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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
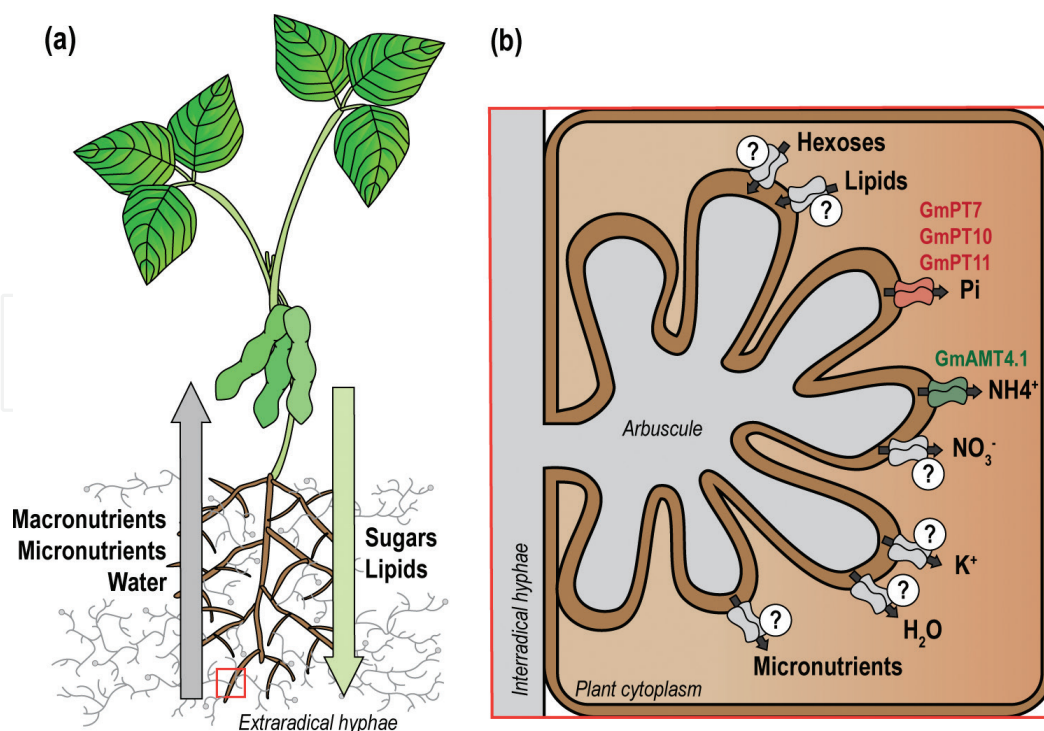

Figure 1.
Overview of the mycorrhizal nutrient uptake pathway in AM roots of soybean plants via the extraradical mycelium of the fungus (a) and the mycorrhizal interface consisting of the fungal arbuscule in root cortical cells surrounded by the periarbuscular membrane of the host (b). Both fungal cell 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 lipochitooligosaccharides, 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 (NH_4^+) 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 NH_4^+ 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. meibomiae)* 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 N_2 into two NH_3.

$$N_2 + 8H^+ + 8e^- + 16ATP = 2NH_3 + H_2 + 16ADP + 16P_i$$
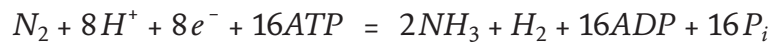
The product of biological N fixation (BNF) is ammonia, which diffuses out of the bacteroids into the acidic symbiosome space and is here protonated to ammonium. The symbiosome membrane is energized by an H^+-ATPase, which pumps protons into the symbiosome space and thereby promotes the uptake of NH_3/ NH_4^+ into the plant cytosol, where NH_4^+ is rapidly assimilated into amino acids, and the ureides, allantoin and allantoic acid [69]. A candidate for the uptake of NH_4^+ 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. RNA*i* 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 N_2. Plants allocate up to 30% of their photosynthetically fixed C to rhizobia [74], which is oxidized in the bacteroids to ATP. The N_2 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 N_2 molecule into two NH_3. 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 N_2 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].
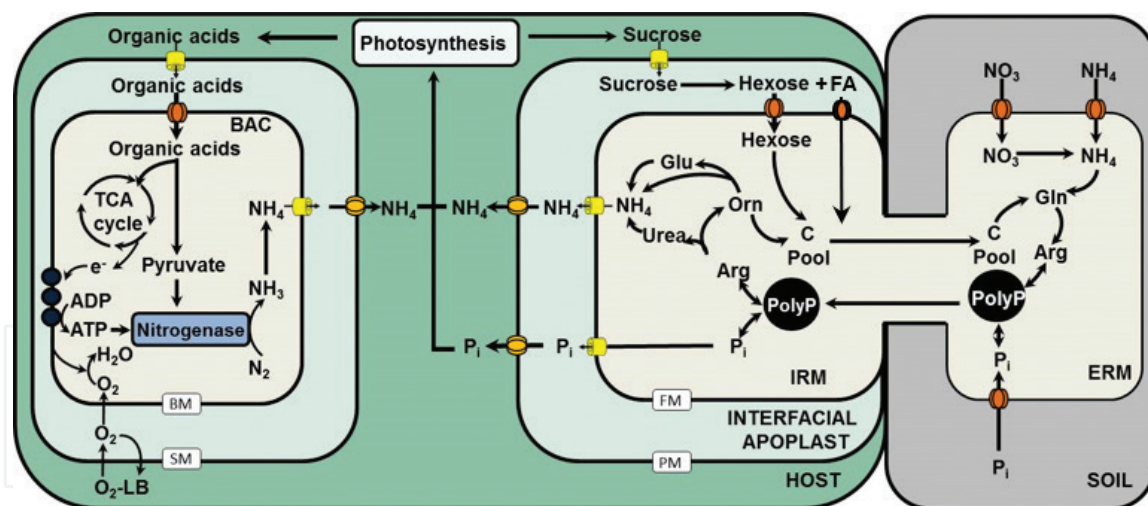
Figure 2.
Transport and nutrient exchange pathways in the symbiosis with N-fixing bacteria (BAC) and AM fungi (IRM and ERM). Abbreviations: BAC, N-fixing bacteroid; BM, Bacteroid membrane; ERM, Extraradical mycelium; FA, Fatty acids; FM, Fungal plasma membrane; IRM, Intraradical mycelium; PM, Periarbuscular 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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References

[1] Turner TR, James EK, Poole PS. The plant microbiome. Genome Biology. 2013;**14**:209. DOI: 10.1186/gb-2013-14-6-209

[2] Vryzas Z. The plant as metaorganism and research on next-generation systemic pesticides – Prospects and challenges. Frontiers in Microbiology. 2016;**7**. DOI: 10.3389/fmicb.2016.01968

[3] Peoples MB, Brockwell J, Herridge DF, Rochester IJ, Alves BJR, Urquiaga S, et al. The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis. 2009;**48**:1-17. DOI: 10.1007/bf03179980

[4] Herridge DF, Peoples MB, Boddey RM. Global inputs of biological nitrogen fixation in agricultural systems. Plant and Soil. 2008;**311**:1-18. DOI: 10.1007/s11104-008-9668-3

[5] Smith SE, Read DJ. Mycorrhizal symbiosis. 3rd ed. New York: Academic Press; 2008. DOI: 10.1016/B978-0-12-370526-6.X5001-6

[6] Santoyo G, Moreno-Hagelsieb G, Del Carmen Orozco-Mosqueda M, Glick BR. Plant growth-promoting endophytes. Microbiological Research. 2016;**183**:92-99. DOI: 10.1016/j.micres.2015.11.008

[7] Püschel D, Janouskova M, Voriskova A, Gryndlerova H, Vosatka M, Jansa J. Arbuscular mycorrhiza stimulates biological nitrogen fixation in two *Medicago* spp. through improved phosphorus acquisition. Frontiers in Plant Science. 2017;**8**. DOI: 10.3389/fpls.2017.00390

[8] Ossler JN, Zielinski CA, Heath KD. Tripartite mutualism: Facilitation or trade-offs between rhizobial and mycorrhizal symbionts of legume hosts. American Journal of Botany. 2015;**102**:1332-1341. DOI: 10.3732/ajb.1500007

[9] Bulgarelli RG, Correia Marcos FC, Ribeiro RV, Lopez De Andrade SA. Mycorrhizae enhance nitrogen fixation and photosynthesis in phosphorus starved soybean (*Glycine max* L. Merrill). Environmental and Experimental Botany. 2017;**140**:26-33. DOI: 10.1016/j.envexpbot.2017.05.015

[10] Bournaud C, James EK, De Faria SM, Lebrun M, Melkonian R, Duponnois R, et al. Interdependency of efficient nodulation and arbuscular mycorrhization in *Piptadenia gonoacantha*, a Brazilian legume tree. Plant, Cell & Environment. 2017. DOI: 10.1111/pce.13095

[11] Wang B, Qiu Y-L. Phylogenetic distribution and evolution of mycorrhizae in land plants. Mycorrhiza. 2006;**16**:299-363. DOI: 10.1007/s00572-005-0033-6

[12] Öpik M, Zobel M, Cantero JJ, Davison J, Facelli JM, Hiiesalu I, et al. Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. Mycorrhiza. 2013;**23**:411-430. DOI: 10.1007/s00572-013-0482-2

[13] Delaux P-M, Radhakrishnan GV, Jayaraman D, Cheem J, Malbreil M, Volkening JD, et al. Algal ancestor of land plants was preadapted for symbiosis. Proceedings of the National Academy of Sciences of the United States of America. 2015;**112**:13390-13395. DOI: 10.1073/pnas.1515426112

[14] Wright DP, Read DJ, Scholes JD. Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L. Plant, Cell and Environment. 1998;**21**:881-891. DOI: 10.1046/j.1365-3040.1998.00351.x

[15] Helber N, Wippel K, Sauer N, Schaarschmidt S, Hause B, Requena N. A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus *Glomus* sp is crucial for the symbiotic relationship with plants. The Plant Cell. 2011;**23**:3812-3823. DOI: 10.1105/tpc.111.089813

[16] Bravo A, Brands M, Wewer V, Dörmann P, Harrison MJ. Arbuscular mycorrhiza-specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development of arbuscular mycorrhiza. New Phytologist. 2017;**214**:1631-1645. DOI: 10.1111/nph.14533

[17] Keymer A, Pimprikar P, Wewer V, Huber C, Brands M, Bucerius SL, et al. Lipid transfer from plants to arbuscular mycorrhiza fungi. eLife. 2017;**6**. DOI: 10.7554/eLife.29107

[18] Luginbuehl LH, Menard GN, Kurup S, Van Erp H, Radhakrishnan GV, Breakspear A, et al. Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. Science. 2017;**356**:1175-1178. DOI: 10.1126/science.aan0081

[19] Ryan MH, Graham JH. Is there a role for arbuscular mycorrhizal fungi in production agriculture? Plant and Soil. 2002;**244**:263-271. DOI: 10.1023/a:1020207631893

[20] Kaiser C, Kilburn MR, Clode PL, Fuchslueger L, Koranda M, Cliff JB, et al. Exploring the transfer of recent plant photosynthates to soil microbes: Mycorrhizal pathway vs direct root exudation. New Phytologist. 2014;**205**:1537-1551. DOI: 10.1111/nph.13138

[21] Heinonsalo J, Jörgensen KS, Haahtela K, Sen R. Effects of *Pinus sylvestris* root growth and mycorrhizosphere development on bacterial carbon source utilization

and hydrocarbon oxidation in forest and petroleum-contaminated soils. Canadian Journal of Microbiology. 2000;**46**:451-464. DOI: 10.1139/cjm-46-5-451

[22] Nuccio EE, Hodge A, Pett-Ridge J, Herman DJ, Weber PK, Firestone MK. An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. Environmental Microbiology. 2013;**15**:1870-1881. DOI: 10.1111/1462-2920.12081

[23] Herman DJ, Firestone MK, Nuccio E, Hodge A. Interactions between an arbuscular mycorrhizal fungus and a soil microbial community mediating litter decomposition. FEMS Microbiology Ecology. 2012;**80**:236-247. DOI: 10.1111/j.1574-6941.2011.01292.x

[24] Brundrett M. Diversity and classification of mycorrhizal associations. Biological Reviews. 2004;**79**:473-495. DOI: 10.1017/s1464793103006316

[25] Garcia K, Doidy J, Zimmermann SD, Wipf D, Courty P-E. Take a trip through the plant and fungal transportome of mycorrhiza. Trends in Plant Science. 2016;**21**:937-950. DOI: 10.1016/j.tplants.2016.07.010

[26] Javot H, Penmetsa RV, Terzaghi N, Cook DR, Harrison MJ. A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. Proceedings of the National Academy of Sciences, USA. 2007;**104**:1720-1725. DOI: 10.1073/pnas.0608136104

[27] Delaux P-M, Séjalon-Delmas N, Bécard G, Ané J-M. Evolution of the plant–microbe symbiotic 'toolkit'. Trends in Plant Science. 2013;**18**:298-304. DOI: 10.1016/j.tplants.2013.01.008

[28] Akiyama K, Hayashi H. Strigolactones: Chemical signals for fungal symbionts and parasitic weeds in plant roots. Annals of Botany. 2006;**97**:925-931. DOI: 10.1093/aobmc1063

[29] Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. The Plant Cell. 2005;**17**: 3489-3499. DOI: 10.1105/tpc.105.035410

[30] Parniske M. Arbuscular mycorrhiza: The mother of plant root endosymbioses. Nature Reviews Microbiology. 2008;**6**:763-775. DOI: 10.1038/nrmicro1987

[31] Hohnjec N, Czaja-Hasse LF, Hogekamp C, Küster H. Pre-announcement of symbiotic guests: Transcriptional reprogramming by mycorrhizal lipochitooligosaccharides shows a strict co-dependency on the GRAS transcription factors NSP1 and RAM1. BMC Genomics. 2015;**16**:994. DOI: 10.1186/s12864-015-2224-7

[32] Smit P, Raedts J, Portyanko V, Debelle F, Gough C, Bisseling T, et al. NSP1 of the GRAS protein family is essential for rhizobial nod factor-induced transcription. Science. 2005;**308**:1789-1791. DOI: 10.1126/science.1111025

[33] Grunwald U, Guo WB, Fischer K, Isayenkov S, Ludwig-Müller J, Hause B, et al. Overlapping expression patterns and differential transcript levels of phosphate transporter genes in arbuscular mycorrhizal, P_i-fertilised and phytohormone-treated *Medicago truncatula* roots. Planta. 2009;**229**: 1023-1034. DOI: 10.1007/s00425-008-0877-z

[34] Li H, Smith FA, Dickson S, Holloway RE, Smith SE. Plant growth depressions in arbuscular mycorrhizal symbioses: Not just caused by carbon drain? New Phytologist. 2008;**178**:852-862. DOI: 10.1111/j.1469-8137.2008.02410.x

[35] Smith SE, Jakobsen I, Gronlund M, Smith FA. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: Interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiology. 2011;**156**:1050-1057. DOI: 10.1104/pp.111.174581

[36] Cely MVT, De Oliveira AG, De Freitas VF, De Luca MB, Barazetti AR, Dos Santos IMO, et al. Inoculant of arbuscular mycorrhizal fungi (*Rhizophagus clarus*) increase yield of soybean and cotton under field conditions. Frontiers in Microbiology. 2016;**7**:720. DOI: 10.3389/fmicb.2016.00720

[37] Afolayan ET, Eguavon MI. Morphological and yield assessment of soybean (*Glycine max* L.) as influenced by arbuscular mycorrhizal fungi and other soil amendments. Journal of Agricultural Science and Food Research. 2017;**8**:193

[38] Isobe K, Higo M, Kondo T, Sato N, Takeyama S, Torigoe Y. Effect of winter crop species on arbuscular mycorrhizal fungal colonization and subsequent soybean yields. Plant Production Science. 2014;**17**:260-267. DOI: 10.1626/pps.17.260

[39] Wang X, Zhao S, Bücking H. Arbuscular mycorrhizal growth responses are fungal specific but do not differ between soybean genotypes with different phosphate efficiency. Annals of Botany. 2016;**18**:11-21. DOI: 10.1093/aob/mcw074

[40] Wang X, Yan X, Liao H. Genetic improvement for phosphorus efficiency in soybean: A radical approach. Annals

of Botany. 2010;**106**:215-222. DOI: 10.1093/aob/mcq029

[41] Liu H, Song F, Liu S, Li X, Liu F, Zhu X. Arbuscular mycorrhiza improves nitrogen use efficiency in soybean grown under partial root-zone drying irrigation. Archives of Agronomy and Soil Science. 2018. DOI: 10.1080/03650340.2018.1493724

[42] Qin L, