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Chapter

Beneficial Plant Microbe Interactions and Their Effect on Nutrient Uptake, Yield, and Stress Resistance of Soybeans

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Abstract

Plants are meta-organisms that are associated with complex microbiomes. Many of the microorganisms that reside on plant surfaces (epiphytes) or within plant tissues (endophytes) do not cause any plant diseases but often contribute significantly to the nutrient supply of their host plant and can help the plant to overcome a variety of biotic or abiotic stresses. The yield potential of any plant depends not only on successful plant traits that improve, for example, the adaptation to low input conditions or other stressful environments but also on the plant microbiome and its potential to promote plant growth under these conditions. There is a growing interest to unravel the mechanisms underlying these beneficial plant microbe interactions because the activities of these microbial communities are of critical importance for plant growth under abiotic and biotic stresses and could lead to the development of novel strategies to improve yields and stress resistances of agronomically important crops. In this chapter, we summarize our current understanding of the beneficial interactions of soybean plants with arbuscular mycorrhizal fungi, nitrogen-fixing rhizobia, and fungal and bacterial endophytes and identify major knowledge gaps that need to be filled to use beneficial microbes to their full potential.

Keywords: arbuscular mycorrhizal symbiosis, biological nitrogen fixation, endophytes, rhizobia, tripartite interactions

1. Introduction

The plant rhizosphere and phyllosphere is colonized by a wide range of epiphytic and endophytic microorganisms, and these microorganisms can establish beneficial, neutral, or detrimental associations of varying intimacy with their host plant. Recent developments in sequencing technologies have enabled us to study the composition and function of plant microbiomes, but these microbiomes are dynamic and differ among different plant tissues and in response to the environment. The microbiome can also be seen as “the second plant genome” and can consist of 10 times more genes than typical plant genomes [1]. Beneficial microorganisms that

are associated with plants hold enormous potential to be developed into microbial fertilizers or microbial pesticides [2] and new biotechnological tools to improve the nutrient efficiency and stress tolerance of crops, and environmental sustainability of agroecosystems. Specific interactions between microbes and plants, such as the *rhizobium*-legume symbioses, are well understood, but the majority of the plant microbiome, and its contribution to the extended phenotype of the host, is not yet well defined.

Soybeans form interactions with nitrogen-fixing rhizobia, and this symbiosis plays a key role not only for the nitrogen (N) nutrition of the plant but also for agricultural productivity since soybean root residues provide N for other plants in crop rotations [3, 4]. Arbuscular mycorrhizal (AM) fungi colonize the root system of the majority of land plants, including soybeans; transfer nutrients such as phosphate (P), N, potassium (K), and other nutrients to their host plants; and improve the resistance of their host plants against abiotic (e.g., drought, salinity, and heavy metals) and biotic stresses [5]. In addition, soybeans are associated with bacterial or fungal endophytes that exhibit a wide range of plant growth promoting capabilities, including the production of phytohormones, an improved N nutrition through biological nitrogen fixation (diazotrophic endophytes), the biosynthesis of ACC (1-aminocyclopropane-1-carboxylate) deaminase, the capability to solubilize phosphate, and also the biosynthesis and release of antimicrobial metabolites or siderophores to inhibit the growth of pathogenic microorganisms [6].

The plant microbiome is a largely unexplored resource of beneficial microorganisms with diverse properties and a hidden potential to manipulate plant growth and success in stressful environments. However, while the symbiosis of soybeans with rhizobia and AM fungi is well characterized, the functional role of endophytes is only known for a limited number of isolates. Our functional understanding of these interactions is mainly based on experiments with individual symbionts, but there is increasing evidence that individual symbionts can also affect the interactions of the plant with other symbionts [7–10]. We summarize here the effects of different beneficial microbes on nutrient uptake, yield, and stress resistance of soybeans and identify knowledge gaps that hinder the application of these interactions to their full potential in soybean production systems.

2. Beneficial plant microbe interactions of soybean plants

2.1 Arbuscular mycorrhizal symbiosis

The arbuscular mycorrhizal (AM) symbiosis is arguably the most important symbiosis on earth and is formed by more than 65% of all known land plant species ($n > 200,000$), including all legumes and many other agronomically important crops, such as wheat, corn, and rice [11]. AM fungi are classified into the fungal subphylum Glomeromycota that consists of less than 350 fungal species [12]. AM fungi co-exist relatively morphologically unaltered with plants for more than 400 million years, and there is evidence that suggests that the AM symbiosis played a critical role for land plant evolution [13].

