptake of its host plant, specifically through the increased expression of the phosphate transporter PiPT [61]. Therefore, next-stage research into endophytic nutrient cycling should focus on phosphate to gain a clearer picture of their overall ecological importance.

### Nonlimiting soil nutrients

Not all nutrients transferred from symbiotic fungi to plants are limited in soils; and both arbuscular mycorrhizal and ectomycorrhizal fungi are able to transfer mineral nutrients, such as sulfur, cesium, zinc, potassium, and copper [62–64]. These minerals enter soil systems in a manner similar to phosphorous; however, large quantities are typically not required by plants and neither are they particularly mobile in soil; as such, they are relatively abundant in most ecosystems [65].

In arbuscular mycorrhizal fungi, the transfer of non-limiting and limiting soil nutrients is linked such that the overall efficiency of total mineral transfer is dependent on the relative abundance of limited nutrients. For example, examination of the plant genetics underlying fungal nutrient transfer revealed that, during mycorrhizal association, several *M. truncatula* sulfate transporters (MtSULTRs) were upregulated, indicating that root colonization by fungi increased the rate of plant sulfur absorption [66]. However, the efficiency of sulfur uptake in plant roots was directly tied to phosphate availability, and there was an increase in sulfur transfer only when soil phosphate content was low [62]. A similar relation has been observed between potassium and phosphate in ectomycorrhizal nutrient transfer. The transfer of potassium to cluster pine trees (*Pinus pinaster*) was significantly increased when colonized by the ectomycorrhizal fungi *Hebeloma cylindrosporum* [67]. However, expression of the fungal potassium transporter HcTrk1 was correlated with a decrease in the phosphate transporter HcPT1 [67].

There is little information available on the ability of endophytes to transfer nonlimiting soil nutrients to their plant hosts. Historically, endophytes were viewed as asymptomatic plant colonizers with no nutrient transfer capabilities; however, we now know that this is not the case

[19,20,46,47]. Future research on fungal endophytes should focus on the extent of endophyte-mediated nutrient cycling as well as on their overall ecological impact.

### Reciprocal exchange: trading carbon for nutrients

In soil ecosystems, readily available carbon is limited, particularly with respect to fungi, which are incapable of breaking down complex organic compounds. Plants fix carbon and offer easily metabolizable carbohydrates to fungi that have evolved to colonize plant roots and offer, in exchange, limiting nutrients to the plant [68].

This reciprocity has been observed in several mycorrhizal fungi. *Glomus intraradices* transfers phosphorus to the roots of wild carrot (*Daucus carota*) and barrel clover in exchange for carbon [69]. An increase in the efflux of carbon from the carrot roots that was available to the fungus resulted in an increase in phosphate transfer to the plant [69]. Conversely, a decrease in available carbon slowed the overall rate of phosphate transfer [69]. These results are significant because they not only indicate a close symbiotic relation between the plant and the fungus, but also reveal that the fungus and plant are capable of sensing their immediate environments and actively participating in the reciprocal exchange of nutrients.

A similar relation has been observed with regard to nitrogen transfer, with nitrogen being transferred to carrots by *G. intraradices* in exchange for photosynthetically fixed carbon [70]. Here again, nitrogen transfer to the plant was reliant on plant carbon provided to the fungus [70]. Furthermore, *G. intraradices* would not transfer nitrogen until plant sugars had initially been transferred to the fungus [70]. Thus, reciprocal nutrient exchange stabilizes the cooperative relation between the symbiotic partners.

The mechanisms that control carbon transport to intracellular mycelia have yet to be elucidated; however, plant sugar transporters and enzymes have a key role in the movement of plant-derived carbohydrates to the fungus. In *Glomus* spp., a high-affinity monosaccharide transporter, MST2, has been characterized [71]. The expression of this transporter correlates with expression patterns of the mycorrhizal phosphate transporter, PT4, indicating that the amount of carbon received by the fungal symbiont is directly related to the phosphate transfer efficiency [71]. Plant sugar transporters also show elevated expression during mycorrhizal colonization. In the model mycorrhizal association between *M. truncatula* and *G. intraradices*, increased expression of *MtSucS1*, a plant sucrose synthase gene, has been reported surrounding internal hyphae and arbuscules [19]. Furthermore, the expression of a family of *M. truncatula* sucrose transporters, MtSUTs, has been shown to increase in mycorrhizal-colonized roots [19].

