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# **The Role of the Mycorrhizal Symbiosis in Nutrient Uptake of Plants and the Regulatory Mechanisms Underlying These Transport Processes**

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Additional information is available at the end of the chapter

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## **1. Introduction**

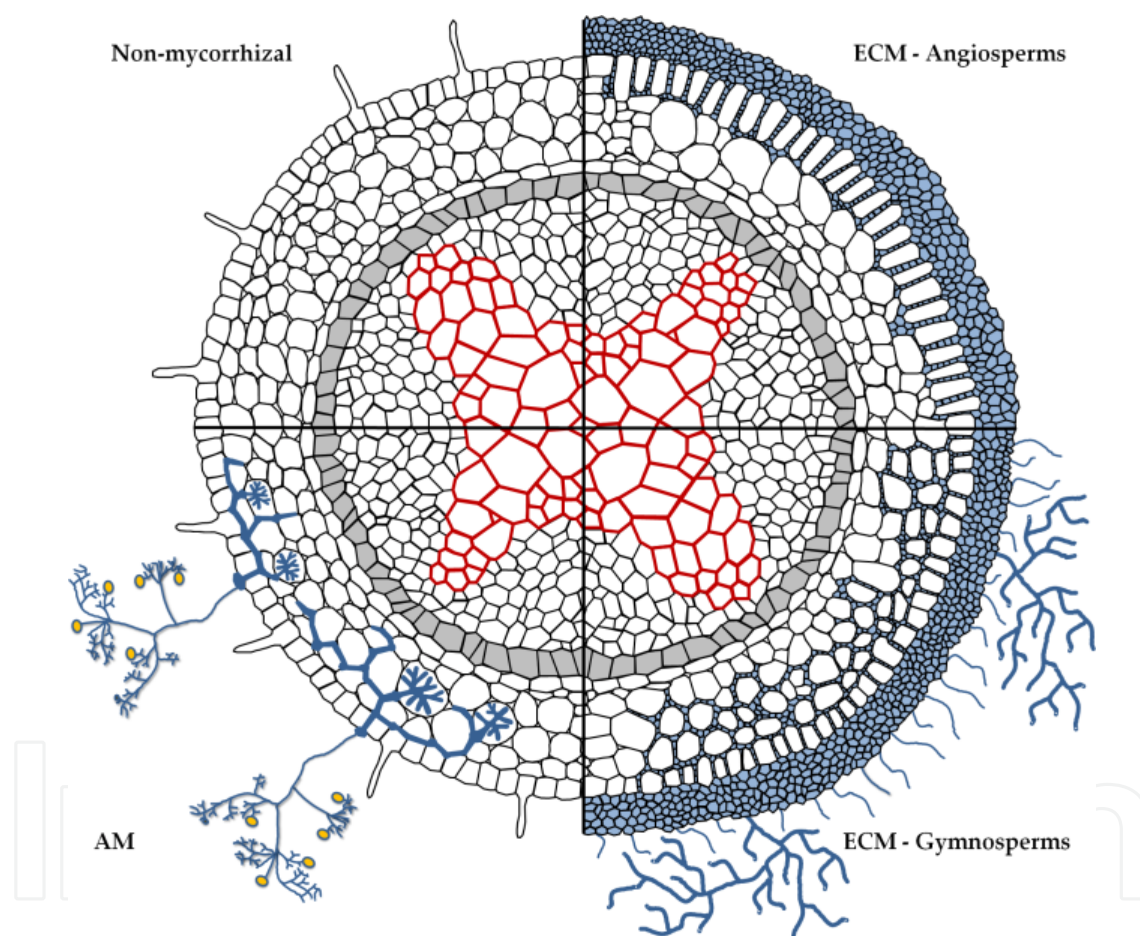
The mycorrhizal symbiosis is arguably the most important symbiosis on earth. Fossil records indicate that arbuscular mycorrhizal interactions evolved 400 to 450 million years ago [1] and that they played a critical role in the colonization of land by plants. Approximately 80 % of all known land plant species form mycorrhizal interactions with ubiquitous soil fungi [2]. The majority of these mycorrhizal interactions is mutually beneficial for both partners and is characterized by a bidirectional exchange of resources across the mycorrhizal interface. The mycorrhizal fungus provides the host plant with nutrients, such as phosphate and nitrogen, and increases the abiotic (drought, salinity, heavy metals) and biotic (root pathogens) stress resistance of the host. In return for their beneficial effect on nutrient uptake, the host plant transfers between 4 and 20% of its photosynthetically fixed carbon to the mycorrhizal fungus [3]. In contrast to mutually beneficial mycorrhizal interactions, some mycoheterotrophic plants (approximately 400 plant species from different plant families, such as bryophytes, pteridophytes, and angiosperms) rely on mycorrhizal fungi for their carbon supply. These plants have lost their photosynthetic capabilities and parasitize mycorrhizal fungi that are associated with neighbor autotrophic plants.

Primary focus of this chapter is on mutually beneficial ectotrophic and arbuscular mycorrhizal interactions, because of their high economic and ecological significance and their application potential. Arbuscular mycorrhizal fungi colonize the roots of many agriculturally important food and bioenergy crops and could serve as 'biofertilizers and bioprotectors' in environmentally sustainable agriculture. Ectomycorrhizal fungi on the other hand colonize a smaller number of plant species, but play as symbiotic partners of tree

and shrub species a key role in forest ecosystems [4], and could be a critical component in phytoremediation and/or revegetation applications [5, 6].

## 2. Structural diversity of mycorrhizal interactions

Arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) associations differ in their structural characteristics and in the plant and fungal species that they involve. In AM roots the fungus penetrates intercellularly and intracellularly into the root cortex, whereas in ECM roots the fungus only penetrates intercellularly into the root cortex. Figure 1 illustrates the main structural differences between AM and ECM associations of angiosperms or gymnosperms, which are discussed in greater detail below.



**Figure 1.** Structural characteristics of arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM) roots of gymnosperms or angiosperms.

### 2.1. Arbuscular mycorrhizal interactions

Arbuscular mycorrhizas are the most common form of mycorrhizal interactions. They are formed by a wide variety of host plants (approximately 65% of all known land plant species) [2], including many agriculturally important crop species, such as soybean, corn, rice, and wheat. All AM fungi have been classified into the separate fungal phylum Glomeromycota [7],