It is long known that AM fungi can increase the nutrient uptake of their host plant and are able to deliver substantial amounts of P, N, K, sulfur (S), and trace elements, such as copper (Cu) and zinc (Zn) to the plant. Many AM fungi also provide non-nutritional benefits for their host that are critical for plant survival or fitness and improve, for example, the resistance of plants against abiotic (e.g., drought, heavy metal, and salinity) and biotic (pathogens) stresses [5]. In return for these benefits, host plants transfer up to 20–25% of their photosynthetically

derived carbohydrates to the fungal symbiont [14]. It was generally believed that carbon is transferred to the fungus in the form of hexoses [15], but recent evidence suggests that also fatty acids can move across the mycorrhizal interface to the fungal partner (**Figure 1**) [16–18].

AM fungi are ubiquitous in soils and can account for up to 50% of the microbial biomass in soils [19]. AM fungi form extensive hyphal networks in soils, and the extraradical mycelium (ERM) of the fungus acts as an extension of the root system and increases the nutrient absorbing surface of the root. The ERM with its mycorrhizosphere (interface between fungal hyphae and the soil) acts as an important conduit between microbial communities and the host plant [20] and can provide soil microbial communities with plant-derived carbon (C) inputs in large distance from the root. The mycorrhizosphere represents in soils an important ecological niche for diverse microbial communities that are specifically adapted to this mycorrhizosphere. According to estimates, the bacterial density in the mycorrhizosphere is 4–5 times higher than in the plant rhizosphere [21]. However, the presence of AM fungal mycelia does not only lead to quantitative but also to qualitative changes in the microbial community composition in soils [22]. The presence of AM fungal hyphae plays an important role in the bacterial community assembly during decomposition [22] and affects the access of members of these microbial communities to C sources during decomposition [23].

Within the host root, the fungus can not only spread intercellularly but also penetrate the root cortex intracellularly, and it forms here highly branched specialized structures called arbuscules that are separated from the plant symplast by the plant periarbuscular membrane [24]. Some AM fungal species also form vesicles, thick-walled, lipid-containing storage organs in the roots. Arbuscules are the site of nutrient exchange between the plant and the fungus, and both the fungal cell

