

Review

Role of Arbuscular Mycorrhizal Fungi in the Nitrogen Uptake of Plants: Current Knowledge and Research Gaps

Heike Bücking * and Arjun Kafle

Biology and Microbiology Department, South Dakota State University, Brookings, SD 57007, USA;
E-Mail: arjun.kafle@sdstate.edu

* Author to whom correspondence should be addressed; E-Mail: heike.buecking@sdstate.edu;
Tel.: +1-605-688-5463; Fax: +1-605-688-5624.

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Abstract: Arbuscular mycorrhizal (AM) fungi play an essential role for the nutrient uptake of the majority of land plants, including many important crop species. The extraradical mycelium of the fungus takes up nutrients from the soil, transfers these nutrients to the intraradical mycelium within the host root, and exchanges the nutrients against carbon from the host across a specialized plant-fungal interface. The contribution of the AM symbiosis to the phosphate nutrition has long been known, but whether AM fungi contribute similarly to the nitrogen nutrition of their host is still controversially discussed. However, there is a growing body of evidence that demonstrates that AM fungi can actively transfer nitrogen to their host, and that the host plant with its carbon supply stimulates this transport, and that the periarbuscular membrane of the host is able to facilitate the active uptake of nitrogen from the mycorrhizal interface. In this review, our current knowledge about nitrogen transport through the fungal hyphae and across the mycorrhizal interface is summarized, and we discuss the regulation of these pathways and major research gaps.

Keywords: ammonium; arginine; interface; mycorrhiza; nitrate; nutrient transport; organic nitrogen; periarbuscular membrane; phosphate; plant microbe interaction

1. Introduction

The arbuscular mycorrhizal (AM) symbiosis plays a key role for the nutrient uptake of more than 60% of land plants, including many important crop species such as wheat, corn, and soybean [1]. AM fungi are ubiquitous and can account for up to 50% of the microbial biomass in soils [2]. The extraradical mycelium (ERM) of the fungus acts as an extension of the root system and increases the uptake of phosphate (P), nitrogen (N), sulfur, and magnesium but also of trace elements, such as copper and zinc. In addition, AM fungi also provide non-nutritional benefits to their host plant, as they improve the resistance of plants against several abiotic (drought, salinity, heavy metals) and biotic (pathogens, herbivores) stresses [3]. AM fungi therefore play a key role in the survival and fitness of plants and act as “ecosystem engineers” of plant communities [4]. However, the benefits for the plant are not free of charge, and the plant transfers between 4% to 22% of its assimilated carbon (C) to the AM fungus [5]. It has been suggested that the substantial C costs are responsible for the variability of mycorrhizal growth responses that have been described. These growth responses can range from highly beneficial to detrimental and follow a mutualism to parasitism continuum [6–8].

AM fungi belong to the fungal phylum Glomeromycota, and are obligate biotrophs that are unable to complete their life cycle without the carbon supply from their host. This dependency on the host and the observation that many host plants suppress their mycorrhizal colonization particularly under high nutrient supply conditions has led to the overall assumption that the host plant is in control of the symbiosis [9]. However, the long co-evolution of about 400 to 450 million years for both partners in the AM symbiosis also allowed the fungus to improve its strategies to control the nutrient transport to the host despite its obligate biotrophic life cycle [10,11]. It has been suggested that carbon to nutrient exchange in the AM symbiosis is controlled by biological market dynamics and that reciprocal reward mechanisms ensure a “fair trade” between both partners in the AM symbiosis [10].

AM fungi and their plant partners form a complex network of many-to-many interactions; each host plant is colonized by communities of AM fungi and fungal individuals colonize multiple host plants simultaneously and interconnect plants by common mycorrhizal networks (CMNs). These many-to-many interactions allow both partners in the symbiosis to choose among multiple trading partners but also force both partners to compete with other partners for nutrient or carbon resources [12,13]. CMNs play a key role for the long distance transport of nutrients, water, stress chemicals and allelochemicals and allow the interconnected host plants to “communicate” with other plants within their CMN [14–18]. CMNs have also been discussed as a pathway for the transport of N from donor to recipient plants [19]. It is clear that CMNs affect the survival and fitness, behavior and competitiveness of the plants and fungi that are linked via these networks, but our current understanding about how the nutrient or infochemical allocation among plants within a CMN is controlled, is very limited [11,20].

The contribution of AM fungi to the N nutrition of their host plant is still under debate [21], and it has been suggested that higher N contents in mycorrhizal plants are just a consequence of an improved supply with P [22]. However, it is clear that AM fungi transfer N to their host, and plants are able to take up N from the mycorrhizal interface. This review summarizes our current knowledge about N uptake, metabolism, and transport in the AM symbiosis and discusses major research gaps in our understanding.

2. Mycorrhizal Roots Have Two Uptake Pathways for Nutrients

Mycorrhizal roots have two uptake pathways for nutrients: the plant uptake pathway (PP) and the mycorrhizal uptake pathway (MP; Figure 1). The PP involves the uptake of nutrients via high- or low affinity uptake transporters in the epidermis or root hairs. Particularly for nutrients with a low mobility in the soil (e.g., P), the uptake via the PP is often limited by the development of depletion zones around the roots. By contrast, the MP involves the uptake by high affinity nutrient transporters in the ERM, followed by the translocation along the hyphae to the intraradical mycelium (IRM) in the root cortex, and the uptake from the mycorrhizal interface by mycorrhiza-inducible plant uptake transporters [23]. However, a plant is simultaneously colonized by communities of AM fungi that can differ in their efficiency with which their MP contributes to the total uptake of nutrients by the plant. The uptake and transport of nutrients via both pathways and their contribution to the nutrient supply of the plant has so far primarily been studied for P [21,23–25], but both pathways also play a role in the N uptake by plants.

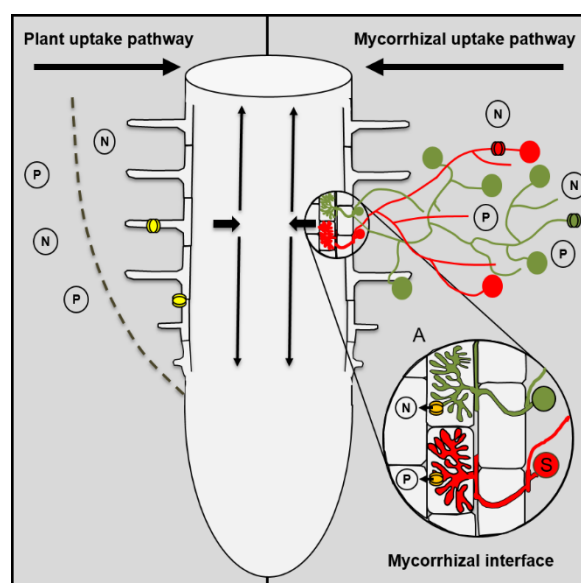


Figure 1. Plant uptake and mycorrhizal uptake pathway. Plants can take up nutrients by transporters that are located in epidermis or root hairs (yellow symbols) or via the mycorrhizal uptake pathway that comprises the uptake of nutrients by fungal transporters in the extraradical mycelium (red or green symbols), the transport through the hyphae from the ERM to the IRM (see mycorrhizal interface), and the uptake from the mycorrhizal interface by mycorrhiza-inducible plant transporters in the periarbuscular membrane (orange symbols). Indicated by the red and green fungal structures is the colonization of one host root by multiple fungal species that can differ in their efficiency with which they are able to take up nutrients from the soil and transfer these nutrients to their host.