There is little information available on nutrient reciprocity in endophytic fungi. However, it has been shown that carbon exchange may be required for EIPF root association. A raffinose transporter (Mrt) was found to be crucial for rhizospheric competence in *Metarhizium* and a gene knockout led to poor growth in soybean root exudate [72]. *Metarhizium* also produces an extracellular invertase, which when knocked out, led to poor growth in root exudate but improved the root-colonizing ability of the fungus [73]. It was hypothesized that the invertase

knockout caused a reduction in carbon catabolite repression resulting in increased production of plant cell wall-degrading depolymerases that subsequently allowed for root colonization.

Several nonlimiting soil nutrients are transferred to plants through fungal intermediaries. Despite the lack of knowledge surrounding the transfer of these nutrients, it has been observed that, with respect to certain micronutrients, transfer is dependent on plant reciprocity [66,74]. However, whether there is any reciprocity with regard to the transfer of plant carbon to fungal endophytes in exchange for soil nutrients has yet to be elucidated.

### Applications

Although arbuscular mycorrhizal, ectomycorrhizal, and endophytic fungi are ubiquitous in soils, specific applications of these fungi in agricultural fields, with respect to increasing plant productivity and survival, remain intangible. The inability to culture mycorrhizal fungi effectively in the laboratory is a major impediment to producing mycorrhizal fungi on an industrial scale for commercial applications, and large-scale culturing on plants is a cumbersome process that yields inconsistent results [75]. Recently, some of these inconsistencies have been alleviated through novel culturing techniques [75]; however, advances in the study of mycorrhizal genetics could lead to the creation of easily manipulated laboratory strains for study as well as for industrial applications.

Regardless of laboratory difficulties, there is great potential for the industrial application of mycorrhizal fungi. In agricultural and horticultural settings, mycorrhizal fungi have been shown to improve water-uptake efficiency, nutrient acquisition, and the survival rate of young crops [2]. By increasing the presence of mycorrhizal fungi in industrial soils, there should be further increases in positive, fungal-conferred benefits to plants.

EIPF have been manufactured on an industrial scale for the treatment of insect pests [76]. *Metarhizium* and *Beauveria* are of particular interest because they infect a large range of insects and have nitrogen transfer capabilities [43,44,77,78]. These fungi live in a wide range of hosts, their genomes have been sequenced, and they are amenable to traditional molecular genetic techniques, such as gene knockouts. As such, these fungi may provide insight into the roles of fungal genes in reciprocal nutrient exchanges, particularly if there are homologous transfer genes in mycorrhizal fungi.

### Concluding remarks

Despite the integral role that plant-associating fungi have in the cycling of soil nutrients, they remain relatively enigmatic from a research perspective. Although several studies have been conducted on the nutrient transfer capabilities of mycorrhizal fungi, a combination of their inability to be effectively cultured and their recalcitrance to traditional genetic manipulation leaves a paucity of information on these fungi [79]. Endophytes have the potential to be an important model for the study of plant root interactions. Unlike mycorrhizal fungi, several endophytes can be easily cultured in the laboratory, and stable transformation systems are available [22,23]. Through the

use of novel methodology and by using EIPF as a model, there is the potential to characterize genes involved in fungal nutrient transfer. The next stage of research needs to focus not only on genetics, but also on understanding the complex relations that exist in soil with respect to fungal population structure, nutrient availability, and microbial competition. Both mycorrhizal and endophytic fungi naturally associate with economically important crops; thus, understanding and evaluating their ecological roles in agricultural settings is integral to downstream application. The increased presence of either mycorrhizal fungi or EIPF in industrial fields would potentially increase crop yield and, in the case of EIPF, protect crops from harmful insect pests on a broad scale. Overall, the application of optimized root-colonizing, and nutrient cycling fungi into agricultural and horticultural settings could increase fertilizer use efficiency, limit run-off contamination, decrease total energy inputs into agroecosystems, and increase crop production, all of which are fundamental goals towards sustainable and environmentally viable agriculture.