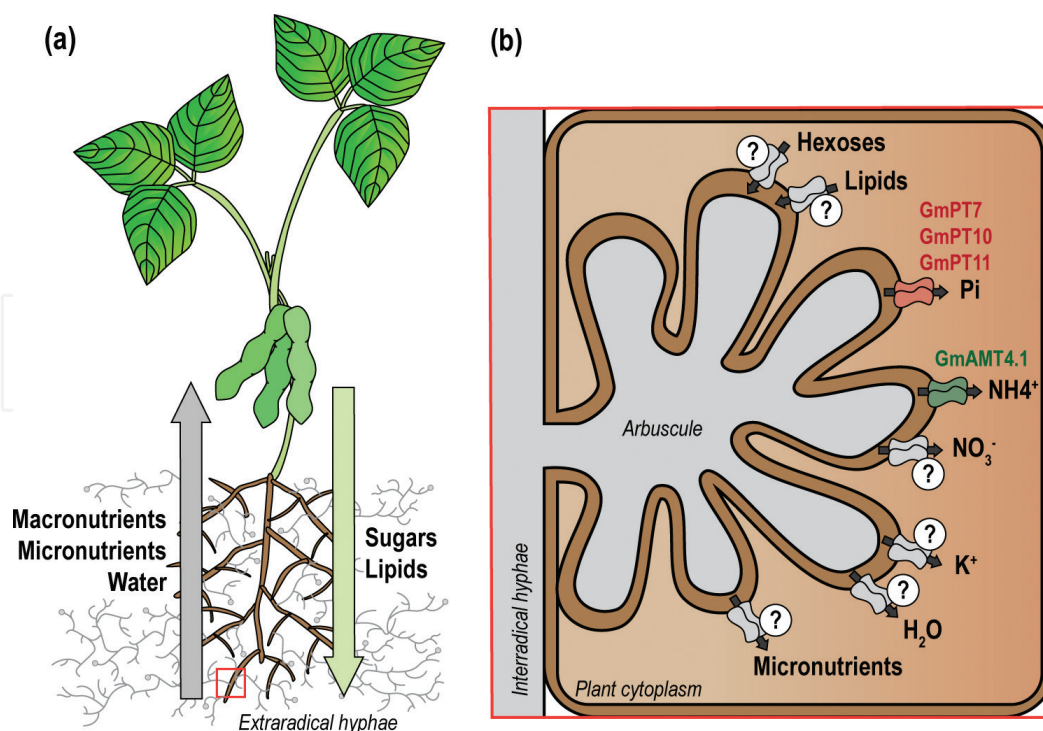


Figure 1. Overview of the mycorrhizal nutrient uptake pathway in AM roots of soybean plants via the extraradical mycelium of the fungus (a) and the mycorrhizal interface consisting of the fungal arbuscule in root cortical cells surrounded by the periarbuscular membrane of the host (b). Both fungal cell membrane and plant periarbuscular membrane are characterized by the presence of mycorrhiza-specific transporters that play a critical role for the nutrient exchange across the mycorrhizal interface of soybean plants (e.g., *GmPT7* or *GmAMT4.1*, see also below).

membrane and the plant periarbuscular membrane are characterized by the presence of specific transport proteins that play a critical role for the resource exchange between both partners (**Figure 1**) [15, 25, 26].

The colonization of host roots by AM fungi is based on a molecular dialog between both partners that facilitates partner recognition and triggers responses in both partners that are critical for the establishment of the symbiosis [27]. After fungal spore germination, an extensive hyphal branching in close proximity to host roots can be observed that is triggered by strigolactones and other compounds in root exudates [28]. After attachment to the host root surface and the differentiation of a fungal hyphopodium, the fungus penetrates the root, spreads with the help of a prepenetration apparatus [29], and forms arbuscules in the cells of the root cortex. This process is initiated by the release of lipochitoooligosaccharides, or Myc factors, by the fungus that are perceived by specific receptors on the host root surface and trigger a cascade of molecular responses in the host root. The pathway is called the common symbiotic signaling pathway (CSSP), since similar responses can be observed after the perception of rhizobial Nod factors [27, 30]. A key role for the perception of fungal Myc or Nod factors by the rhizodermis plays the membrane-bound receptor-like kinase SYMRK that activates the mevalonate (MVA) biosynthetic enzyme HMGR1(3-hydroxy-3-methylglutaryl CoA reductase 1). A second set of CSSP proteins is located in the nuclear pore complex and includes the three nucleoporins NUP133, NUP85, and NENA; the ATP-powered Ca^{2+} pump MCA8; and cation channels encoded by CASTOR and POLLUX involved in the strong Ca^{2+} oscillations in the nucleus of rhizodermal cells that can be observed shortly after Myc factor perception. Another set of proteins is located in the nucleoplasm and decodes these Ca^{2+} signals [30, 31]. A Ca^{2+} /calmodulin-dependent protein kinase (CCaMK) phosphorylates with the help of calmodulin CYCLOPS, which then regulates gene expression either directly or through GRAS transcription factors such as NSP1, NSP2, and RAM1 [30–32]. The elucidation of the CSSP is mainly based on studies in the model legumes *Medicago truncatula* or *Lotus japonicus*, but the fact that the proteins of the CSSP are highly evolutionary conserved, and even present in plants that are unable to form AM interactions, suggests that this pathway is also established in soybeans.

Mycorrhizal plants have two pathways that are involved in the nutrient uptake from the soil: the “plant pathway” via high- and low-affinity transporters in root epidermis and root hairs or the “mycorrhizal pathway” that first involves the uptake of nutrients via the ERM of the fungus, transport to the arbuscules, and then the uptake by the plant from the interfacial apoplast through specialized transporters in the periarbuscular membrane. In response to the colonization with AM fungi, transporters that are involved in the plant pathway are often down-regulated, while mycorrhiza-specific transporters in the periarbuscular membrane are induced [33], indicating that there is a shift in the nutrient acquisition strategy and that the mycorrhizal pathway can become the dominant pathway for nutrient uptake [34, 35].

2.1.1 Importance of arbuscular mycorrhizal fungi for yield and nutrient uptake of soybeans

Under both greenhouse and field conditions, increases in nutrient content, yield, and overall fitness of soybeans in response to an AM colonization can be observed [36, 37], and soybean yields are significantly correlated to the colonization of the roots with AM fungi [38]. Many reports clearly demonstrate the positive effects of AM fungi on the nutrient uptake of soybeans and here particularly on the uptake of phosphorus (P) and nitrogen (N) [39–41]. However, the effects can differ greatly among AM fungi. Our own studies demonstrated, for example, that while

the AM fungus *Rhizophagus irregularis* can increase the P nutrition of soybeans with low or high P acquisition efficiency, *Glomus custos* had no effect and *Glomus aggregatum* even led to slight growth depressions under medium P supply conditions [39].