Ectomycorrhizal roots are enclosed by a more or less densely arranged fungal sheath that can represent a significant apoplastic barrier and restricts the nutrient uptake of ectomycorrhizal roots via the PP [26,27]. However, in contrast to ectomycorrhizal roots, AM roots are structurally unaltered and can theoretically use both pathways for nutrient uptake. Previously, it was generally believed that the PP is not affected by the symbiosis and that both uptake pathways act additively. This led to the overall

assumption that the uptake via the MP was negligible in cases in which the mycorrhizal growth responses were neutral and the mycorrhizal plants did not differ in their P contents from non-mycorrhizal plants. However, there is increasing evidence that this view is oversimplified, and that mycorrhizal plants differ in their nutrient acquisition strategy from non-mycorrhizal plants [21,23,28].

Multiple studies have demonstrated that even in non-responsive plants the MP can contribute significantly to the P uptake of the plant [24,29]. Plant P transporters that are involved in the uptake via the PP are down-regulated in response to the AM symbiosis [30,31], while mycorrhiza-specific transporters that are involved in the P uptake from the mycorrhizal interface are induced [32–34]. The suppression of the PP by the AM symbiosis can also lead to negative mycorrhizal growth responses when the reduction in the P uptake via the PP is not fully compensated for by an increase in the P uptake via the MP. This can cause an overall reduction in total P uptake and lead to P deficiency of the plant [21]. AM fungi differ in their capability to suppress the PP [31]. A strong suppression of the PP will shift the ratio between the two uptake pathways towards the MP and will result in a higher mycorrhizal dependency of the host.

Whether the N uptake via the PP is affected by the AM symbiosis is currently unknown. However, similar to what has previously been described for P, there are indications that plant ammonium (NH_4^+) or nitrate (NO_3^-) transporters are down-regulated [35,36], while mycorrhiza-inducible N transporters are up-regulated by the AM symbiosis [36–39]. Arbuscules are prematurely degraded when the mycorrhiza-inducible P transporter *PT4* is not expressed [40]. Recently, it was shown that the arbuscular life span in *Medicago* plants is not only influenced by *PT4* but also by the mycorrhiza-inducible NH_4^+ transporter *AMT2;3* [41]. There is increasing evidence that suggests that the N and P uptake and transport are tightly linked in the AM symbiosis and that the transport of both nutrients controls mycorrhizal functioning (see also below). More studies are necessary to understand the role that both uptake pathways play for the N uptake, and how the activity of both pathways is regulated.

Contribution of the Arbuscular Mycorrhizal Symbiosis to Plant Nutrition

While the positive effect of the AM symbiosis on P nutrition is long known [3,21], the contribution of AM fungi to the N nutrition of their host plant is still under debate [23]. The mobility of the inorganic N sources nitrate (NO_3^-) and ammonium (NH_4^+) in the soil is relatively high, and the rhizosphere is less likely to become N depleted. Unlike for P, where the access of the ERM to P sources beyond the root depletion zone is clearly advantageous, it is expected that the ERM does not increase the access to the N resources in the soil. Recent studies, however, suggest that the NH_4^+ uptake system of AM fungi has a five times higher affinity for NH_4^+ than typical uptake systems of plants, what would enable the fungus to take up NH_4^+ from the soil even under low N supply conditions [42].

In the literature, negative [43], neutral [44] or positive [45,46] effects of the AM symbiosis on N nutrition have been reported (for review [47]), and several authors postulated that an improved N status of AM plants is only the consequence of an improved P nutrition [22]. Hawkins and George [44], for example, reported that the hyphal N supply was not sufficient to sustain an adequate N nutrition of a host plant under N limitation. By contrast, other studies have shown that AM fungi can increase the N acquisition of plants compared to non-mycorrhizal controls that were supplied with additional P [48]. Recent results demonstrated that the ability of AM fungi to improve the N nutrition of their host is

relatively widespread within the Glomeromycota, but that there is a high intraspecific diversity between different isolates of one AM fungal morphospecies [46]. The authors reported that out of the 31 fungal isolates that were tested, the colonization with six fungal isolates led on average to a mycorrhizal growth response of 191% and a 2.4-fold increase in the N tissue concentration of *Medicago sativa*. These positive effects were independent from the contribution of these fungal isolates to P supply. There is increasing evidence that a pathway for N through the fungal hyphae to the host plant exists, even if the percentage contribution to total N nutrition of the host plant can vary considerably and is context dependent [23,46]. In AM root organ cultures, 21% of the total N in the roots were taken up by the ERM [49]; and in similar experiments even higher proportions were observed [50]. In maize, 75% of the N in the leaves were taken up by the ERM of an AM fungus [51].

In several plant species mycorrhiza-inducible NO_3^- [52] or NH_4^+ [36,38,39,41,53], transporters have been identified that are able to facilitate the uptake of N sources from the mycorrhizal interface. Recent results demonstrated that both P and N are important determinants for the AM symbiosis and that the colonization of the plant host is controlled by feedback mechanisms between both nutrients [12]. For example, both, P and N starvation of the plant induce a nutrient stress transcriptome that is favorable for AM colonization. Under P and N stress, plant defense genes are down-regulated, while genes that are involved in the strigolactone biosynthesis are up-regulated [54]. Strigolactone serves as an important signal for AM fungi in the soil and stimulates hyphal branching during the presymbiotic growth stage [55]. High P availabilities often reduce the AM colonization of the plant, but N starvation triggers a signal that promotes AM colonization and reverses the inhibitory effects of high P availabilities on AM colonization [41,56]. However, the recovery of AM colonization did not lead to increased N levels in these plants [56]. When the fungal P transport to *Medicago truncatula* plants is inhibited by the suppression of the mycorrhiza-inducible plant P transporter *MtPt4*, arbuscules are prematurely degraded, and the symbiosis is not maintained by the host [40]. However, AM fungi can escape premature degeneration of their arbuscules in *MtPt4* mutants under N starvation [41,57], and it has been demonstrated that the expression of mycorrhiza-induced plant P transporters are not critical for the AM symbiosis as long as the plant is grown under low N conditions [41]. All these results clearly demonstrate that N is transferred through the fungal hyphae to the host, and that N transport to the host may play a critical role for the maintenance and the nutrient efficiency of the AM symbiosis.