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### References

- Bonfante, P. and Genre, A. (2010) Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. *Nat. Commun.* 1, 48
- Parniske, M. (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat. Rev. Microbiol.* 6, 763–775
- Azcon-Aguilar, C. and Barea, J.M. (1997) Applying mycorrhiza biotechnology to horticulture: significance and potentials. *Sci. Hortic. Amsterdam* 68, 1–24
- Clark, R.B. and Zeto, S.K. (2000) Mineral acquisition by arbuscular mycorrhizal plants. *J. Plant Nutr.* 23, 867–902
- Peterson, R.L. et al., eds (2004) *Mycorrhizas: Anatomy and Cell Biology*, CABI
- Fukasawa, Y. et al. (2009) Effects of attack of saprobic fungi on twig litter decomposition by endophytic fungi. *Ecol. Res.* 24, 1067–1073
- Slippers, B. and Wingfield, M.J. (2007) Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biol. Rev.* 21, 90–106
- Sasan, R.K. and Bidochka, M.J. (2012) The insect pathogenic fungus *Metarhizium robertsii* (Clavicipitaceae) is also an endophyte that stimulates plant root development. *Am. J. Bot.* 99, 101–107
- Klironomos, J.N. (2000) Host-specificity and functional diversity among arbuscular mycorrhizal fungi. In *Microbial Biosystems: New Frontiers* (Bell, C.R. et al., eds), pp. 845–851, Atlantic Canada Society for Microbial Ecology
- Saikkonen, K. (2007) Forest structure and fungal endophytes. *Fungal Biol. Rev.* 21, 67–74
- Smith, S.E. and Read, D.J., eds (1997) *Mycorrhizal Symbiosis*, Academic Press
- Helgason, T. and Fitter, A.H. (2009) Natural selection and the evolutionary ecology of the arbuscular mycorrhizal fungi (Phylum Glomeromycota). *J. Exp. Bot.* 60, 2465–2480
- Javot, H. et al. (2007) A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. *Proc. Natl. Acad. Sci. U.S.A.* 104, 1720–1725
- Genre, A. et al. (2008) Prepenetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago truncatula* and *Daucus carota*. *Plant Cell* 20, 1407–1420
- Genre, A. et al. (2005) Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *Plant Cell* 17, 3489–3499
- Gaziz, R. and Chaverri, P. (2010) Diversity of fungal endophytes in leaves and stems of wild rubber trees (*Hevea brasiliensis*) in Peru. *Fungal Ecol.* 3, 240–254
- Porras-Alfaro, A. and Bayman, P. (2011) Hidden fungi, emergent properties: endophytes and microbiomes. *Annu. Rev. Phytopathol.* 49, 291–315
- Rodriguez, R.J. et al. (2009) Fungal endophytes: diversity and functional roles. *New Phytol.* 182, 314–330
- Behie, S.W. et al. (2012) Endophytic insect-parasitic fungi translocate nitrogen directly from insects to plants. *Science* 336, 1576–1577
- Behie, S.W. and Bidochka, M.J. (2014) Ubiquity of insect-derived nitrogen transfer to plants by endophytic insect-pathogenic fungi: an additional branch of the soil nitrogen cycle. *Appl. Environ. Microbiol.* 80, 1553–1560
- Smith, S.E. and Smith, F.A. (2012) Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia* 104, 1–13