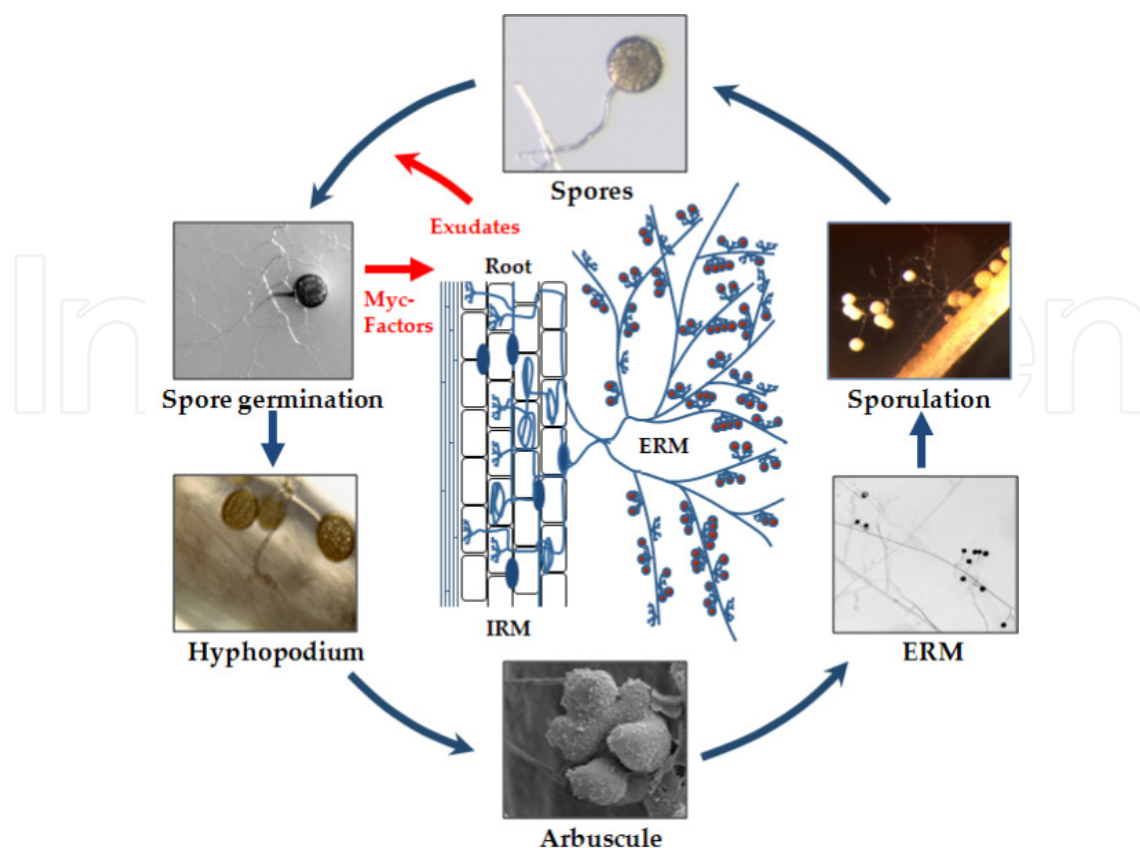
which is composed of approximately 150 fungal species [1] with a high genetic and functional diversity within each species. AM fungi are classified into three classes (Archaeosporomycetes, Glomeromycetes, and Paraglomeromycetes), and the five orders: Archaeosporales (e.g. *Geosiphon pyriformes*, *Archaeospora trappei*), Diversisporales (e.g. *Scutellospora calospora*, *Acaulospora laevis*, *Entrophospora infrequens*), Gigasporales (e.g. *Gigaspora margarita*, *G. rosea*), Glomerales (e.g. *Glomus intraradices*, *G. mosseae*, *G. geosporum*) and Paraglomerales (e.g. *Paraglomus occultum*, *P. laccatum*). This group of fungi is unique due to its age, lifestyle and genetic make-up. AM fungi may have evolved over 1000 million years ago and can be seen as living fossils because they co-exist relatively morphologically unaltered with plants for more than 400 million years [8]. The symbiosis is frequent in all early diverging lineages of the major plant clades. Non-mycorrhizal species or other mycorrhizal types developed in plant lineages of more recent origin. This suggests that this symbiosis is the ancestral form of mycorrhizal interactions and that it played a critical role in the evolution of land plants [1]. In comparison, the symbiosis with nitrogen-fixing Rhizobia bacteria evolved much later (approximately 60 million years ago), and this symbiosis is restricted to only one plant clade.

AM fungi are coenocytic and hyphae and spores contain hundreds of nuclei [9]. The polymorphic nature of these nuclei and the relatively large genome of these fungi has made genome sequencing and annotation of this important group of fungi particularly challenging [8, 10], but recently the first transcriptome of the AM fungus *Glomus intraradices* became available [11]. They are asexual, but an exchange of genetic material between closely related fungi via anastomosis has been observed.

#### 2.1.1. Structural characteristics of arbuscular mycorrhizal roots and fungal life cycle

AM fungi are obligate biotrophs and rely on their autotrophic host to complete their life cycle and to produce the next generation of spores (Figure 2). The spores are able to germinate without the presence of a host, but the spores respond with an increase in hyphal branching and metabolic activity to root exudates [12-14]. Plant roots release for example strigolactones that are able to induce pre-symbiotic growth of AM fungal spores [15].

On the host root surface, AM fungi form a specific appressorium – the hyphopodium. Fungal hyphae emerging from this hyphopodium penetrate into the root through the prepenetration apparatus, which guides the fungal hyphae through the root cells toward the cortex. In the cortex the hyphae enter the apoplast, and grow laterally along the root axis, and penetrate into inner root cortical cells. In ‘typical’ AM associations of the ‘Arum type’ enters the fungus the cell by small hyphal branches that by continuous dichotomous branching develop into characteristic highly branched arbuscules (Figure 2, 3d). By contrast, in ‘Paris type’ mycorrhizas spreads the fungus primarily from cell to cell and develops extensive intracellular hyphal coils that sometimes show an arbuscular like branching [1]. The fungus does not enter the plant symplast and is excluded from the host cytoplasm by the enlarged periarbuscular membrane (PAM) of the host. Some fungi also form vesicles, fungal storage organs in the root apoplast.



**Figure 2.** Life cycle of an AM fungus and the different steps during AM development.

Despite its coenocytic nature, the mycelium that is formed within the root, the intraradical mycelium (IRM) differs morphologically and functionally from the extraradical mycelium (ERM), the mycelium that grows into the soil. The ERM absorbs nutrients from the soil and transfers these nutrients to the host root. The IRM on the other hand releases nutrients into the interfacial apoplast and exchanges them against carbon from the host. The fungus uses these carbon resources to maintain and to enlarge the ERM, for cell metabolism (e.g. active uptake processes, nitrogen assimilation), and for the development of spores, which are able to initiate the colonization of a next generation of host plants (Figure 2, 3a).

### 2.1.2. Colonization of the root with arbuscular mycorrhizal fungi

Similar to Nod factors that play an important role in root nodulation, AM fungi release Myc factors that lead to an expression of plant symbiosis related genes and prepare the root for AM symbiosis. One active Myc factor has been identified as lipochitooligosaccharide [16]. Nod factors are also lipochitooligosaccharides and have a similar composition. It has been suggested that Nod factors developed from Myc factors, and that the functions of Myc and Nod factors overlap [17]. This is also supported by the fact that AM and rhizobial symbiosis share parts of the same signal transduction pathway - the so-called common symbiosis pathway. So far seven genes (SYM genes) of the common symbiosis pathway have been identified that are required for both root symbioses (Table 1).



Gene	Predicted gene function
<i>SYMRK</i>	Leucine-rich receptor-like kinase that plays an essential role for root endosymbioses with Rhizobia bacteria, AM fungi and <i>Frankia</i> bacteria, and is involved in the signal transduction to the cytoplasm after the perception of Nod or Myc factors [8, 18].
<i>NUP85/</i> <i>NUP133</i>	Putative components of the nuclear pore complex that are involved in the transport of macromolecules through the nuclear envelope [8].
<i>CASTOR/</i> <i>POLLUX</i>	Cation channels in the nuclear envelope that are essential for the perinuclear calcium spiking after the perception of Nod or Myc factors [8, 19].
<i>CCaMK</i>	Calcium and calmodulin-dependent protein kinase with three calcium binding motifs that acts as sensor of the nuclear calcium signatures and is involved in the phosphorylation of <i>CYCLOPS</i> [8, 20].
<i>CYCLOPS</i>	Protein with unknown function that acts as phosphorylation target of <i>CCaMK</i> downstream of the nuclear calcium spiking and is presumably the branchpoint of the common SYM pathway [8, 20].

**Table 1.** Genes of the common symbiosis pathway and their predicted function

One common signaling component is the receptor-like kinase *SymRK* that is involved in the direct or indirect perception of fungal or rhizobial signals and transduces the signal through its intracellular kinase domain to the cytoplasm [17, 18]. The two nucleoporins *NUP85* and *NUP133* act downstream and could be involved in the transport of *CASTOR* and/or *POLLUX* to the inner nuclear envelope. *CASTOR* and *POLLUX* are ion channels that are involved in the oscillation of the calcium concentration in the nucleus and perinuclear cytoplasm (calcium spiking) that can be observed shortly after the plant perceives signals from its root symbionts [8, 19]. The calcium-calmodulin dependent protein kinase *CCaMK* with its calmodulin- and calcium-binding domains is localized in the nucleoplasm and acts likely as the sensor of the calcium signatures that are induced by the perception of Myc or Nod factors. *CCaMK* is known to phosphorylate the last identified SYM gene *CYCLOPS*, which encodes a protein with no sequence similarity to proteins with known function. *CYCLOPS* contains a functional nuclear localization signal and a carboxy-terminal coiled-coil domain and it has been suggested that *CYCLOPS* represents a branch point in the common SYM pathway. Infection threat formation and arbuscular development are *CYCLOPS* dependent, but nodule organogenesis is *CYCLOPS* independent [20].

In contrast to the SYM pathway, little is known about the cellular and molecular re-programming that is required for the intracellular colonization and the development of arbuscules in cortical cells. In *Petunia hybrida* *pam1* mutants (*penetration and arbuscule morphogenesis 1*) fungal hyphae are able to penetrate into the cells, but the intracellular accommodation of the arbuscules is defective and intracellular hyphae are rapidly degraded [21]. *Pam1* is a homologue of the *VAPYRIN* gene in *Medicago truncatula* and *VAPYRIN* mutants show a similar phenotype. The *PAM1* protein shows an affinity to tonospheres, mobile structures that are associated with the tonoplast, and that can also be found in the

vicinity of intracellular hyphae in mycorrhizal roots. The first physical contact between both partners leads to a local upregulation of two CAAT-box binding transcription factors (*MtCbf1* and *MtCbf2*) in arbusculated cells of *Medicago truncatula*. Both transcription factors are able to interact with a large range of promoters, and could play a role in the sequential re-programming of root tissues during the establishment of an AM symbiosis [22].

### 2.1.3. The mycorrhizal interface in arbuscular mycorrhizal associations

Critical for the mutualism in the AM symbiosis is the bidirectional exchange of nutrients across the mycorrhizal interface. The interface between the fungus and the host includes the PAM and the fungal plasma membrane, the fungal cell wall and the periarbuscular space between the fungal cell wall and the PAM. The PAM differs in its protein composition from the plant plasma membrane of non-arbusculated cells and is characterized by mycorrhiza-inducible transporters that facilitate the uptake of nutrients from the mycorrhizal interface. One of these transporters is *Pt4*, a high affinity phosphate (P) transporter that is only expressed in mycorrhizal roots and that is involved in the acquisition of P delivered by the fungus [23, 24]. A high-affinity ammonium ( $\text{NH}_4^+$ ) transporter (*AMT2;2*) is also localized in the PAM. This transporter is exclusively expressed in arbusculated cells of mycorrhizal roots, but not in root nodules [25]. In contrast to other high affinity  $\text{NH}_4^+$  transporters of plants, *AMT2;2* of *Lotus japonicus* (*LjAMT2;2*) transfers  $\text{NH}_3$  instead of  $\text{NH}_4^+$  and it has been suggested that the transporter takes up the positively charged  $\text{NH}_4^+$  from the mycorrhizal interface and releases uncharged  $\text{NH}_3$  into the plant cytoplasm. The detection of mycorrhiza-inducible sulphate transporters in AM roots suggests that also sulphate is transferred from the AM fungus to the host across the mycorrhizal interface [26, 27]. The transport of carbon from the host to the fungus is driven by a monosaccharide transporter in the fungal arbuscular membrane (*MST2*) [28]. This transporter takes up glucose but also other monosaccharides, such as xylose, what indicates that the fungus can also use cell wall sugars of the plant as alternative carbon source.

## 2.2. Ectomycorrhizal interactions

There are approximately 7000 to 10000 fungal species and 8000 plant species that form ectomycorrhizal (ECM) associations [29]. The number of plant species is relatively small (approximately 3%), but the group includes plants with high global and economic importance due to the disproportionate large terrestrial land surface that these plants cover, and as main producers of timber. The plant species include wooden perennials, trees or shrubs from cool, temperate boreal or montane forests, but also species from arctic alpine shrub communities [1, 4]. However, most of these plant species are not exclusively colonized by ECM fungi. Many species, such as *Populus* (see Figure 3d), *Salix*, *Betula* and *Fagus* also form AM interactions, and there are indications that the AM symbiosis is the common mycorrhizal form of this taxon [1].

ECM fungi are relatively closely related to saprotrophic fungi and mainly belong to the Basidiomycota (e.g. *Amanita muscaria*, *Hebeloma cylindrosporum*, *Laccaria bicolor*, *Paxillus*

*involutus*, *Pisolithus tinctorius*, *Suillus bovinus*, *Xerocomus badius*), but also include some Ascomycota (e.g. *Cenococcum geophilum*, *Tuber borchii*, *Scleroderma hypogaeum*) [1]. The switch from the presumably ancestral saprotrophic to the symbiotic behavior developed convergently in several fungal families during evolution. In contrast to AM fungi, many ECM fungi can be grown in axenic culture without a host, and this has allowed screening of their ability to use different carbon or nutrient sources [30]. ECM fungi have a dual life style and are considered to be facultative saprotrophs. In the soil they are highly competitive in nutrient acquisition and secrete a number of hydrolytic enzymes that allow them to degrade litter polymers, and to use organic nutrient sources [4]. At the same time they live within plant roots as symbionts and this requires a set of adaptation mechanisms to avoid plant parasitism. ECM fungi have for example lost their ability to degrade plant cell wall polysaccharides (cellulose, pectins, and pectates), and this restricts their penetration into the root to the intercellular spaces [31].

### 2.2.1. Colonization of the root with ectomycorrhizal fungi

Typical for ECM roots are changes in the root morphology, such as the dichotomous branching of lateral roots, e.g. in pines (Figure 3b), the production of a large number of root meristems and as a result an extensive root branching (Figure 3c), the inhibition of root hair formation, and the enlargement of cortical cells. Many of these morphological effects can be observed prior to colonization and can be interpreted as a preparation of the plant to increase root symbiosis.

Prior to the establishment of a functional ECM root and similar to the processes during AM development, there is an exchange of signals and cross-talk between both partners. The fungal tryptophan betaine hypaphorine has been shown to trigger reduced root hair elongation and swelling of the root hair tip and a stimulation of short root formation [32]. ECM fungi also produce phytohormones, including auxins, cytokinins, abscisic acid and ethylene, and it has been shown that the changes in the root morphology are caused by an overproduction of auxin in ECM fungal hyphae and changes in the endogenous hormone levels in the roots. The effect of ECM fungi on lateral root formation is independent from the plant's ability to form ECM associations. The ECM fungus *Laccaria bicolor* can induce lateral root formation also in *Arabidopsis thaliana*, a non-mycorrhizal plant, and the effect is correlated to an accumulation of auxin in the root apices [33]. The auxin accumulation in the root tips and/or other fungal signals could stimulate basipetal auxin transport and lateral root primordia formation by an induction of plant genes involved in auxin transport and signaling.

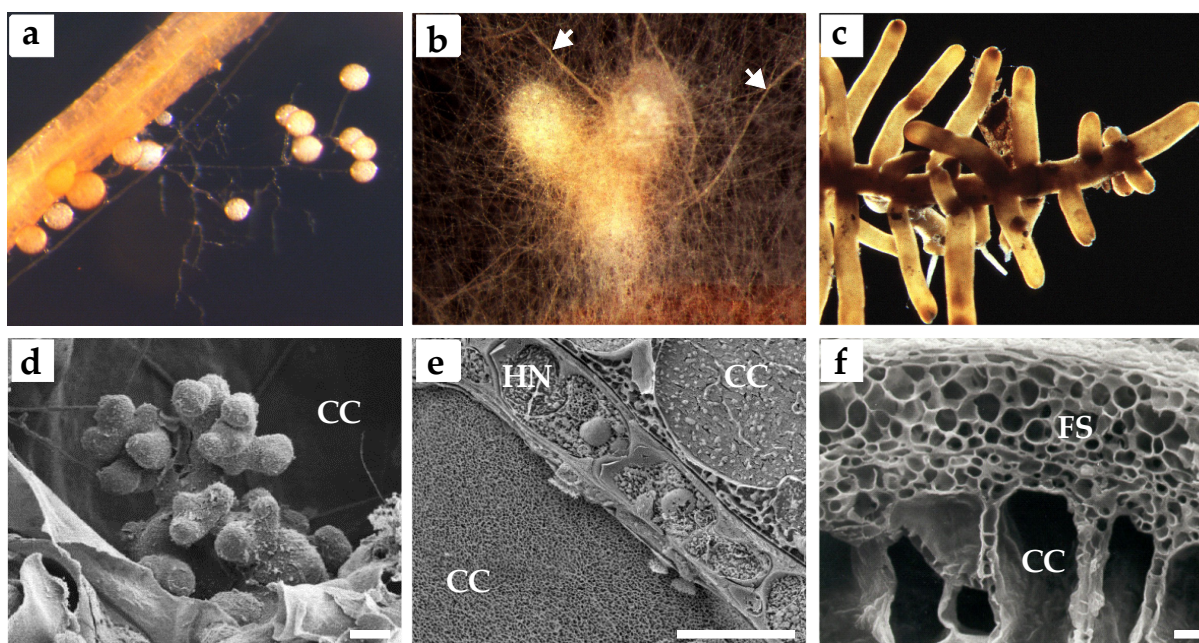
The fungal partner responds to root exudate components, such as rutin and zeatin, with a stimulation in hyphal growth and branching and growth towards the root and an accumulation of hypaphorine [32, 34]. In response to host signals, ECM fungi also release effector proteins into the rhizosphere, such as the *MYCORRHIZAL INDUCED SMALL SECRETED PROTEIN 7* (*MiSSP7*) of *Laccaria bicolor*. This fungal protein targets the plant nucleus after its uptake, and alters plant gene expression [35]. *MiSSP7* has been shown to be crucial for the establishment of the ECM symbiosis and resembles effector proteins of



pathogenic fungi, and bacteria with similar function. A transcriptional response of the host can be observed within hours after an initial contact between both partners has been established. Plant genes encoding proteins involved in stress and defense response, as well as genes involved in signal transduction and communication, and water uptake are upregulated in response to the presence of an ECM fungus in the rhizosphere [36].