Some of the observed differences among these AM fungi seem to be related to the impact of the AM fungus on plant P transporter expression. Fourteen genes of the Pht1 family have been identified in soybeans [42], and three of these transporters show high expression levels in AM roots [43]. While the colonization of the roots with *R. irregularis* led to the downregulation of *GmPt4*, a high-affinity P uptake transporter that is presumably involved in the uptake of P from the soil via the plant uptake pathway was the expression of *GmPt9*, and *GmPt10* upregulated in AM roots. *GmPt9* and *GmPt10* cluster with the mycorrhiza-inducible P transporters *OsPt11* of *Oryza sativa* (rice) and *MtPt4* of *Medicago truncatula* that play a critical role for the P uptake from the mycorrhizal interface [26, 44]. *GmPt9* was upregulated by *G. aggregatum* and *R. irregularis*, but *GmPt10* was only upregulated by *R. irregularis*, indicating that this transporter is involved in the P uptake from the interface and that *GmPt10* expression can serve as an indicator for mycorrhizal P benefits in soybean plants. *GmPt7*, another soybean P transporter, shows a high expression in cells with mature and active arbuscules but is not expressed in cells with collapsed and degenerated arbuscules, suggesting that this transporter may also play a role for the P transport across the AM interface. However, *GmPt7* is not a mycorrhiza-specific transporter and is also expressed in columella cells of root caps and in lateral root primordia of nonmycorrhizal roots [45]. Similarly, out of the 16 ammonium (NH₄⁺) transporters of soybean, five transporters are mycorrhiza-inducible, and one of them, *GmAMT4.1*, is specifically expressed in arbusculated cells (**Figure 1**), indicating that this transporter could be involved in the NH₄⁺ transport across the AM interface [46]. There is evidence from the model legume *Medicago truncatula* that AM fungi can also improve the acquisition of other macronutrients such as potassium (K) or sulfur [47, 48]. K deficiency is a common problem in soybeans and can lead to yellowing of the leaves, stunted growth, and reduced yields and can become particularly severe under drought stress. Although transcriptional and physiological responses to K deprivation have been studied in other legumes [49], whether AM fungi also play a role in the K acquisition of soybean plants is not yet known.

2.1.2 Importance of arbuscular mycorrhizal fungi for the stress resistance of soybeans

AM fungi can also increase the resistance of soybeans against other abiotic stresses such as drought, salinity, or soil contaminations. It is known for several decades that the AM colonization can improve the tolerance of soybeans against drought [50]. AM fungi can influence leaf water potential, solute accumulation, and oxidative stress of soybeans under drought stress [51] and delay nodule senescence triggered by water deprivation [52]. In mycorrhizal soybeans, plasma membrane aquaporins were down-regulated in response to drought stress, and this could reduce the permeability of membranes for water and contribute to water conservation [53]. In addition, both fungal and plant mitogen-activated protein kinases (MAPKs) are upregulated in AM soybean plants under drought stress. MAPK cascades are known to regulate many cellular processes in response to various stimuli, including abiotic and biotic stresses [54]. AM fungi also improve the tolerance of soybeans against salinity. AM plants had a higher biomass and proline concentrations in roots, but reduced proline and Na concentrations in the shoot under salt stress. When the fungus was pretreated with NaCl, the alleviating effects were even stronger, indicating that the acclimation of the fungus to salinity may play a role for the stress response [55]. AM fungi can also improve the tolerance of soybeans against arsenic [56] and aluminum [57] by reducing the uptake of these toxic metals.

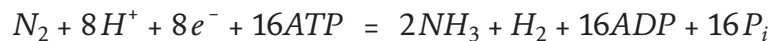
Soybean yield and productivity is also threatened by many fungal or bacterial diseases and soil inhabiting nematodes. Soybean cyst nematodes (SCNs, *Heterodera glycines*), brown spot (*Septoria glycines*), charcoal rot (*Macrophomina phaseolina*), rot and stem rot (*Phytophthora sojae*), and soybean rust (*Phakopsora pachyrhizi* and *P. meibomiaae*) are among the most important pathogens of soybeans and cause substantial yield losses in the U.S. [58]. SCNs are often responsible for hidden yield losses, since soil infestations remain often undetected until they become severe. SCN can spread easily from field to field via soil movements with machinery, wind, or by humans and can now be detected in 90% of the soybean producing states in the U.S. [59]. SCN infestations can lead to yield losses of more than 30% and are responsible for about \$ 1.5 billion in soybean crop damage each year in the U.S. AM fungi can protect soybeans against a wide range of pathogens, including fungi, bacteria, nematodes, or insects [60] and reduce the SCN egg population in soils by 70% [61]. The positive impact of AM fungi on biotic stresses has been attributed to the overall positive effect on nutrient uptake and a damage compensation effect, the competition for root space and soil nutrients, induced systemic resistance (ISR), and altered rhizosphere interactions. In addition, AM fungi form extensive hyphal networks in soils and can connect plants of the same or of different plant species by common mycelial networks (CMNs). CMNs play an important role in the plant-to-plant communication and can transfer infochemicals and warning signals from infested plants to uninfested plants and stimulate defense reactions in these plants [62].

2.2 Nitrogen-fixing symbiosis with rhizobia

Most legume plants are able to interact with N-fixing bacteria called rhizobia that are able to reduce atmospheric dinitrogen (N_2) into ammonia (NH_3) in specialized root nodules. The symbiosis evolved in legumes between 25 and 50 million years ago [63, 64] and plays an important role for plant nitrogen (N) nutrition. Rhizobia can contribute up to 70% to the total N nutrition, grain legumes can gain up to 300 kg N, and legume trees (e.g., *Acacia* sp.) up to 600 kg N per ha and year from these interactions [4, 65]. Free living rhizobia produce Nod factors that are perceived by plant roots and act as triggers for the common symbiotic signaling pathway (CSSP, see above). Nod factors are also lipochitooligosaccharides that are composed of chitin chains with various lipid modifications. Chitin is the main constituent of fungal but not of bacterial cell walls, and the functional and structural similarities between Nod and Myc factors have led to the assumption that rhizobia adopted the evolutionary far more ancient (~ 450 million years) CSSP to establish this endosymbiotic interaction with legumes [66]. Nod factors stimulate the curling of root hairs, and entrapped bacteria within these curls are transported within infection threads to the inner zone of developing root nodules. Inside of cortical cells, the rhizobia divide and multiply, and are released into vesicles, called symbiosomes, in which they differentiate to fully functional bacteroids. One or more differentiated bacteroids are surrounded by the plant symbiosome membrane, which represents a barrier by which the host plant can control the movement of solutes to the bacteroids through specialized transporters or channels [67].

Bacteroids express the nitrogenase complex that consists of six protein subunits (two each of NifH, NifD, and NifK), two $[4Fe-4S]$, two (Fe_8S_7) iron-sulfur clusters, and two iron-molybdenum cofactors (Fe_7MoS_9N) called FeMoco, which catalyze the N_2 reduction to NH_3 [68]. The nitrogenase metallocenters are all oxygen-labile and must operate in an environment with a low level of free oxygen, and nodules provide their bacterial symbionts with this oxygen-reduced environment for optimum N fixation [69]. N fixation by bacteroids is a highly energy consuming process, and

rapid respiration in the bacteroids is necessary to produce the 16 ATP required for the conversion of each atmospheric N₂ into two NH₃.



The product of biological N fixation (BNF) is ammonia, which diffuses out of the bacteroids into the acidic symbiosome space and is here protonated to ammonium. The symbiosome membrane is energized by an H⁺-ATPase, which pumps protons into the symbiosome space and thereby promotes the uptake of NH₃/NH₄⁺ into the plant cytosol, where NH₄⁺ is rapidly assimilated into amino acids, and the ureides, allantoin and allantoic acid [69]. A candidate for the uptake of NH₄⁺ from the symbiosome space is NOD26, which was first identified in soybeans [70]. NOD26 belongs to the major intrinsic protein/aquaporin (MIP/AQP) channel family and is exclusively localized in the symbiosome membrane [67]. The ureides, allantoin and allantoic acid, serve as the dominant long-distance transport molecule for N from the root nodules to the shoots [71, 72]. Cortex cells and the vascular endodermis of nodules express *GmUPS1-1* and *GmUPS1-2*, which play a role for the transport of allantoin and allantoic acid out of the root nodules to the sink organs. RNAi knockouts of these proteins accumulate ureides in the root nodules and show a reduced N transport to the shoots [73].

BNF is an energy expensive process, which requires 16 ATP to fuel the reduction of one N₂. Plants allocate up to 30% of their photosynthetically fixed C to rhizobia [74], which is oxidized in the bacteroids to ATP. The N₂ fixation rate of rhizobia is higher when the nodules receive more C, suggesting that the allocation of C to nodules is a limiting factor for BNF. Transgenic *Medicago sativa* plants that overexpress a sucrose phosphate synthase, a key enzyme for sucrose biosynthesis in plants, show higher C contents in nodules, more and larger nodules per plant, and an enhanced nitrogenase activity of the root nodules [75]. Free-living rhizobia can grow on a variety of different sugars, including mono- and disaccharides, but the absence of transporters for these sugars in bacteroids suggests that rhizobia in symbiosis take up dicarboxylates and here particularly malate from the symbiosome space. The C₄-dicarboxylate transport system that is localized in the inner bacteroid membrane is encoded by the *dctA* gene, has a high mobility for malate, and is essential for symbiotic nitrogen fixation [76]. Although the mechanisms of N fixation and assimilation are well documented, key steps are still unknown. For example, little is known about the C metabolism inside nodules, the regulatory steps that control the C export to rhizobia, and the proteins involved in the C and N transport between partners. Recent evidence in the model legumes *M. truncatula* and *Lotus japonicus* suggests that sucrose transporters from the Sugar Will Eventually be Exported Transporter (SWEET) family could be involved in the sucrose efflux from the phloem toward nodulated cells [77].

2.2.1 Significance of rhizobia for soybean agriculture

According to estimates, soybeans with their rhizobia populations fix around 20 million tons of N each year, and this has an enormous influence on agricultural productivity, not only on soybeans, but also on other crops in crop rotation systems [3, 4]. Soybean residues in the soil enrich the soil with N, improve soil organic matter, and can lead to yield increases in non-legume crops that follow soybeans. Crop rotations or intercropping systems of cereals with legumes can result in higher crop yields without fertilizer additions [78]. However, conventional agricultural management practices and other anthropogenic factors can have a negative impact on rhizobial function. In addition, excessive tillage, applications of higher N fertilizer

dosages, and extended fallow periods can also have detrimental effects on rhizobia populations in soils. As a consequence, integrating this symbiosis more efficiently in modern agricultural practices is crucial to limit the amount of fertilizers used and make agriculture more environmentally sustainable. Exploring ecologically best fitted ecoregions for soybeans and best adapted soybean cultivars will help farmers to produce more yield with reduced inputs. Rhizobial strains differ in their efficacy in symbiosis with different soybean cultivars, and the input of N into agricultural systems can be increased by the inoculation of legumes with optimized rhizobia for different environments [65]. The development of better inoculation strategies and specifically adapted rhizobia for different soybean cultivars could reduce the dependency of farmers on agrochemicals and enhance food security [65].

2.3 Tripartite symbiosis with arbuscular mycorrhizal fungi and rhizobia

In natural environments, legume roots form tripartite interactions and are simultaneously colonized by both AM fungi and rhizobia [7, 79]. Tripartite interactions have been shown to improve plant productivity, seed yield, P and N acquisition, and photosynthetic rates [10, 80, 81]. The rhizobial nitrogenase complex requires at least 16 ATP to reduce one N₂ molecule into two NH₃. Consequently, nodules act as strong P sinks in legume root systems to provide sufficient P resources to the bacteroids for optimum BNF [79, 82]. Since AM fungi are able to improve the P nutrition of legume plants, AM fungi can increase the BNF by root nodules by at least 50% [10]. Nonmycorrhizal soybean plants have lower nodule numbers and weights and particularly under low P supply lower N fixation rates [7, 83]. AM fungi can also provide their hosts with microelements that are essential for N₂ fixation, including zinc, iron, manganese, and molybdenum [84, 85].

AM fungi and rhizobial bacteria can act synergistically and can improve plant productivity, seed yield, and grain quality [7, 10, 81]. However, the prior inoculation by either rhizobia or AM fungi can also reduce the subsequent colonization by the other symbiont [86]. Plants control the extent of root colonization by both symbionts by an autoregulatory mechanism, possibly to limit the high C costs associated with these interactions [83, 87]. Whether AM fungi and rhizobia interact antagonistically or synergistically depends on the environmental context [81] and the compatibility between symbiotic partners [10, 88]. For example, the rhizobial strain STM 7183 is more compatible with the AM fungus *Rhizophagus clarus* and leads to higher nodulation rates, nitrogenase activities, and plant growth responses than STM 7282 [10]. Similarly, plant productivity and seed yields of nodulated soybeans were higher when the plants were co-inoculated with the AM fungus *Rhizophagus irregularis* than with *Acaulospora tuberculata* or *Gigaspora gigantea* [88]. Soybean cultivars also differ in their ability to benefit from their microbial communities [89]. Consequently, the symbiotic efficiency should be integrated into soybean breeding programs, and AM fungi and N-fixing bacteria with high compatibility should be identified to improve the productivity and stress resistance of soybeans and other legumes.

Both interactions are costly, and the host plant allocates up to 20% of its photosynthetically fixed C to its fungal [14, 90] and up to 30% to its N-fixing symbionts (**Figure 2**) [74]. C acts as an important trigger for symbiotic functioning, and a reduction in the C fluxes to the symbionts decreases BNF by rhizobia [91], and P and N uptake and transport by AM fungi [92–94]. Considering the high C costs of these symbioses for the host, plants are under a selective pressure to strongly regulate the C fluxes to both root symbionts, but these control mechanisms are currently poorly understood. Resource exchange between host and AM fungi is controlled by a reciprocal reward mechanism that is driven by biological market dynamics [95].

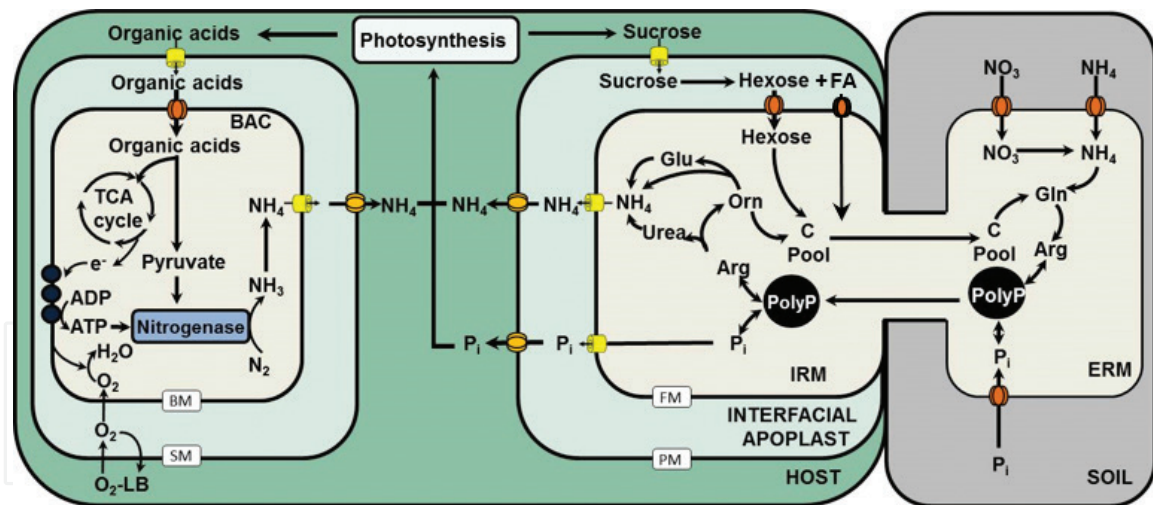


Figure 2. Transport and nutrient exchange pathways in the symbiosis with N-fixing bacteria (BAC) and AM fungi (IRM and ERM). Abbreviations: BAC, N-fixing bacteroid; BM, Bacteroid membrane; ERM, Extraradical mycelium; FA, Fatty acids; FM, Fungal plasma membrane; IRM, Intraradical mycelium; PM, Periarbuscular membrane; SM, Symbiosome membrane.

Our own results recently demonstrated that similar mechanisms may also control the resource to C exchange in tripartite interactions; *Medicago* plants allocate C to the different root symbionts in tripartite interactions in response to nutrient demand conditions; and the AM fungus becomes a stronger competitor for C resources from the host, when the fungal partner has access to N [79].

AM fungi have stronger effects on plant gene expression than rhizobia [96], but our current understanding of the molecular mechanisms involved in the C allocation to individual root symbionts is limiting. An overexpression of a leaf sucrose phosphate synthase of *M. sativa* increases starch production, allowing the plant to allocate more photosynthates to root nodules and consequently improved nitrogenase activity and overall plant growth [75]. There is evidence that suggests that sucrose transporters (SUTs) could be involved in the regulation of beneficial C fluxes toward the fungal symbiont [97], and the expression of *MtSUT2* and *MtSUT4-1* has been shown to be positively correlated to the C allocation to different symbiotic partners in tripartite interactions [79]. *MtSWEET1b* and *MtSWEET6* of the Sugars Will Eventually be Exported Transporter family (SWEET) are highly expressed in AM roots, and preferentially transport hexoses such as glucose, and could be involved in the transport of hexoses or fatty acids across the mycorrhizal interface to the fungal partner [79, 98]. *MtSWEET11* is specifically expressed in root nodules and could be involved in the sugar distribution within root nodules, but loss-of-function mutants indicate that *MtSWEET11* is not essential for BNF [99]. A better understanding of these processes is critical because it may be key to improve the resource exchange between plants and symbionts and ultimately to enhance productivity of agronomically important legumes.

2.4 Symbiosis with endophytic bacteria or fungi

Endophytes are defined as organisms that live inside plant hosts for at least part of their lives, without causing apparent disease symptoms in the host as a result of this colonization [100]. Fungal and bacterial endophytes are nearly ubiquitous across all groups of vascular plants [101], but there is a large biological diversity among endophytes, and it is not rare for some plant species to host hundreds of different endophytic species [102]. Fungal endophytes have been shown to enhance growth and seed production or protect against environmental stresses such as

drought or P deficiency or provide defense against herbivory through the synthesis of various biologically active metabolites, such as alkaloids. In soybeans diverse communities of fungal endophytes can be found, and several of these endophytes have plant growth-promoting capabilities and enhance, for example, soybean growth in nickel- or copper-contaminated soils by reducing the levels of stress-related phytohormones such as abscisic acid and jasmonic acid [103]; or increase glutathione activities and thereby reduce oxidative stress [104]. The inoculation of soybean plants with fungal endophytes can also lead to higher shoot biomasses, chlorophyll contents, and photosynthetic rates compared to noninoculated soybeans under salt stress and decrease the abundances of SCN in soils [105].

Soybeans host also a diverse group of bacterial endophytes, and many endophytic bacteria have plant growth-promoting capabilities [106], such as the ability to produce plant growth hormones, or ACC (1-aminocyclopropane-1-carboxylate) deaminase, solubilize phosphate, or release antimicrobial metabolites or siderophores that can inhibit the growth of pathogenic microorganisms. ACC deaminase reduces the levels of ethylene, an important stress hormone in plants. Several endophytic bacteria are also diazotrophs and have like rhizobia bacteria the ability to fix N. Bacterial endophytes also interact with rhizobia bacteria and can enhance root nodulation and activity, and as a consequence, the N content of soybean plants [107]. The dual inoculation with rhizobia and a salt-tolerant bacterial endophyte led to synergistic responses and promoted the fitness of soybean plants under salt stress [108].

3. Important research gaps and future challenges

Beneficial plant microbe interactions with AM fungi, rhizobia, or bacterial and fungal endophytes have enormous potential to improve plant growth and nutrient uptake in stressful environments and to increase the environmental sustainability of soybean agriculture. However, while the beneficial effects of AM fungi and rhizobia on soybean productivity are long known, the effect of only a small number of endophytes is currently known. The plant microbiome is a still unexplored resource of microorganisms with a so far hidden potential to promote plant growth and success under abiotic or biotic stress conditions, and with unknown effects on the plant phenotype.

The obligate lifestyle of AM fungi has made for a long time the production of fungal inoculum in large quantities difficult, but the development of sterile transgenic root organ cultures has led to an increased commercialization of AM fungal inocula for the utilization in agroecosystems [109]. Although increases in yield and biomass have been reported in different crops after inoculation with these inocula [36, 110], in other studies, inconsistent or neutral effects were observed [111]. AM fungi differ in the benefit that they provide for their host plant [112], and mycorrhizal growth responses are highly context dependent. Several factors can alter the success of AM fungal inoculation in agroecosystems, including plant/fungal compatibility, the degree of competition with the native microbial population, or timing of inoculation [113]. All these aspects need to be taken into consideration to find the most adapted and specific conditions for an efficient use of AM fungal inocula in a given field or for a certain crop. Our current understanding of the effect of beneficial plant microbes on soybeans is mainly based on studies with single symbiont, but plant productivity and stress resistance in agroecosystems depend on diverse microbial communities and the interactions among the different microorganisms in these communities. Identifying and characterizing the molecular mechanisms responsible for the functioning of different plant microbe interactions

is crucial to harness these symbiotic microorganisms in agroecosystems. Currently, most knowledge is gathered on model legumes, such as *Medicago truncatula*, but the information about soybeans is limited. However, the accumulation of genomic and transcriptomic data, along with the development of molecular tools such as stable transformations, e.g., [114], CRISPR-Cas9 system [115], or mutant populations, will provide us with a better understanding of these interactions in soybeans.

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Conflict of interest

The authors have no conflict of interest.

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