- Doidy, J. et al. (2012) The *Medicago truncatula* sucrose transporter family: characterization and implication of key members in carbon partitioning towards arbuscular mycorrhizal fungi. *Mol. Plant.* 5, 1346–1358
- Javot, H. et al. (2011) *Medicago truncatula* mtpt4 mutants reveal a role for nitrogen in the regulation of arbuscule degeneration in arbuscular mycorrhizal symbiosis. *Plant J.* 68, 954–965
- Mukherjee, S. et al. (2010) Development of a transformation system in the swainsonine producing, slow growing endophytic fungus, *Undifilum oxytropis*. *J. Microbiol. Methods* 81, 160–165
- Sebastianes, F.L. et al. (2012) Genetic transformation of *Diaporthe phaseolorum*, an endophytic fungus found in mangrove forests, mediated by *Agrobacterium tumefaciens*. *Curr. Genet.* 58, 21–33
- Fang, W. et al. (2004) *Agrobacterium tumefaciens*-mediated transformation of *Beauveria bassiana* using an herbicide resistance gene as a selection marker. *J. Invertebr. Pathol.* 85, 18–24
- Kiers, E.T. et al. (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* 333, 880–882
- Boring, L.R. et al. (1988) Sources, fates, and impacts of nitrogen inputs to terrestrial ecosystems: review and synthesis. *Biogeochemistry* 6, 119–159
- Yoneyama, K. et al. (2007) Nitrogen deficiency as well as phosphorus deficiency in sorghum promotes the production and exudation of 5-deoxystrigol, the host recognition signal for arbuscular mycorrhizal fungi and root parasites. *Planta* 227, 125–132
- Field, C. and Mooney, H.A. (1986) The photosynthesis–nitrogen relationship in wild plants. In *On the Economy of Plant Form and Function* (Givnish, T.J., ed.), pp. 25–55, Cambridge University Press
- Steppe, T.F. (1996) Consortial N<sub>2</sub> fixation: a strategy for meeting nitrogen requirements of marine and terrestrial cyanobacterial mats. *FEMS Microbiol. Ecol.* 21, 149–156
- Van Der Heijden, M.G.A. et al. (2007) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* 11, 296–310
- Hodge, A. and Fitter, A.H. (2010) Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. *Proc. Natl. Acad. Sci. U.S.A.* 107, 13754–13759
- Sinsabaugh, R.L. (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biol. Biochem.* 42, 391–404
- Schimel, J.P. and Bennett, J. (2004) Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85, 591–602
- Nygren, C.M.R. et al. (2007) Detection of extracellular protease activity in different species and genera of ectomycorrhizal fungi. *Mycorrhiza* 17, 241–248
- Ngwene, B. et al. (2013) Influence of different mineral nitrogen sources (NO<sub>3</sub><sup>-</sup>-N vs. NH<sub>4</sub><sup>+</sup>-N) on arbuscular mycorrhiza development and N transfer in a *Glomus intradices*–cowpea symbiosis. *Mycorrhiza* 23, 107–117
- Tian, C. et al. (2010) Regulation of the nitrogen transfer pathway in the arbuscular mycorrhizal symbiosis: gene characterization and the coordination of expression with nitrogen flux. *Plant Physiol.* 153, 1175–1187
- Mian, I.A. et al. (2009) The importance of ammonium mobility in nitrogen-impacted unfertilized grasslands: a critical reassessment. *Environ. Pollut.* 157, 1287–1293

- 40 Perez-Tienda, J. *et al.* (2011) GintAMT2, a new member of the ammonium transporter family in the arbuscular mycorrhizal fungus *Glomus intraradices*. *Fungal Genet. Biol.* 48, 1044–1055
- 41 Kobae, Y. *et al.* (2010) Localized expression of arbuscular mycorrhiza-inducible ammonium transporters in soybean. *Plant Cell Physiol.* 51, 1411–1425
- 42 López-Pedrosa, A. *et al.* (2006) GintAMT1 encodes a functional high-affinity ammonium transporter that is expressed in the extraradical mycelium of *Glomus intraradices*. *Fungal Genet. Biol.* 43, 102–110
- 43 Javelle, A. *et al.* (2003) Molecular characterization, function and regulation of ammonium transporters (Amt) and ammonium-metabolizing enzymes (GS, NADP-GDH) in the ectomycorrhizal fungus *Hebeloma cylindrosporium*. *Mol. Microbiol.* 47, 411–430
- 44 Kempainen, M.J. and Pardo, A.G. (2013) *LbNrt* RNA silencing in the mycorrhizal symbiont *Laccaria bicolor* reveals a nitrate-independent regulatory role for a eukaryotic NRT2-type nitrate transporter. *Environ. Microbiol. Rep.* 5, 353–366
- 45 Jargeat, P. *et al.* (2003) Characterization and expression analysis of a nitrate transporter and nitrite reductase genes, two members of a gene cluster for nitrate assimilation from the symbiotic basidiomycete *Hebeloma cylindrosporium*. *Curr. Genet.* 43, 199–205
- 46 Usuki, F. and Narisawa, K. (2007) A mutualistic symbiosis between a dark septate endophytic fungus, *Heteroconium chaetospora*, and a nonmycorrhizal plant, Chinese cabbage. *Mycologia* 99, 175–184
- 47 Upson, R. *et al.* (2009) Nitrogen form influences the response of *Deschampsia antarctica* to dark septate root endophytes. *Mycorrhiza* 20, 1–11
- 48 Behie, S.W. *et al.* (2013) Nutrient transfer to plants by phylogenetically diverse fungi suggests convergent evolutionary strategies in rhizospheric symbionts. *Commun. Integr. Biol.* 6, e22321
- 49 Koegel, S. *et al.* (2013) The family of ammonium transporters (AMT) in *Sorghum bicolor*: two AMT members are induced locally, but not systemically in roots colonized by arbuscular mycorrhizal fungi. *New Phytol.* 198, 853–865
- 50 Gaude, N. *et al.* (2012) Arbuscule-containing and non-colonized cortical cells of mycorrhizal roots undergo a massive and specific reprogramming during arbuscular mycorrhizal development. *Plant J.* 69, 510–528
- 51 Ruttner, K.C. (2003) The global phosphorus cycle. *Treatise Geochem.* 8, 585–643
- 52 Vasquez, P. *et al.* (2000) Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biol. Fert. Soils* 30, 460–468
- 53 Smith, S.E. and Smith, F.A. (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. *Annu. Rev. Plant Biol.* 62, 227–250
- 54 Plassard, C. and Dell, B. (2010) Phosphorus nutrition of mycorrhizal trees. *Tree Physiol.* 30, 1129–1139
- 55 Smith, S.E. *et al.* (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiol.* 156, 1050–1057
- 56 Harrison, M.J. and Van Buuren, M.L. (1995) A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. *Nature* 378, 626–629
- 57 Maldonado-Mendoza, I.E. *et al.* (2001) A phosphate transporter gene from the extra-radical mycelium of an arbuscular mycorrhizal fungus *Glomus intraradices* is regulated in response to phosphate in the environment. *Mol. Plant Microbe Interact.* 14, 1140–1148
- 58 Benedetto, A. *et al.* (2005) Expression profiles of a phosphate transporter gene (GmosPT) from the endomycorrhizal fungus *Glomus mossae*. *Mycorrhiza* 15, 620–627
- 59 Taty, M. *et al.* (2009) Two differentially regulated phosphate transporters from the symbiotic fungus *Hebeloma cylindrosporium* and phosphorus acquisition by ectomycorrhizal *Pinus pinaster*. *Plant J.* 57, 1092–1102
- 60 Loth-Pareda, V. *et al.* (2011) Structure and expression profile of the phosphate Pht1 transporter gene family in mycorrhizal *Populus trichocarpa*. *Plant Physiol.* 156, 2141–2154
- 61 Yadav, V. *et al.* (2010) A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant. *J. Biol. Chem.* 285, 26532–26544
- 62 Sieh, D. *et al.* (2013) The arbuscular mycorrhizal symbiosis influences sulfur starvation responses of *Medicago truncatula*. *New Phytol.* 197, 606–616
- 63 Gyuricza, V. *et al.* (2010) Effect of potassium and phosphorus on the transport of radiocesium by arbuscular mycorrhizal fungi. *J. Environ. Radioact.* 101, 482–487
- 64 Cartmill, A.D. *et al.* (2008) Arbuscular mycorrhizal fungi enhance tolerance of vinca to high alkalinity in irrigation water. *Sci. Hortic.* 115, 275–284
- 65 Nord, E.A. and Lynch, J.P. (2009) Plant phenology: a critical controller of soil resource acquisition. *J. Exp. Bot.* 60, 1927–1937
- 66 Casieri, L. *et al.* (2012) Transcriptional response of *Medicago truncatula* sulphate transporters to arbuscular mycorrhizal symbiosis with and without sulphur stress. *Planta* 235, 1431–1447
- 67 Garcia, K. *et al.* (2014) Potassium nutrition of ectomycorrhizal *Pinus pinaster*: overexpression of the *Hebeloma cylindrosporium* HcTrk1 transporter affects the translocation of both K<sup>+</sup> and phosphorus in the host plant. *New Phytol.* 201, 951–960
- 68 Smith, F.A. *et al.* (2009) More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses. *New Phytol.* 182, 347–358
- 69 Fang, W. and St Leger, R.J. (2010) Mrt, a gene unique to fungi, encodes an oligosaccharide transporter and facilitates rhizosphere competency in *Metarhizium robertsii*. *Plant Physiol.* 154, 1549–1557
- 70 Liao, X. *et al.* (2013) *Metarhizium robertsii* produces and extracellular invertase (MrINV) that plays a pivotal role in rhizospheric interactions and root colonization. *PLoS ONE* 8, 1–11
- 71 Bücking, H. and Shachar-Hill, Y. (2005) Phosphate uptake, transport and transfer by the arbuscular mycorrhizal fungus *Glomus intraradices* is stimulated by increased carbohydrate availability. *New Phytol.* 165, 899–912
- 72 Fellbaum, C.R. (2012) Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis. *Proc. Natl. Acad. Sci. U.S.A.* 109, 2666–2671
- 73 Helber, N. *et al.* (2011) A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus *Glomus* sp is crucial for the symbiotic relationship with plants. *Plant Cell* 23, 3812–3823
- 74 Cairney, J.W.G. (2012) Extramatrical mycelia of ectomycorrhizal fungi as moderators of carbon dynamics in forest soil. *Soil Biol. Biochem.* 47, 198–208
- 75 Ceballos, I. *et al.* (2013) The *in vitro* mass-produced model mycorrhizal fungus, *Rhizophagus irregularis*, significantly increases yields of the globally important food security crop cassava. *PLoS ONE* 8, e70633
- 76 Kassa, A. (2008) Whey for mass production of *Beauveria bassiana* and *Metarhizium anisopliae*. *Micol. Res.* 112, 583–591
- 77 Roberts, D.W. and Hajek, A.E. (1992) Entomopathogenic fungi as bioinsecticides. In *Frontiers in Industrial Mycology* (Leathan, G.F., ed.), pp. 144–159, Chapman & Hall
- 78 Meyling, N.V. *et al.* (2011) Below and above ground abundance and distribution of fungal entomopathogens in experimental conventional and organic cropping systems. *Biol. Control* 59, 180–186
- 79 Parniske, M. (2004) Molecular genetics of the arbuscular mycorrhizal symbiosis. *Curr. Opin. Plant Biol.* 7, 414–421
- 80 Cappellazzo, G. (2008) Characterization of an amino acid permease from the endomycorrhizal fungus *Glomus mosseae*. *Plant Physiol.* 147, 429–437
- 81 Allen, J.W. and Shachar-Hill, Y. (2009) Sulfur transfer through an arbuscular mycorrhiza. *Plant Physiol.* 149, 549–560
- 82 Zuccaro, A. *et al.* (2011) Endophytic life strategies decoded by genome and transcriptome analyses of the mutualistic root symbiont *Piriformospora indica*. *PLoS Pathog.* 7, e1002290
- 83 Kempainen, M.J. *et al.* (2009) fHANT-AC genes of the ectomycorrhizal fungus *Laccaria bicolor* are not repressed by l-glutamine allowing simultaneous utilization of nitrate and organic nitrogen sources. *Environ. Microbiol. Rep.* 2, 541–553
- 84 Mansouri-Bauly, H. *et al.* (2006) Sulfur uptake in the ectomycorrhizal fungus *Laccaria bicolor* S238N. *Mycorrhiza* 16, 421–427