### 2.2.2. Structural characteristics of ectomycorrhizal roots

An established ECM symbiosis is characterized by three structural components: the hyphal sheath or mantle, the Hartig net (in later passages of this text sometimes also referred to as intraradical mycelium or IRM), and the extraradical mycelium. The hyphal sheath or mantle encloses the root completely. The structural composition of the mantle is very diverse and can range from relatively thin, loosely arranged assemblages of hyphae to very thick, multilayered and pseudoparenchymatous mantles (Figure 1, 3bcf). The surface of the mantle can be compact and smooth (Figure 3c) or rough with numerous emerging hyphae and hyphal strands or rhizomorphs (Figure 3b). The fungal sheath is involved in nutrient storage and controls the nutrient transfer to the host. The fungal mantle can represent a significant apoplastic barrier [37, 38] and thereby creates a closed interfacial apoplast, in which the conditions can be controlled by both partners.



**Figure 3.** Morphological characteristics of AM (a, d) and ECM (b, c, e, f) roots. Images of the outer root morphology (a-c) and scanning electron microscopical images of fungal structures within the root (d-f). (a) AM root of *Daucus carota* colonized with *Glomus intraradices* with fungal spores and ERM; (b) dichotomous ECM pine root colonized by *Suillus bovinus* with rhizomorphs (arrows); (c) ECM root of beech colonized by an unidentified fungus with extensive root branching; (d) arbuscule of an AM fungus within the cortical cell (CC) of an ECM root of *Populus tremuloides*; (e) Hartig net (HN) region and mycorrhizal interface in an ECM root of *Populus tremuloides*; (f) ECM root with epidermal Hartig net and radially elongated epidermis cells and fungal sheath (FS).

The Hartig net plays the key role in the nutrient transfer between both partners. The Hartig net is formed by hyphae that penetrate into the root cortex intercellularly (Figure 1, 3e). The penetration depth of the Hartig net differs between angiosperms and gymnosperms. Most angiosperms develop an epidermal Hartig net and confine the penetration of the Hartig net to the outer epidermis, which is often radially elongated (Figure 1, 3f). By contrast, the Hartig net in gymnosperms normally encloses several layers of cortical cells and sometimes extends up to the endodermis (Figure 1)[1].

The extraradical mycelium (ERM) of the fungus acts as an extension of the root system and it has been estimated that the ERM of the fungus *Pisolithus tinctorius* can represent 99% of the nutrient-absorbing surface length of pine roots [39]. The ERM of ECM fungi can account for 32% of the total microbial biomass and 700-900 kg ha<sup>-1</sup> in forest soils [40, 41]. The ERM can have a relatively simple organization with individual hyphae with similar structure that grow into the soil (mainly in ascomycetes) or can be differentiated into singular hyphae and rhizomorphs (Figure 3b). Rhizomorphs are aggregates of hyphae which grow in parallel and whose organization level can range from simple assemblages of undifferentiated and loosely woven hyphae to complex aggregations of hyphae with structural and functional differentiations [42].

### 2.2.3. The mycorrhizal interface in ectomycorrhizal associations

Transport studies suggest that in ECM associations nutrients are exchanged simultaneously across the same interface [43]. The interface includes the plasma membranes and cell walls of both partners and the interfacial matrix between both partners. The plant transfers photosynthates as sucrose from source to sink organs and ECM roots act as strong carbon sinks in mycorrhizal root systems. It is generally accepted that in contrast to phytopathogenic fungi or ericoid mycorrhizal fungi, AM and ECM fungi are not able to use sucrose as a carbon source, and that they take up simpler sugars, such as glucose or fructose, from the mycorrhizal interface. The presence of invertase genes in fungal genomes is correlated with the nutritional mode and in contrast to other plant-associated fungi, such as pathogens, or endophytes, there are no indications that AM or ECM fungi possess invertase genes [44] or have invertase activity [30]. Consequently, mycorrhizal fungi rely on the invertase activity of the host in the interfacial apoplast for sucrose hydrolysis. Sucrose hydrolysis makes the hexoses glucose and fructose available for the fungus and it has been suggested that glucose is mainly taken up by hyphae of the Hartig net and fructose mainly by hyphae of inner mantle layers [45]. Compared to the ERM, fungal hexose transporters are up-regulated in ECM roots, indicating that the fungus in symbiosis takes up carbon primarily from the mycorrhizal interface [46].

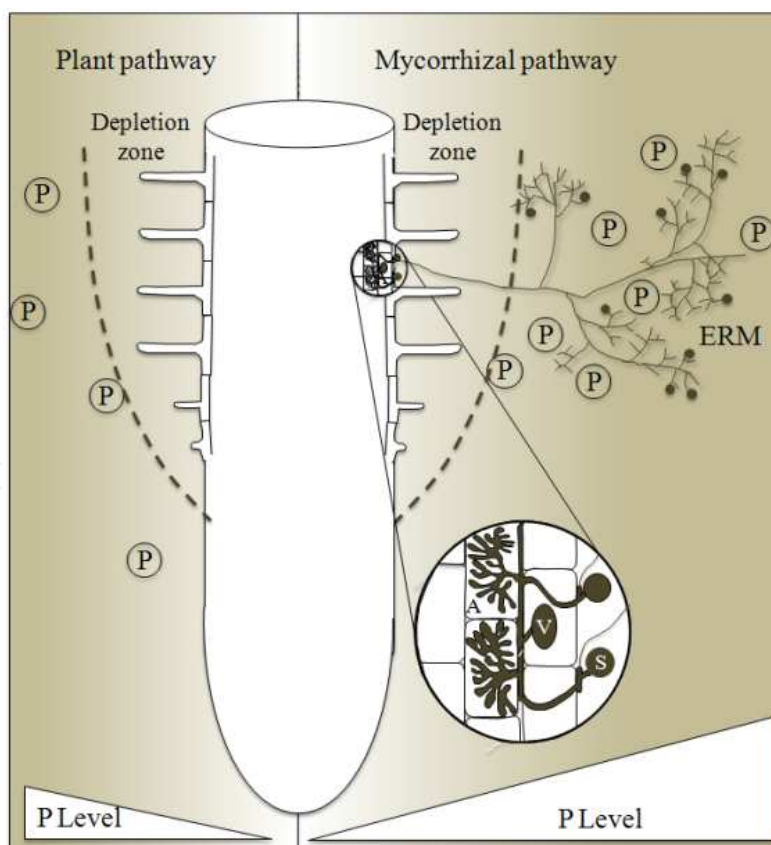
The high affinity NH<sub>4</sub><sup>+</sup> importer *AmAMT2* of *Amanita muscaria* is up-regulated in the ERM, but down-regulated in Hartig net and the fungal sheath [47]. The high expression of this transporter in the ERM suggests a high capability of the ERM for NH<sub>4</sub><sup>+</sup> uptake. The low expression level in the Hartig net on the other hand indicates that NH<sub>4</sub><sup>+</sup> can serve as a potential nitrogen source that is delivered by the mycorrhizal fungus to the host. A low

expression level of this  $\text{NH}_4^+$  importer in the Hartig net would reduce the re-absorption of  $\text{NH}_4^+$  by the fungus from the interfacial apoplast and increase the net transport of  $\text{NH}_4^+$  to the host. The potential transport of  $\text{NH}_4^+$  across the ECM interface is also supported by the presence and upregulation of plant high affinity  $\text{NH}_4^+$  importers in ECM roots [48].

### 3. Discussion

#### 3.1. Nutrient uptake pathways in arbuscular mycorrhizal or ectomycorrhizal roots

Mycorrhizal plants can take up nutrients from the soil via two pathways: the 'plant pathway' that involves the direct uptake of nutrients from the soil by the root epidermis and its root hairs or the 'mycorrhizal pathway' that involves the uptake of nutrients via the ERM of the fungus and the transport to the Hartig net in ECM interactions or to the IRM in AM interactions, and the uptake by the plant from the interfacial apoplast. The uptake of nutrients from the soil via the plant pathway, however, is often limited by the low mobility of nutrients in the soil. The mobility of for example phosphate (P) is so low that its uptake leads rapidly to the development of depletion zones around the roots and limits the further P uptake via the plant pathway to the low rate of diffusion [49].



**Figure 4.** P uptake of the plant via the plant pathway or mycorrhizal pathway. Abbreviations: Extraradical mycelium of the fungus (ERM), vesicles (V) and spores (S) of the arbuscular mycorrhizal fungus.



AM and ECM roots differ in their structural characteristics and this has implications for the nutrient uptake pathways in AM or ECM plants (Figure 1). AM roots do not form a fungal sheath and can theoretically use both pathways for nutrient uptake. It has previously been suggested that in the AM symbiosis both uptake pathways act additively. This led to the assumption that the uptake via the mycorrhizal pathway can be neglected when for example the P availability in the soil is high and mycorrhizal plants not always show a positive growth response. This view, however, is now being questioned [50-52], and it has been argued that the mycorrhizal pathway can dominate the total P uptake and that the true contribution of the mycorrhizal pathway to total P uptake can be “hidden” [52]. It has been demonstrated that even in non-responsive wheat plants, 50% of the plant’s P can be taken up via the mycorrhizal pathway [53]. This indicates that mycorrhizal plants change their nutrient acquisition strategy and that even under high P availabilities in the soil, the mycorrhizal fungus can still contribute significantly to the P uptake of the plant. Plant P transporters that are involved in the uptake via the plant pathway are down-regulated in response to the AM symbiosis [54, 55], while mycorrhiza-specific transporters that are involved in the P uptake from the mycorrhizal interface are induced [23, 24, 56].

However, the contribution of the plant or mycorrhizal pathway to total P uptake also depends on the plant and fungal species. *Glomus intraradices* has been shown to suppress the expression of plant P transporters of the plant pathway the most, whereas *G. mosseae* had the least effect [55]. In tomato, almost 100% of the plant’s P was taken up by *G. intraradices* via the mycorrhizal pathway, but the contribution of *Gigaspora rosea* to total P uptake was much lower [57]. *Glomus caledonium* always showed a high P uptake and transfer independent on the plant species, but *Glomus invernaium* only transferred significant amounts of P to the host plant flax [58]. This indicates that the contribution of the mycorrhizal pathway to nutrient acquisition also depends on fungal specific effects on the activity of the plant pathway and on the efficiency with which both partners interact and exchange nutrients across the mycorrhizal interface.

By contrast, in ECM tree species such as *Pinus*, the majority of the root surface is composed of roots that do not contribute to nutrient acquisition, such as the condensed tannin zone or the cork zone. Root zones that are active in nutrient acquisition such as the non-mycorrhizal white or ECM roots represent only 2% or 16% of the total root length, respectively [59]. ECM roots are characterized by a more or less dense fungal sheath that surrounds the mycorrhizal root completely. If the fungal mantle is impermeable to nutrient ions, the underlying root tissue would be isolated from the soil solution, and these roots would exclusively rely on the mycorrhizal pathway for nutrient uptake. Whether the fungal sheath represents an apoplastic barrier depends on the ECM fungal species and the structure and properties of the mantle. Some fungi for example have been shown to express and to release hydrophobins during ECM development [60]. Hydrophobins are small hydrophobic proteins that are involved in the adhesion of hyphae to surfaces, but can also increase the water repellency of the fungal sheath, and thereby make the sheath impermeable for water and nutrient ions [61, 62]. The fungal mantle of *Hebeloma cylindrosporum* for example is

impermeable to sulfate [63]. The fungal sheath of *Pisolithus tinctorius* and of *Suillus bovinus* is not completely impermeable to nutrient ions, but it seems likely that under normal soil conditions when plant and fungus compete for nutrients, the passive movement of nutrients through the fungal sheath of ECM roots is restricted and that a significant part of the nutrient uptake of these ECM roots is under fungal control [37, 63]. Under consideration that only 2% of the root surface of pines is non-mycorrhizal, and that the ERM of an ECM fungus can represent up to 99% of the nutrient-absorbing surface length of pine roots [39], ECM tree species such as pines are considered to be highly dependent on their fungal symbionts [64, 65], and it can be assumed that the mycorrhizal pathway plays in ECM root systems an even more significant role for nutrient uptake than in AM root systems (Table 2).

Characteristic	ECM symbiosis	AM symbiosis
Fungal life style	Facultative saprotroph	Obligate biotroph
Structural components	Mantle, Hartig net, and ERM with or without rhizomorphs	Arbuscules or intercellular hyphal coils, ERM, vesicles in some types
Penetration	Exclusively intercellularly	Intercellularly and intracellularly
Nutrient uptake pathway	ECM roots represent a significant proportion of the nutrient absorbing surface and nutrient uptake predominately via the mycorrhizal pathway	Theoretically plant and mycorrhizal pathway, but mycorrhizal pathway can dominate nutrient uptake in mycorrhizal roots
Contribution to plant nutrition	Particularly important for N nutrition, but also significant contributions to P nutrition	Particularly important for P nutrition; contributions to N nutrition still under debate
Fungal nutrient resources	Efficient uptake of inorganic and organic nutrient resources	Uptake predominately of inorganic nutrient resources, utilization of organic nutrient resources considered to be small

**Table 2.** Comparison of nutrient uptake mechanisms in ECM or AM interactions.

## 4. Mycorrhizal interactions and phosphate nutrition of plants

### 4.1. Uptake of phosphate from the soil

The positive effect of mycorrhizal fungi on phosphate (P) nutrition is long known and has been attributed to:

- The exploration of large soil volumes by the ERM in which orthophosphate ( $P_i$ ) is scavenged and delivered to plant cortical cells, bypassing the plant pathway for P uptake [66, 67] (Figure 4);
- The small hyphal diameter that allows the fungus to penetrate into small soil cores in search for P, and higher P influx rates per surface unit [66, 68];
- The capability of mycorrhizal fungi to store P in form of polyphosphates, which allows the fungus to keep the internal  $P_i$  concentration relatively low, and allows an efficient transfer of P from the ERM to the IRM [69]; and



- The production and secretion of acid phosphatases and organic acids that facilitate the release of P from organic complexes [70, 71].

Similar to plants, fungi have two uptake systems for P: (1) a high affinity system that works against an electrochemical potential gradient, which takes up  $P_i$  from the soil via proton co-transport [72, 73], and (2) a low affinity system which facilitates the diffusion of  $P_i$  across the fungal plasma membrane [74]. AM and ECM fungi express high affinity P transporters in the ERM that are involved in the P uptake from the soil [75, 76] (Figure 5). The expression of these transporters is regulated in response to the externally available P concentration, and to the P demand of the fungus. Under  $P_i$  starvation the transcript levels generally increase. Interestingly, in the ERM of the ECM fungus *Hebeloma cylindrosporum* two P transporters are expressed, one transporter is up-regulated under low (*HcPT1*), and one transporter is up-regulated under high P supply conditions (*HcPT2*) [76]. The simultaneous expression of two fungal P transporters that respond differently to the P level in the soil, could enable the ERM of the fungus to take up P efficiently also from locally varying P concentrations in the soil (e.g. from nutrient hot spots or from the root rhizosphere with its low  $P_i$  concentrations).

## 4.2. Fungal phosphate metabolism

Orthophosphate ( $P_i$ ) that is absorbed by the ERM can (a) replenish the cytoplasmic, metabolically active  $P_i$  pool; (b) be incorporated into phospholipids, RNA-, DNA- and protein-phosphates; (c) or can be transferred into a storage pool of short- or long-chained polyphosphates (polyP) [77] (Figure 5). Inorganic polyP are linear polymers in which  $P_i$  residues are linked by energy-rich phospho-anhydride bonds. Two types of polyP can be distinguished in mycorrhizal fungi: short chain polyP with a length of up to 20  $P_i$  residues and long chained polyP with more than 20  $P_i$  residues. The average length of short chained polyP in AM fungi has been estimated as 11-20  $P_i$  [78, 79], and of long chained polyP as 190 to 300  $P_i$  residues [80, 81]. Mycorrhizal fungi can rapidly store a significant proportion (more than 60%) of their cellular P as polyP [69, 82, 83]. In the mycorrhizal symbiosis, polyP are involved in:

- P homeostasis in the hyphae and maintenance of low intracellular  $P_i$  levels.** Low  $P_i$  levels in fungal hyphae increase the efficiency with which P can be absorbed and reduce the osmotic stress at high internal P concentrations [79, 84];
- Long-distance transport from the ERM to the IRM.** Based on the high flux rate of P through the hyphae of mycorrhizal fungi<sup>1</sup> [85, 86], it has been suggested that P is transferred mainly as polyP from the ERM to the root [87, 88]. The chain lengths of polyP in the ERM are longer than in the IRM, suggesting that polyP are primarily formed in the ERM and re-mobilized in the IRM [89]. In ECM and AM fungi a motile tubular vacuole system has been identified [90-92], that allows the polyP transport through the hyphae separately from the cytoplasmic compartment and enables the fungus to fine-tune its local cytoplasmic  $P_i$  concentration.

<sup>1</sup> between  $2 \times 10^{-10}$  and  $2 \times 10^{-9}$  mol P cm<sup>-2</sup> s<sup>-1</sup> have been described for AM fungi

- c. **Regulation of P transport.** It has been suggested that mycorrhizal fungi control the intracellular  $P_i$  concentration in the IRM and the P flux to the host by regulating the formation and/or turnover rates of polyP in the IRM or in the Hartig net region [93-95]. Long-chain polyP are mainly involved in long term storage of P, whereas short-chain polyP are correlated to the P transport in the symbiosis [93].
- d. **Cation homeostasis.** PolyP are polyanions and their negative charge is balanced by cations. The cations  $K^+$  and  $Mg^{2+}$  are mainly involved in charge balance [82, 83, 96], but polyP can also serve as trap for toxic cations such as heavy metals [82]. The basic amino acid  $Arg^+$  can also be involved in the charge balance of polyP and it has been suggested that in ECM fungi polyP can also store significant amounts of N [82, 97].

## 5. Mycorrhizal interactions and nitrogen nutrition

### 5.1. Uptake of nitrogen from the soil

Many ecosystems in which the nitrogen (N) availability in the soil is low and the supply with N often limits plant growth are dominated by ECM plant communities. ECM fungi can take up inorganic N sources very efficiently from soils [98, 99], but their capability to utilize organic N sources, and to make these sources available for the host plant, is generally seen as an important factor in the N nutrition of ECM plant species [1]. Many ECM fungi can for example mobilize and utilize amino acids and amides, such as glutamine, glutamate and alanine, which can represent a significant N pool, particularly in acid-organic soils [1]. Some amino acids can be taken up intact, and can directly be incorporated into assimilation pathways and can thereby also represent a significant carbon pool for ECM fungi [100].

By contrast, the contribution of AM fungi to total N uptake of plants is still under debate. The mobility of the inorganic N sources nitrate ( $NO_3^-$ ) and ammonium ( $NH_4^+$ ) in the soil is relatively high, and it has been suggested that the improved N status of AM plants is only the consequence of an improved P nutrition [101]. However, there are numerous reports in which a significant transport of N by AM fungi to their host has been demonstrated. In AM root organ cultures, 21% of the total N in the roots were taken up by the ERM [102]; and in similar experiments even higher proportions were observed [103]. In maize, 75% of the N in the leaves were taken up by the ERM of an AM fungus [104]. It becomes increasingly clear, that the mycorrhizal pathway can play a role in the N nutrition of AM plants, even if the percentage contribution to total N nutrition of the host plant can vary considerably [50]. Compared to ECM fungi, the capability of AM fungi to utilize organic N sources is considered to be relatively low, but some AM fungi are also able to use organic N sources. AM fungi can take up exogenously supplied amino acids [13, 103, 105] and are able to mobilize N from organic nutrient patches and to transfer these N sources to their host [106].

The ERM of AM and ECM fungi can take up the inorganic N sources ammonium ( $NH_4^+$ ) or nitrate ( $NO_3^-$ ) from the soil [99, 103, 105] (Figure 5).  $NH_4^+$  has often been described as the

preferred N source of mycorrhizal fungi, because its uptake is energetically more efficient than the uptake of  $\text{NO}_3^-$  [13, 102, 105]. However, ECM fungi differ in their ability to absorb  $\text{NO}_3^-$  from the soil, and some ECM fungi have been shown to produce a greater biomass when supplied with  $\text{NO}_3^-$  compared to  $\text{NH}_4^+$  [107]. However, a supply of  $\text{NH}_4^+$  leads to a down-regulation of a  $\text{NO}_3^-$  transporter and a nitrate reductase of *Hebeloma cylindrosporum*, what suggests that also ECM fungi generally prefer  $\text{NH}_4^+$  over  $\text{NO}_3^-$  [1, 108, 109].

Several high affinity  $\text{NH}_4^+$  transporters from AM and ECM fungi have been identified. The expression of *AMT1* and *AMT2*, two  $\text{NH}_4^+$  transporters of the ECM fungus *H. cylindrosporum*, is regulated by the exogenous  $\text{NH}_4^+$  supply. The expression of both transporters is up-regulated under low  $\text{NH}_4^+$  supply conditions, but down-regulated in response to an exogenous supply of  $\text{NH}_4^+$ . It has been suggested that the intracellular level of glutamine is responsible for the repression under high supply conditions. In addition to *AMT1* and *AMT2*, a low affinity  $\text{NH}_4^+$  transporter (*AMT3*) is expressed under non-limiting  $\text{NH}_4^+$  supply conditions, which enables the fungus to maintain a basal level of N uptake and assimilation also at high exogenous supply conditions [110]. *GintAMT1*, an  $\text{NH}_4^+$  transporter of the AM fungus *Glomus intraradices* seems to be mainly involved in the uptake of  $\text{NH}_4^+$  by the ERM under low  $\text{NH}_4^+$  availabilities [111]. An exogenous supply of  $\text{NO}_3^-$  stimulates the expression of a fungal  $\text{NO}_3^-$  transporter in the ERM of *G. intraradices* [112]. Similar to the N repression observed for the  $\text{NH}_4^+$  transporters in ECM fungi, the expression of this transporter is repressed by an increase in the internal levels of  $\text{NH}_4^+$  or a downstream metabolite, such as glutamine [113].

## 5.2. Fungal nitrogen metabolism

After its uptake from the soil,  $\text{NO}_3^-$  is converted into  $\text{NH}_4^+$  via nitrate and nitrite reductases in AM [103, 114] and ECM fungi [109]. In AM fungi and most ECM fungi,  $\text{NH}_4^+$  is assimilated via the glutamine synthase/glutamine oxoglutarate aminotransferase (GS/GOGAT) pathway into amino acids [103, 112].

In AM fungi, the amino acids glutamine and arginine (Arg) are major sinks for absorbed N and both amino acids become highly labeled after a supply of  $^{15}\text{NO}_3^-$  or  $^{15}\text{NH}_4^+$  to the ERM [103]. AM fungi assimilate N into Arg via the anabolic arm of the urea cycle and its key enzymes: carbamoyl phosphate synthase, argininosuccinate synthase, and argininosuccinate lyase<sup>2</sup> [112, 115]. The transcript levels in the ERM of these genes respond within hours to an exogenous supply of  $\text{NO}_3^-$  [112], what supports the view that Arg is rapidly assimilated in the fungal ERM of the AM symbiosis. Arg has a low C/N ratio and is positively charged and it has been suggested that polyP play a key role for the transport of N from the ERM to the IRM in the AM symbiosis [103, 115, 116] (Figure 5). Also for ECM fungi a potential role of Arg in N translocation has been discussed.

<sup>2</sup> carbamoyl phosphate synthase (CPS, production of carbamoyl phosphate from  $\text{CO}_2$ , ATP and  $\text{NH}_3$ ); argininosuccinate synthase (ASS, synthesis of argininosuccinate from citrulline and aspartate); and argininosuccinate lyase (AL, conversion of L-argininosuccinate to Arg and fumarate).

However,  $^{15}\text{N}$  labeling experiments suggest, that N can also be transferred as glutamine from the ERM to the IRM in ECM systems [1].

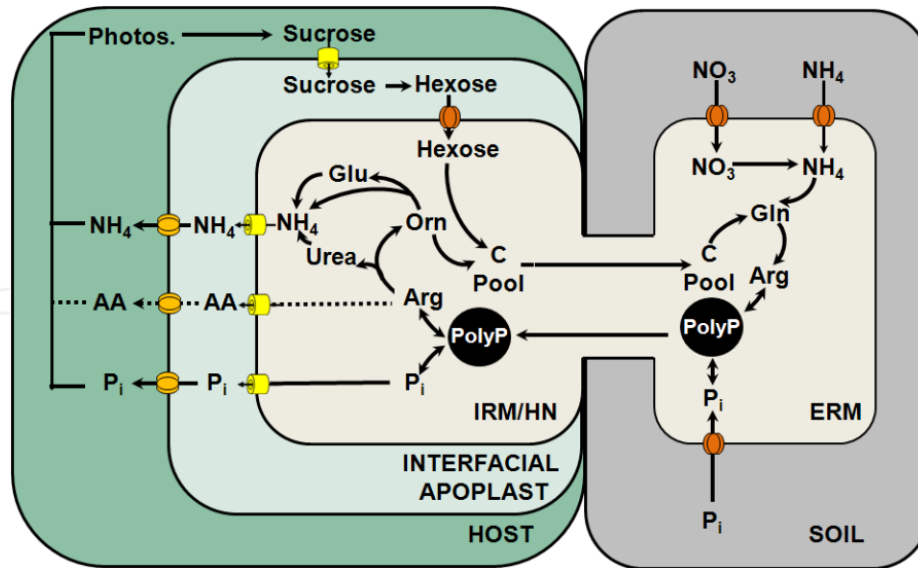
For AM associations, it has been suggested that N is transferred in inorganic form across the mycorrhizal interface. Current models on N transport in the AM symbiosis propose the breakdown of Arg in the IRM via the catabolic arm of the urea cycle into inorganic N, which is subsequently transferred across the mycorrhizal interface to the host [112, 113]. Several genes that are involved in Arg breakdown are highly expressed in the IRM, and are up-regulated in response to a  $\text{NO}_3^-$  supply to the ERM [112]. The catabolic enzymes arginase (*CAR1*) and urease (*URE*)<sup>3</sup> are involved in Arg breakdown, and the activities of both enzymes increase in AM roots after a supply of inorganic N sources to the ERM [113, 116]. A supply of  $\text{NO}_3^-$  to the ERM also results in increasing transcript levels of *CAR1* and *URE* in the IRM and it has been suggested that this may be due to an increase in the internal Arg level in the IRM [112] after a supply of  $\text{NO}_3^-$  to the ERM.

For ECM associations, it has traditionally been believed that amino acids are transferred across the mycorrhizal interface and that N transport from the fungus to the host is in organic form [1]. If organic N is transferred across the mycorrhizal interface, the carbon skeletons of amino acids would contribute to a significant re-flux of carbon from the fungus to the host. The uptake of amino acids from the interface would also require the presence of efficient plant uptake systems for organic N from the mycorrhizal interface, which have not yet been identified. ECM plants on the other hand have been shown to express high affinity  $\text{NH}_4^+$  transporters in the ECM interface [48], what suggests that N could also be transferred as inorganic  $\text{NH}_4^+$  across the mycorrhizal interface. This view is also been supported by the observed down-regulation of fungal  $\text{NH}_4^+$  transporters in fungal sheath and Hartig net, what would reduce the fungal re-absorption of  $\text{NH}_4^+$  from the interface [47]. Whether ECM fungi utilize, similar to AM fungi, the catabolic arm of the urea cycle to release  $\text{NH}_4^+$  in the Hartig net, still needs to be investigated (Figure 5). However, ECM fungi are also able to hydrolyze urea via urease [117]. In ECM roots two fungal aquaporins are highly expressed, that belong to the group of Fps-like aquaglyceroporins [118]. When expressed in yeast, both aquaporins increase the permeability of the membrane for  $\text{NH}_4^+$  but not for urea. Based on this observation, the authors concluded that an aquaporine mediated leakage of urea does not play a significant role for N transport in the ECM symbiosis and that  $\text{NH}_4^+$  is released into the mycorrhizal interface.

### 5.3. Regulation of nutrient transport across the mycorrhizal interface

The molecular mechanisms that are involved in the regulation of P and N transfer across the mycorrhizal interface are still unknown [50]. Models of nutrient transfer across the mycorrhizal interface generally involve an efflux of P and N from the fungal symplast through the fungal plasma membrane into the interfacial apoplast and the active absorption across the plasma membrane by the host plant (Figure 5).

<sup>3</sup> arginase (*CAR1*, Arg breakdown to ornithine and urea); urease (*URE*, hydrolysis of urea to  $\text{CO}_2$  and ammonia)



**Figure 5.** Transport processes in arbuscular and ectomycorrhizal interactions. The model shows the nutrient uptake by the fungal ERM through  $P_i$ ,  $NO_3^-$  or  $NH_4^+$  transporters (red), N assimilation into Arg via the anabolic arm of the urea cycle (only in AM fungi shown) and the conversion of  $P_i$  into polyP in the ERM, transport of polyP from the ERM to the IRM, polyP hydrolysis and release of Arg and  $P_i$  in the IRM or HN, Arg breakdown to  $NH_4^+$  via the catabolic arm of the urea cycle (only in AM fungi shown), facilitated  $P_i$ ,  $NH_4^+$ , and potential amino acid (AA, only in ECM postulated) efflux through the fungal plasma membrane (yellow) into the interfacial apoplast, plant uptake of nutrients from the mycorrhizal interface through mycorrhiza-inducible  $P_i$  or  $NH_4^+$  transporters, stimulation in photosynthesis by improved nutrient supply and facilitated efflux of sucrose through the plant plasma membrane into the interfacial apoplast, sucrose hydrolysis in the interfacial apoplast via an apoplastic plant invertase, and uptake of hexoses by the mycorrhizal fungus through fungal monosaccharide transporters.

The net loss of nutrients from free living fungi is normally regarded as slow, and membrane transport processes are generally favouring fungal re-absorption [77]. Therefore it has been suggested, that in the interface, conditions might exist, that promote the efflux of nutrients from the fungus or reduce the level of competing fungal uptake systems. The following conditions could contribute to a stimulation of nutrient transport across the mycorrhizal interface:

**Development and maintenance of a concentration gradient:** A concentration gradient across the mycorrhizal interface with high concentrations in the IRM and low concentrations in the interfacial apoplast and in cortical cells would maximize the efflux of nutrients through the fungal plasma membrane, and reduce fungal re-absorption. High P concentrations for example within the hyphae of the Hartig net or in the IRM increase the P transfer to the host [83, 119] and reduce fungal re-absorption [75, 120, 121]. There are also indications that the differential expression of plant and fungal uptake transporters in the mycorrhizal interface plays a role in the development of a strong concentration gradient across the mycorrhizal interface. High affinity P and N transporters of ECM and AM fungi are highly expressed in the ERM, but down-regulated in the IRM [47, 122]. This favours the active absorption of nutrients by the ERM, but reduces the re-absorption of nutrients by the IRM. By contrast, mycorrhiza-inducible high affinity plant  $P_i$  [23, 24, 56, 123] and  $NH_4^+$  transporters [25, 48, 124] are localized in plant plasma membranes in the interface region.



The high expression of these transporters facilitates the uptake of resources by the plant and the development of a strong concentration gradient across the interface (Figure 5). Plants express low affinity  $P_i$  transporters that can also act as channels and stimulate  $P_i$  efflux under low exogenous  $P_i$  concentrations [125]. However, whether fungal  $P_i$  transporters in the interface may have similar capabilities is still unknown.

**Carbon as trigger for nutrient transport:** AM fungi are obligate biotrophs and also ECM fungi absorb carbon mainly from the mycorrhizal interface in symbiosis. The carbon from the host provides the required resources for an extension of the ERM, for active uptake or other energy consuming processes, and for the development of new infection units. The supply of carbon by the host has been shown to stimulate the P uptake and transfer in AM [83, 94, 126] and ECM symbiosis [95] and it has been suggested that the  $P_i$  efflux from the IRM could be directly linked to the glucose uptake by the mycorrhizal fungus [127]. The  $P_i$  efflux from the IRM of the AM fungus *Gigaspora margarita* can be stimulated by an external supply of glucose, and its subsequent phosphorylation is coupled to a breakdown of polyP [128]. Also ECM fungi show an increased  $P_i$  efflux after a supply with sucrose under pure culture conditions [121]. There is currently no molecular evidence for a direct linkage between  $P_i$  efflux and carbon supply, but it has been shown that the expression of *MST2* (a monosaccharide transporter of *Glomus sp.*) and *PT4* (the mycorrhiza-inducible P transporter in the PAM) are tightly linked [28].

The carbon supply of the host also stimulates N uptake and transport in the AM symbiosis and triggers changes in fungal gene expression [113]. An increase in the carbon availability stimulates the expression of several genes involved in N assimilation and Arg biosynthesis<sup>4</sup> in the ERM, but reduces the transcript levels of a fungal urease. This increases the levels of Arg in the ERM and stimulates the export of Arg to the IRM (Figure 5). In the IRM, higher carbon availability induces fungal arginase and urease activity, but reduces the transcript levels of genes involved in Arg biosynthesis. The low expression of these enzymes will prevent that  $NH_4^+$ , which is released in the IRM as a result of the increased arginase and urease activities, is re-converted into Arg. This will lead to an increase in the internal  $NH_4^+$  level in the IRM and will facilitate N release into the mycorrhizal interface [113]. An effect of the photosynthetic activity of the host on N uptake and transport has also been observed in ECM birch seedlings [129].

**PolyP hydrolysis:** AM and ECM fungi store significant amounts of P as polyP and it is generally assumed that polyP play an important role in the transport of P and N from the ERM to the IRM [116] (Figure 5). An active hydrolysis of polyP in the IRM would release  $P_i$  and Arg (for subsequent breakdown into  $NH_4^+$  by the catabolic arm of the urea cycle) into the fungal cytoplasm and would facilitate the efflux into the mycorrhizal interface. AM and ECM fungi regulate the nutrient transport to the host by the accumulation or remobilization of polyP and it has been shown that the carbon supply of the host plant can trigger polyP hydrolysis [83, 94, 95, 113]. It has been proposed that polyP synthesis and breakdown could

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<sup>4</sup> N assimilation: glutamine synthetase, glutamate synthase; Arg biosynthesis: carbamoyl-phosphate synthase glutamine chain, argininosuccinate synthase, argininosuccinate lyase

be regulated by the activity of H<sup>+</sup>-ATPases in the fungal tonoplast [130] or by a differential regulation of the involved enzymes.

**Effects on membrane permeability.** The accumulation of particular ions (e.g. H<sup>+</sup>, K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>) in the interfacial apoplast could lead to a depolarization of the transmembrane electric potential and to an opening of ion channels [131, 132]. Monovalent cations, such as Na<sup>+</sup> and K<sup>+</sup>, have been shown to stimulate P<sub>i</sub> efflux from ECM fungal hyphae in pure culture experiments and could also have an effect on fungal re-absorption [121]. Lyso-phosphatidylcholine (LPC) acts in AM roots as a lipophilic signal, that induces the expression of mycorrhiza-inducible P transporters and there are indications for an extracellular localization and production of LPC. LPC leads to a rapid extracellular alkalinization of tomato cells in suspension-cultures [133] and could have similar effects also on the fungal membrane potential at the mycorrhizal interface. AM and ECM fungi in symbiosis have been shown to express aquaporins [118, 134]. Aquaporins are integral membrane channels that facilitate the concentration gradient-driven water and/or solute transport through the plasma membrane. In root nodules, aquaporins have been shown to be involved in the NH<sub>4</sub><sup>+</sup> flux through the symbiosome membrane that encloses the N fixing bacteroids [135]. In ECM roots, they could facilitate the transport of NH<sub>4</sub><sup>+</sup> through the fungal plasma membrane into the interfacial apoplast and in combination with a reduced re-absorption increase the net transport of NH<sub>4</sub><sup>+</sup> to the host [118]. Aquaporins could also stimulate the efflux of other nutrients, such as phosphate, through the fungal plasma membrane into the mycorrhizal interface [136].

## 6. Conclusions

It has been hypothesized that mycorrhizal growth responses follow a mutualism – parasitism continuum [137] and that the outcome of the symbiosis primarily depends on cost (carbon) to benefit (nutrient gain) ratios. When the nutrient availability in the soil is high, growth depressions in AM plants have been observed [e.g. 138, 139], and it was generally assumed that the negative growth responses are the result of the high carbon costs of the symbiosis for the host plant that are not counterbalanced by a net gain in P. Alternatively, it has been suggested that negative growth responses in AM interactions could also be the result of a reduced P uptake via the plant pathway which is not compensated for by an increase in P uptake via the mycorrhizal pathway, leading to an overall reduction in total P uptake and P deficiency for the plant [52]. Similarly, it has been proposed that for ECM plants carbon is an excess rather than a costly resource and that the outcome of the symbiosis for the host is primarily dependent on the nutrient acquisition efficiency of the ECM fungus [140].

Carbon to P exchange processes in the AM symbiosis are driven by biological market dynamics and both partners reciprocally reward beneficial partners with more resources [94]. AM fungi differ in their efficiency with which they suppress the plant nutrient uptake pathway [55]. AM fungi are completely dependent on their host plant for their carbon supply, and it is interesting to speculate that the suppression of the plant pathway could be

more a fungal-driven than a host-motivated response. A strong suppression of the plant pathway will shift the ratio between both uptake pathways towards the mycorrhizal pathway and will result in a higher mycorrhizal dependency of the host. A high mycorrhizal dependency increases the carbon allocation to the root system [141], and this will make more carbon available for the fungus, which in return has been shown to trigger P and N transport in the AM symbiosis [83, 94, 113, 126]. This is also consistent with the observation that the N transport to the host was reduced when the fungus had access to an additional carbon source [113], and the mycorrhizal dependency of the fungus was reduced. This indicates that the fungus is more in control of the symbiosis than previously been suggested and that mycorrhizal fungi can gain an advantage in the symbiosis by adjusting their nutrient transfer to the host.

The question arises, whether and how the host plant is able to control the symbiosis. Arbuscules in the AM symbiosis undergo in the host cell a cycle of growth, degradation, senescence and recurrent growth, and the typical life span of arbuscules is only 8.5 days [142]. The life span of arbuscules has been shown to depend on their ability to deliver nutrients to the host and is regulated by the host plant demand. A high supply of the plant with P, leads in roots of *Petunia hybrida* to malformed arbuscules with a low branching pattern. P acts systemically and even a relatively small increase in the P level in the shoot, has a large effect on the AM colonization [143]. This effect was not the result of transcriptional changes in SYM genes, but was correlated to a decrease in the expression of the mycorrhiza-inducible P transporter (Pt4) in the PAM. Pt4 expression plays a critical role for the AM symbiosis and when MtPt4 (Pt4 of *Medicago truncatula*) is not expressed, arbuscules are prematurely degraded and the fungus is unable to proliferate within the host [144]. Interestingly, in MtPt4 mutants under N deficiency the degeneration of arbuscules is suppressed [145], what suggests that plant and fungus can change their resource exchange dynamics according to nutrient demand and supply conditions. The host driven regular turnover of arbuscules seems to provide the AM plant with an instrument to remove and 'to penalize' inefficient AM fungal symbionts [8], and to respond with transformations in its intracellular colonization to short term changes in the exogenous nutrient supply conditions.

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