3. Nitrogen Uptake by Arbuscular Mycorrhizal Fungi

3.1. Uptake of Inorganic N Sources

Nitrate (NO_3^-) is the dominant form of N that is available to plants and fungi in most agricultural soils, whereas ammonium (NH_4^+) predominates in many undisturbed or very acidic soils, where NO_3^- can be almost entirely absent. The ERM of AM fungi can take up NH_4^+ [58–62] and NO_3^- [62,63], but NH_4^+ is generally preferred, because it is energetically more efficient than NO_3^- [49,50,62,64] (see also Table 1). Based on pH changes induced by the ERM, when hyphae were supplied with NO_3^- or NH_4^+ , it has been hypothesized that the NO_3^- uptake by the hyphae is active and coupled to an H^+ -symport mechanism, while NH_4^+ is taken up by an antiport mechanism with a net H^+ efflux [65,66]. Similar to plants and other fungi, AM fungi have high-affinity and low-affinity uptake systems for NH_4^+ . The low

K_m value of $2.53 \pm 0.25 \mu\text{M}$ for the high affinity uptake system of *Rhizophagus irregularis* demonstrates that the fungus is able to take up NH_4^+ from soils with very low concentrations [42]. K_m values of high affinity NH_4^+ uptake systems of plants are typically higher than the estimated K_m value for the fungal NH_4^+ uptake system [42,67,68].

Table 1. Demonstrated metabolic pathways of nitrogen (N) in arbuscular mycorrhizal (AM) fungi. Shown are the fungal species or the tissues that were tested. Fungal species were renamed according to the reclassification of the Glomeromycota [69]. Species abbreviations and authorities: *Acla*—*Acaulospora laevis* (Gerd. & Trappe), *Clet*—*Claroideoglossum etunicatum* (W. N. Becker & Gerd.; previously *Glomus etunicatum*), *Fumo*—*Funneliformis mosseae* (T. H. Nicolson & Gerd., previously *Glomus mosseae*), *Gima*—*Gigaspora margarita* (W. N. Becker & I. R. Hall), *Giro*—*Gigaspora rosea* (T. H. Nicolson & N. C. Schenck), *Glfa*—*Glomus fasciculatum* (Gerd. & Trappe), *Glma*—*Glomus macrocarpus* (Tul. & C. Tul.), *Glve*—*Glomus versiforme* (P. Karst., S. M. Berch), *Rhcl*—*Rhizophagus clarus* (T. H. Nicolson & N. C. Schenck, previously *Glomus clarum*), *Rhir*—*Rhizophagus irregularis* (N.C. Schenck & G. S. Sm.), *Rhma*—*Rhizophagus manihotis* (R. H. Howeler, Sieverd. & N. C. Schenck).

Pathway/Enzyme	Fungal Species	Tissue				References
		Spore	ERM	AM	IRM	
Inorganic N uptake						
NH ₄ ⁺ uptake	<i>Fumo, Rhir, Acla, Gima</i>	+	+	nd	nd	[50,58,60–62,64,70,71]
NH ₄ ⁺ transporter	<i>Rhir</i>	+	+	+	+	[53,72,73]
NO ₃ [−] uptake	<i>Glfa, Rhir, Fumo</i>	+	+	nd	nd	[62–65,70,71]
NO ₃ [−] transporter	<i>Rhir</i>	+	+	+	+	[71,72,74–76]
Nitrate permease	<i>Rhir</i>	+	+	+	+	[76]
Organic N uptake						
	<i>Acla, Rhir, Gima</i>	nd	+/−	nd	nd	[60]
	<i>Fumo</i>	nd	+	+	nd	[45,58]
		nd	−	nd	nd	[77]
		+	+	+	+	[72]
Amino acid transporter		+	+	+	+	[72]
Amino acid permease	<i>Fumo</i>	−	+	+	nd	[78]
Peptide transporter	<i>Rhir</i>	nd	+	+	+	[79]
Alanine	<i>Rhir</i>	−	nd	nd	nd	[71]
Arginine	<i>Rhir</i>	−	+	nd	nd	[50,71]
Cysteine	<i>Rhir</i>	−	+	nd	nd	[80]
Glycine	<i>Rhir, Fumo, un</i>	+	+	nd	nd	[62,71]
Glutamate	<i>Fumo, Rhir</i>	+	+	nd	nd	[62,71]
Glutamine	<i>Fumo, Rhir</i>	+	+	nd	nd	[71,81]
Methionine	<i>Rhir</i>	−	+	nd	nd	[80]
Ornithine	<i>Rhir</i>	+	nd	nd	nd	[71]
Urea ⁴	<i>Gima</i>	+	+	nd	nd	[71,82]

Table 1. Cont.

Pathway/Enzyme	Fungal Species	Tissue				References
		Spore	ERM	AM	IRM	
Nitrate reduction						
Nitrate reductase ¹	<i>Glfa, Rhir, Fumo, Glma, Glsp</i>	+	nd	+	nd	[72,83–86]
Nitrate reductase (NADH)	<i>Un, Fumo</i>	+	+	+	nd	[52,87–89]
Nitrate reductase (NADPH)	<i>Rhir</i>	nd	+	+	+	[52,90]
Nitrite reductase	<i>Rhir</i>	nd	+	+	+	[72,84]
GDH pathway						
GDH ¹	<i>Clet</i>	nd	nd	+	+	[91]
GDH (NADH)	<i>Fumo, Rhir</i>	+	+	+	+	[71,72,92]
GDH (NADPH)	<i>Glfa</i>	nd	nd	+/–	nd	[83,93]
GS/GOGAT pathway						
Glutamine synthetase	<i>Glfa, Fumo, Rhir</i>	+	+	+	+	[71,72,74,75,81,83,84,93,94]
Glutamate synthase (NADH)	<i>Rhir</i>	+	+	+	+	[64,72,74,75,84,94]
Glutamate synthase (NADPH)	<i>Glfa, Rhir</i>	nd	nd	+	+	[72,83]
Amino acid biosynthesis						
Transaminases	<i>Glfa</i>	nd	nd	+	nd	[83,95]
Asparagine synthase	<i>Rhir</i>	+	+	+	+	[72]
Arginine biosynthesis						
Carbamoyl-P synthase	<i>Rhir</i>	+	+	+	+	[72,74,75]
Argininosuccinate synthase	<i>Rhir</i>	+	+	+	+	[72,74,75]
Argininosuccinate lyase	<i>Rhir</i>	+	+	+	+	[72,74,75]
Arginine breakdown						
Arginase	<i>Rhir</i>	+	+	+	+	[72,74,75]
Urease	<i>Rhcl, Rhir</i>	+	+	+	+	[72,74,75]
Urease accessory protein	<i>Rhir</i>	+	+	+	+	[71,72,75,94]
Ornithine aminotransferase	<i>Rhir</i>	+	+	+	+	[71,72,74,75,94]
Polyamine biosynthesis						
Ornithine decarboxylase	<i>Rhir</i>	+	+	+	+	[72,74,75]
N uptake from interface						
	<i>Medicago truncatula</i>	na	na	+	na	[39,41]
Plant NH ₄ ⁺ transporter	<i>Lotus japonicus</i>	na	na	+	na	[38]
	<i>Glycine max</i>	na	na	+	na	[36]
	<i>Sorghum bicolor</i>	na	na	+	na	[37]

Abbreviations: AM—arbuscular mycorrhizal roots; ERM—extraradical mycelium; IRM—intraradical mycelium; na—not applicable; nd—not determined.

Despite the fungal preference for NH₄⁺, Hawkins and George [70] reported that when NH₄⁺ was the sole N source for mycorrhizal plants, root and shoot biomass, hyphal length densities and N transport via the hyphae to the plant were lower than after NO₃⁻ supply. Valentine *et al.* [96] also reported, that NH₄⁺ fed AM roots had lower numbers of vesicles and arbuscules than NO₃⁻ supplied roots. An excess of NH₄⁺ as sole N source is often considered to be toxic for plants, and inhibits root growth [97]. NH₄⁺ assimilation occurs in the root, while the assimilation of NO₃⁻ is predominantly foliar [98,99]. When NH₄⁺ is the sole N source, the assimilation of NH₄⁺ could increase the consumption of C skeletons

in the root, and reduce the carbon availability for the fungus. Hawkins and George [70] found that NH_4^+ reduced the hyphal length in the soil, but not the number of arbuscules, and assumed that high concentrations of NH_4^+ could also have a direct deleterious effect on the ERM.

Transcriptome studies revealed the expression of several fungal NH_4^+ and NO_3^- transporters in spores, ERM, and IRM [72]. The expression of *GintAMT1*, an NH_4^+ transporter of the AM fungus *Rhizophagus irregularis* (previously *Glomus intraradices*) is induced by low additions of NH_4^+ to the medium (in the presence of relatively high concentrations of NO_3^- in the medium) but suppressed under high NH_4^+ supply. This suggests that the expression of this transporter is substrate inducible, and is regulated by the NH_4^+ supply and fungal NH_4^+ status [73]. By contrast, the NH_4^+ transporter *GintAMT2* is constitutively expressed in the ERM under N limiting conditions, and transitory induced when different N sources are added to the N deficient ERM [53]. The differential localization of high transcript levels of these transporters in colonized roots suggests that both transporters may differ in their role for N uptake and transport. The high expression levels of *GintAMT1* in the ERM indicate that this transporter could be primarily involved in the NH_4^+ acquisition of fungal hyphae from the soil. By contrast, *GintAMT2* is particularly expressed in the IRM, suggesting that this transporter could play a role in the re-uptake of NH_4^+ by the fungus from the symbiotic interface [53].

An exogenous supply of NO_3^- stimulates the expression of a fungal NO_3^- transporter in the ERM of *Rhizophagus irregularis* (also known as *Rhizophagus intraradices* or previously *Glomus intraradices*) [74]. However, the expression of this NO_3^- transporter is repressed by an increase in the internal levels of NH_4^+ or of a downstream metabolite, such as glutamine [75]. Nitrate transporters in many organisms are suppressed when more preferred N sources, such as NH_4^+ become available. This process is known as N catabolite repression, and this regulatory system also controls the expression of NH_4^+ transporters in other fungi [100]. In ectomycorrhizal fungi, the expression of high affinity NH_4^+ transporters is suppressed by high levels of intracellular glutamine [101], and NO_3^- transporter proteins in the yeast *Hansenula polymorpha* are degraded in response to glutamine [102]. GATA transcription factors have been shown to be involved in N catabolite repression and in the promoter sequence of the NH_4^+ transporter *GintAMT2* two GATA core sequences have been identified [53]. The expression profiles of AM fungal NO_3^- and NH_4^+ transporters under different N availabilities suggest that N catabolite repression could also operate in AM fungi and could control the N uptake by AM fungi from the soil.

3.2. Uptake of Organic N by Hyphae

Organic N can represent in many soils a significant proportion of total soil N [103], but the obligate biotrophic life cycle of AM fungi has led to the overall assumption that AM fungi are unable to utilize organic N sources. Several studies, however, have demonstrated that the hyphae of AM fungi grow into organic patches of varying complexity and transfer ^{15}N from these organic patches to their host plant [104,105], and that this can lead to higher plant N contents [106]. When the fungus had access to organic patches that were labelled with ^{15}N and ^{13}C , the fungal ERM got only enriched with ^{15}N , but not with ^{13}C . This confirms that AM fungi do not have saprophytic capabilities and that the fungus acquires ^{15}N from these organic patches likely as a decomposition product [105]. However, even if AM fungi themselves do not act as decomposers, AM fungi accelerate the N mineralization from organic matter [107] and affect the carbon flow through soil microbial communities during decomposition [108].

Plant roots release fixed carbon into their rhizosphere, and this carbon flux into the root rhizosphere acts as an important trigger for decomposition processes and nutrient cycling in soils [109]. AM fungi form an extensive network of hyphae in soils (ERM), and the mycorrhizosphere, the large interface between the fungal hyphae and the soil, could play a similar role than the rhizosphere for decomposition and nutrient cycling in soils. The ERM of the AM fungus acts as a conduit between decomposing microbial communities and the host plant, and provides decomposers with plant-derived carbon inputs and transfers decomposition products to the plant. The mycorrhizosphere represents in soils an important ecological niche and provides nutritionally favorable conditions for diverse microbial communities, and it has been suggested that the presence of AM fungal hyphae plays an important role in the bacterial community assembly during decomposition [110].

Compared to ectomycorrhizal fungi, the capability of AM fungi to utilize organic N sources is considered to be relatively small but also AM fungi are able to take up organic N sources from the soil, such as amino acids (Table 1). Free amino acids can represent an important N source in soils, and AM fungi can take up several amino acids, e.g., aspartic acid, serine [111], glycine, glutamic acid [62,112], glutamine [81], cysteine or methionine [80]. Some amino acids are also taken up by germinating spores during the presymbiotic growth stage of the fungus [71]. Transcriptome studies have shown that fungal amino acid transporters are expressed in the ERM [72]. Cappellazzo and co-authors characterized an amino acid permease of the AM fungus *Funnelliformis mosseae* (previously *Glomus mosseae*) and found that this amino acid transporter binds preferably neutral, nonpolar and hydrophobic amino acids [78]. While some amino acids such as glycine, serine and glutamine competed strongly with proline for this transporter, positively charged amino acids such as arginine, histidine and lysine were only poor competitors. The mycorrhizal colonization of *Sorghum bicolor* led to an increase in the uptake of phenylalanine, methionine, asparagine, tryptophane, and cysteine, but also increased the uptake of the charged amino acids arginine, lysine, and histidine [113]. Other studies demonstrated that arginine can be taken up by the ERM and that arginine is not metabolized during its transport from the ERM to the IRM [50]. This suggests that multiple transporters are involved in the amino acid uptake by the ERM.

Recently, the putative dipeptide transporter *RiPTR2* has been identified in the AM fungus *Rhizophagus irregularis* [79]. Short peptides can represent a greater proportion of N in soils than free amino acids, and this transporter facilitated in complementation assays the uptake of several dipeptides. This suggests that this transporter could play a role in the fungal uptake of small peptides from the soil. However, the transcript levels of this transporter were higher in the IRM than in the ERM, suggesting that *RiPTR2* could also play a role in the reabsorption of peptides from the interfacial apoplast [79].

4. Nitrogen Assimilation and Transport by Arbuscular Mycorrhizal Fungi

4.1. Nitrate Reduction in Arbuscular Mycorrhizal Fungi

After NO_3^- is taken up by plants or fungi, NO_3^- is first reduced to nitrite by a nitrate reductase. Both NADH and NADPH can act as reductant for nitrite formation [52], but, in AM fungi, nitrate reductase activity is mainly driven by NADPH [90]. Nitrate reductase activity in both roots [44,83,84,87] and shoots [83,87,95] of AM plants is generally higher than in non-mycorrhizal control plants (for more information, see also Table 1). In nonmycorrhizal plants, NO_3^- reduction primarily takes place in the

leaves, while in mycorrhizal plants NO_3^- is predominantly reduced in the roots [88,90]. A gene that encodes a fungal nitrate reductase is expressed in spores, ERM and IRM, but the transcript levels are particularly high in the IRM. This could indicate that NO_3^- that is not directly assimilated in the ERM can also be reduced in the fungal tissue within the host root [72,85,90].

The second step in N assimilation is the conversion of nitrite into NH_4^+ by nitrite reductase. A gene encoding a putative fungal nitrite reductase of the AM fungus *Rhizophagus irregularis* shows particularly high expression levels in spores and in the IRM [72]. This is consistent with the high transcript levels of a fungal nitrate reductase in the IRM of AM roots [72]. The expression of *tbnir1*, the nitrite reductase of the ectomycorrhizal (ECM) fungus *Tuber borchii*, is induced by NO_3^- but suppressed when more preferred N sources, such as NH_4^+ or glutamine become available [114]. It has been demonstrated in ECM roots that the activity of the fungal nitrite reductase controls the expression of the plant nitrite reductase gene. When the plant is colonized with a fungal wild type strain, the expression of the plant nitrite reductase is repressed, but the expression increased in roots that were colonized with nitrate reductase deficient fungal strains [115]. This is consistent with the finding that, in mycorrhizal roots, the transcript levels of plant nitrate reductase are lower than in non-mycorrhizal control plants [52,90] and suggests that the expression of the plant nitrite reductase is suppressed by the transfer of reduced N compounds from the fungus to the host [115].

4.2. Nitrogen Assimilation into Amino Acids in Arbuscular Mycorrhizal Fungi

Since excess N in its reduced form NH_4^+ is toxic for cells due to its ability to uncouple respiration, NH_4^+ is rapidly assimilated into amino acids [116]. Two pathways can be involved in the assimilation of NH_4^+ by fungi: the NAD(P)-glutamate dehydrogenase (GDH) or the glutamine synthetase—glutamate synthase (GS-GOGAT) pathway. GDH uses NH_4^+ and 2-oxoglutarate to produce glutamate (Glu), while in the GS-GOGAT pathway, first glutamine (Gln) is produced from Glu and NH_4^+ by glutamine synthetase, and then Gln and 2-oxoglutarate are converted by glutamate synthase (also known as glutamine oxoglutarate aminotransferase) into two molecules of Glu. In the cytoplasm of ascomycetes and basidiomycetes activities of NAD and NADP-dependent GDH can be detected [117]. However, Smith *et al.* [93] found no increased GDH activity in AM roots, and also in the ERM of *Rhizophagus irregularis* no GDH activity could be detected [49]. A putative NAD-dependent GDH gene of *R. irregularis* is down-regulated in the ERM in response to either NO_3^- or NH_4^+ supply, suggesting that GDH plays in AM fungi a more catabolic role rather than a role in N assimilation [49,71,83,94].

NH_4^+ is predominately assimilated via the GS-GOGAT pathway in AM fungi [64,81,83,94]. GS and GOGAT activities have been shown to be significantly higher in roots and shoots of AM plants [83,84,93,95] (see also Table 1), and the decrease in the levels of free amino acids in the ERM of the AM fungus *R. irregularis* after incubation with the GOGAT inhibitor albizzine confirms the activity of a functional GS-GOGAT pathway in AM fungi [64]. Tian and co-authors [74] identified two different functional GS isoforms of *R. irregularis*, *GiGS1* and *GiGS2*. *GiGS1* has a lower K_m than *GiGS2* and is constitutively expressed at high levels in the ERM, while *GiGS2* is strongly induced by an addition of NO_3^- to the ERM. This suggests that *GiGS1* is the main functional enzyme for N assimilation at low N availabilities, and that *GiGS2* may play a more significant role for N assimilation under high N supply conditions [74]. Breuninger and co-authors [81] also found that the fungal GS genes of *Funneliformis mosseae* (*GmGln1*)

and *R. irregularis* (*GiGln1*) are constitutively expressed but that the GS activities in the ERM are modulated in response to different N availabilities. Based on this observation, the authors concluded that the fungal GS activity is not controlled on a transcriptional level, but is subjected to post-transcriptional regulation [81]. However, the GS transcript that was examined by these authors has a closer sequence similarity to *GiGS1* (see above), and only *GiGS2* has been shown to be strongly up-regulated after NO_3^- supply [74].

Consistent with the GS-GOGAT pathway, Gln becomes highly labelled when $^{15}\text{NH}_4^+$ is supplied to AM fungi, and represents one of the major N sinks [50,71,83,95,118]. Gln plays a central role in N metabolism (1) as key N donor; (2) as precursor of many essential metabolites such as nucleic acids, amino sugars, and other amino acids, such as histidine, tyrosine and asparagine; and (3) as key effector for N metabolite repression and regulator of genes involved in N metabolism [67,101,102,119,120]. Due to these important functions, the free levels of Gln in AM fungi are tightly controlled [71]. In addition to Gln, Glu, asparagine (Asn), aspartate (Asp) and alanine (Ala) are abundant free amino acids in germinating spores [71], in the ERM [64] or in AM roots [50,95,118]. Ornithine, serine and glycine are also detectable but in much lower concentrations [64].

Arginine levels (Arg) were not determined in several of the earlier investigations [64,84,95], but more recent studies demonstrate that Arg is the most abundant free amino acid and can represent more than 90% of the total free amino acids in the ERM [118]. Arg levels of up to $200 \text{ nM} \cdot \text{mg}^{-1}$ dry weight have been reported in the ERM [50]. Due to its low carbon-to-nitrogen ratio of 6:4, Arg plays an important role for N storage and N transfer from the ERM to the IRM [50,121]. For example, Arg serves as a N storage molecule in quiescent spores and its catabolic breakdown during spore germination provides the N and C skeletons for the biosynthesis of other amino acids or proteins for the presymbiotic growth of the AM fungus [71].

4.3. Nitrogen Transport from the Extraradical Mycelium to the Intraradical Mycelium

The ERM takes up N from the soil in a significant distance from the root, and transfers N from the ERM to the IRM. The transport of N through the hyphae of the AM symbiosis can be very fast and flux rates similar to those of P have been observed [121,122]. Fungal vacuoles often contain polyphosphates (polyP) and basic amino acids in equimolar concentrations [123–125], and it has been suggested that N could move in the form of Arg with fungal polyP from the ERM to the IRM [50,94,121]. PolyP are negatively charged polyanions and the basic amino acid Arg could serve together with other cations such as K^+ and Mg^{2+} as counter charge and contribute to the required charge balance [126]. Studies on the N transport in the AM symbiosis of *Agropyron repens*, however, also suggest that in addition to Arg, other amino acids such as Gln or Glu could be involved in the translocation of N from the ERM to the IRM [127].

Gene expression studies are consistent with the biosynthesis of Arg in the ERM. Shortly after NO_3^- supply, the transcript levels of a fungal carbamoyl-phosphate synthetase (CPS), argininosuccinate synthase (ASS), and argininosuccinate lyase (AL) are induced in the ERM (Figure 2). All of these enzymes are involved in Arg biosynthesis; CPS catalyzes the formation of carbamoylphosphate from CO_2 , ATP, and NH_3 , which is converted together with ornithine to citrulline and P_i by an ornithine transcarbamoylase (OTC). Citrulline and aspartate are converted to argininosuccinate (AS) by ASS, and AL converts

argininosuccinate to fumarate and Arg. In contrast, in the IRM a fungal arginase (CAR1) and urease (URE) are up-regulated that are involved in the catabolic breakdown of Arg [74]. The biosynthesis of Arg in the ERM followed by the subsequent breakdown of Arg in the IRM are spatially separated but synchronized processes, and confirm the function of the anabolic (ERM) and catabolic part (IRM) of the urea cycle in the AM symbiosis (Figure 2). The synchronization of these processes suggests that Arg plays an important role in the N translocation from the ERM to the IRM [50,74,121].

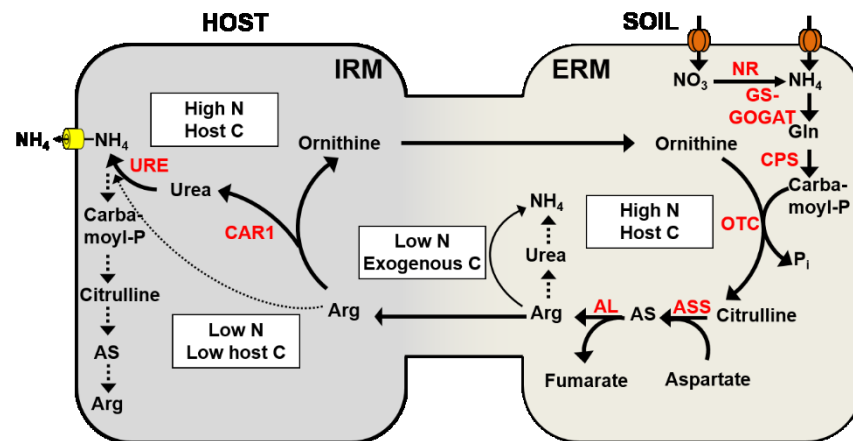


Figure 2. Function and regulation of the anabolic and catabolic arm of the urea cycle in the ERM or the IRM, respectively. The activities of the following enzymes are indicated in red: nitrate reductase/nitrite reductase (NR), glutamine synthetase—glutamate synthase (GS-GOGAT), carbamoyl-phosphate synthetase (CPS), ornithine transcarbamoylase (OTC), argininosuccinate synthase (ASS), argininosuccinate lyase (AL), arginase (CAR1), and urease (URE). Favored pathways are indicated by bold lines, suppressed pathways by dotted lines. White boxes provide additional information about the putative regulation of these pathways.

The C supply of the host plant plays a significant role in the regulation of these processes [12,75]. An increase in the C supply from the host plant led to an up-regulation of genes that are involved in Arg biosynthesis (AL, ASS, CPS), but to a down-regulation of a fungal urease (URE) in the ERM [75]. This will lead to increasing levels of Arg in the ERM (stimulation in biosynthesis and reduced catabolic breakdown) and stimulate the transfer of Arg from the ERM to the IRM. In contrast, in the IRM, an increase in the fungal arginase and urease activity and a down-regulation of genes involved in Arg biosynthesis were observed. This will favor the catabolic breakdown of Arg but prevent the re-assimilation of the released NH_4^+ in the IRM [75]. This demonstrates that the host plant with its C supply is able to regulate fungal gene expression and to trigger fungal N transport. However, when the fungus had access to an exogenous C source (acetate supply to the ERM), the transcript levels of genes involved in N assimilation and Arg biosynthesis were reduced, but the expression of a fungal arginase was induced in the ERM. This could indicate that the Arg transport from the ERM to the IRM is suppressed when the fungus has access to an exogenous carbon source and is not solely dependent on its host for its carbon supply [75].

The active hydrolysis of polyP will release P_i and Arg (for subsequent breakdown into NH_3 and NH_4^+ by the catabolic arm of the urea cycle) into the fungal cytoplasm of the IRM and will facilitate the efflux of P and of N 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 triggers polyP hydrolysis [10,75,128,129]. The regulation of polyP and Arg biosynthesis in the ERM and of polyP and Arg breakdown in the IRM would allow the fungus to control its P and N transport across the mycorrhizal interface.

4.4. Nitrogen Transport across the Mycorrhizal Interface

In Figure 3, a model of the current N transport pathway and its associations to the P and C flux in the AM symbiosis is shown. According to this model, the N transport pathway in the AM symbiosis includes the following steps: (1) uptake of P and of N by the fungal ERM through P_i , NO_3^- or NH_4^+ transporters; (2) N assimilation into Arg via the anabolic arm of the urea cycle and conversion of P_i into polyP; (3) transport of Arg into the fungal vacuole and binding to polyP; (4) transport of polyP from the ERM to the IRM; (5) polyP hydrolysis and release of Arg and P_i in the IRM; (6) Arg breakdown to NH_4^+ via the catabolic arm of the urea cycle; (7) facilitated P_i , NH_4^+ , and potential amino acid efflux into the interfacial apoplast; (8) plant uptake from the interface through mycorrhiza-inducible P_i or NH_4^+ transporters; (9) stimulation in photosynthesis by improved nutrient supply and facilitated efflux of sucrose into the interfacial apoplast; (10) sucrose hydrolysis via an apoplastic plant invertase, and uptake of hexoses by the AM fungus through fungal monosaccharide transporters.

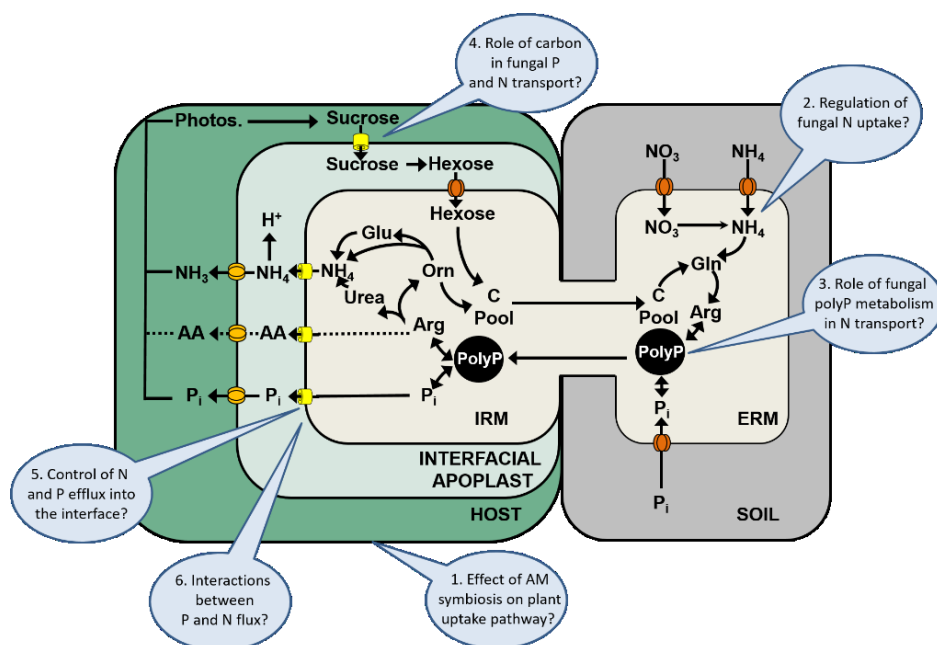


Figure 3. Model of the N and P transport in the AM symbiosis. The model is based on previous models presented in the literature [50,74,75,94]. The model shows the nutrient uptake by the fungal ERM through P_i , NO_3^- or NH_4^+ transporters (red), N assimilation into Arg and the conversion of P_i into polyP, the transport of polyP from the ERM to the IRM, polyP hydrolysis and the release of Arg and P_i in the IRM, Arg breakdown to NH_4^+ via the catabolic arm of the urea cycle, and the P_i , NH_4^+ , and potential amino acid efflux (yellow) into the interfacial apoplast, and the plant uptake from the interface through mycorrhiza-inducible P_i or NH_4^+ transporters. The blue ovals indicate gaps in our current understanding of these processes.

Previous models suggested that N is transferred from the fungus to the host in form of amino acids, but this would lead to a substantial reflux of C from the obligately biotrophic fungus to the host. The uptake of organic N from the mycorrhizal interface would also require the presence of e.g., amino acid transporters in the periarbuscular membrane that could facilitate an uptake of organic N from the mycorrhizal interface. In *Lotus japonicus* a mycorrhiza-inducible plant amino acid transporter (*LjLHT1.2*) has been identified that shows a high expression in arbusculated cells but also in non-colonized cells of the root cortex. The expression of this amino acid transporter in the periarbuscular membrane could indicate that this transporter is involved in the uptake of amino acids from the mycorrhizal interface [130]. However, the expression of this transporter in non-colonized cells suggests that the transporter could also play a role for the re-absorption of amino acids by the cortical cells.

Based on the elucidation of the N transport pathway [74,75,94] (see above and Figure 3), and the identification of several mycorrhiza-inducible NH_4^+ transporters in the periarbuscular membrane [36–38], newer models of N transport in the AM symbiosis now conclude that NH_4^+ is the main form in which N is transferred into the mycorrhizal interface and is taken up by the host. Studies after labeling with ^{13}C also indicate that the resulting CO_2 or HCO_3^- from the breakdown of Arg is not transferred to the plant and remains within the fungal compartment [50,94].

The release of nutrients from free living fungi is normally regarded as slow, and membrane transport processes are generally favoring fungal re-absorption [131]. Therefore, it is likely that conditions at the mycorrhizal interface exist that facilitate the efflux of nutrients from the fungus or reduce the level of competing fungal uptake systems. It is currently unknown how the N transport from the fungal hyphae into the mycorrhizal interface is facilitated, but several different mechanisms have been discussed. For example, in arbusculated cells and in the ERM of maize roots, two functional fungal aquaporins have been identified that are particularly expressed under drought stress [132]. Aquaporins are integral membrane channels that facilitate the concentration gradient-driven water transport through the plasma membrane. However, in addition to their function as water channels, aquaporins have also been shown to facilitate the diffusion across membranes of low molecular weight neutral solutes such as glycerol, ammonia, and carbon dioxide [133]. This has led to the hypothesis that aquaporins could play a role in the leakage of NH_4^+ into the interface [134]. The continuous breakdown of Arg in the IRM and the re-assimilation of NH_4^+ in the root cells will lead to a concentration gradient that could facilitate the movement of NH_4^+ into the mycorrhizal interface, and reduce the re-absorption of NH_4^+ by the AM fungus.

Plant NH_4^+ transporters in the periarbuscular membrane compete with fungal transporters for the NH_4^+ that has been released into the interfacial apoplast. The expression of fungal NH_4^+ transporters in arbusculated cells indicates that the fungus is also able to re-absorb released NH_4^+ , and the fungus could modulate the amount of N that is delivered to the host by the expression of these transporters in the IRM [53]. Several plant NH_4^+ transporters have been identified that are induced in arbusculated cells during the AM symbiosis and that have been implicated in the NH_4^+ uptake from the mycorrhizal interface [37–39,41]. One of these mycorrhiza-inducible NH_4^+ transporters has been identified in *Lotus japonicus* (*LjAMT2;2*) [38]. Interestingly, when *LjAMT2;2* is expressed in oocytes of *Xenopus laevis* the uptake of NH_4^+ does not result in a flow of current. Based on this observation, the authors concluded that the transporter binds NH_4^+ externally but transfers uncharged NH_3 across the periarbuscular membrane. It is unlikely that this high-affinity transporter could recruit NH_3 from the

interfacial apoplast, because NH_4^+ should be the dominant form of $\text{NH}_3/\text{NH}_4^+$ in the interfacial apoplast due to the assumed low pH conditions in this compartment (pK_a of protonation 9.25) [38].

Recently, two plant NH_4^+ transporters of *Medicago truncatula* (*AMT2;3* and *AMT2;4*) have been identified that play a role for the arbuscular life span [41]. Arbuscules are prematurely degraded in mutants in which *pt4*, the mycorrhiza-inducible plant P transporter that is critical for the uptake of P from the mycorrhizal interface, is not expressed [40,57]. This premature degeneration of arbuscules is suppressed by an expression of *AMT2;3* when the plant is grown under N stress, but not by *AMT2;4*. However, only *AMT2;4* has been shown to be a functional NH_4^+ transporter and was able to support in complementation assays the growth of a yeast NH_4^+ transporter mutant [41]. This suggests that *AMT2;3* and *AMT2;4* differ in their function, and that *AMT2;3* could play more a sensing or signaling function that is not present in other AMT transporters [41]. It has been hypothesized that some mycorrhiza-inducible nutrient transporters that are localized in the periarbuscular membrane could act as transceptors, transporter-like proteins with a receptor function [135].

5. Conclusions

In recent years, significant progress has been made in the elucidation of the N transport pathway and the identification of fungal or plant genes that are involved in the N transport from the ERM via the IRM to the host. In this review, we summarized our current understanding of N transport and its regulation in the AM symbiosis that is primarily based on ^{13}C and ^{15}N labeling studies and more recent data on fungal and plant gene expression. However, despite the progress that has been made, many critical questions are still unanswered and should be addressed in future studies. Some of these major research gaps are also indicated in Figure 3, and are listed below.

5.1. Effect of the Arbuscular Mycorrhizal Symbiosis on the Plant Uptake Pathway

There is increasing evidence that the AM symbiosis changes the nutrient uptake strategy of the plant, and that P transporters of the plant uptake pathway are downregulated in response to the AM symbiosis [31]. It has been shown that even in non-responsive plants, the uptake via the mycorrhizal pathway can dominate the overall P uptake by the plant [29]. Whether and how the plant uptake pathway for N is affected by the colonization with AM fungi is currently unknown, but there is evidence that some plant N transporters are downregulated in colonized roots [35,36].

5.2. Regulation of Fungal N Uptake

Fungal N uptake systems have a high affinity for the uptake of N from the soil [42], but how the expression of these transporters is regulated is largely unknown. The transcript levels of some of these transporters are substrate inducible, and regulated by the NH_4^+ supply and/or fungal NH_4^+ status [73]. However, our current understanding of the N transport pathways in the AM symbiosis, is still limited by the fact that these transport pathways were only studied in a small number of fungal species (see also Table 1), and by the lack of molecular tools in AM research. There is evidence that there is a high intraspecific diversity in the efficiency with which fungi are able to improve the N nutrition of their

host [46]. So far, the transcriptome and genome of only one AM fungal species is known [72,76], and attempts to develop fungal mutants for research studies were unsuccessful.

5.3. Role of Fungal PolyP Metabolism in N Transport

PolyP play a significant role for the P storage in fungal hyphae, for the long distance transport of P and other nutrients from the ERM to the IRM, and in the regulation of the nutrient transport to the host [10,126,129]. There are indications that the polyP metabolism may play a role in the efficiency with which AM fungi are able to provide nutritional benefits to their host [46]. The processes that are involved in fungal P_i homeostasis and polyP biosynthesis or remobilization have so far mainly been studied in yeasts (e.g., [136]), but the regulatory mechanisms that control the rates of polyP turnover in mycorrhizal fungal hyphae are largely unknown.

5.4. Role of Carbon in Fungal P and N Transport

Some studies have demonstrated that the carbon supply of the host acts as an important trigger for P and N transport in the AM symbiosis [10,12,75]. However, there are also other reports in which the carbon to nutrient resource exchange was not directly correlated [13]. Currently, we have only limited information how the carbon to nutrient exchange across the mycorrhizal interface is regulated [47]. It has been shown that the compatibility between host plants and AM fungi plays a significant role for the nutritional benefits in the AM symbiosis [25], but it is currently unknown why some AM fungi are only beneficial to certain host plant species, despite their ability to colonize a variety of host plants.

5.5. Control of Fungal N and P Efflux into the Interface

The processes by which nutrients are released into the mycorrhizal interface, or the mechanisms that control this efflux of nutrients through the fungal plasma membrane, are currently unknown. However, a better knowledge of these processes is key to understand the variability or context-dependency of nutritional benefits or mycorrhizal growth responses that have been described for the AM symbiosis.

5.6. Interactions between P and N Flux

There is a growing body of evidence that suggests that there are interactions between the P and N transport in the AM symbiosis [121] and that the maintenance of the AM symbiosis is more determined by the sum of nutritional benefits that are provided via the mycorrhizal interface [54,56,57,137]. It has been speculated that the transport of P or NH_4^+ through the respective mycorrhiza-inducible transporters in the periarbuscular membrane is not only important for the uptake of these nutrients from the mycorrhizal interface but also initiates signaling that controls the arbuscular life span [41]. Currently, the processes by which plants control the symbiosis in order to maximize their nutritional gains or control their C costs are largely unknown. These considerations are also complicated by the fact that, in addition to P and N, other nutrients are also transferred from the fungus to the host and that the symbiosis also provides a variety of non-nutritional benefits to host plants [3]. However, the transport of other nutrients in the AM symbiosis is under-researched and it is currently unknown how other nutrient fluxes or non-nutritional benefits contribute to the C costs of the symbiosis for the host plant.

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Author Contributions

The manuscript was primarily written by Heike Bücking with contributions by Arjun Kafle.

Conflicts of Interest

The authors declare no conflict of interest.

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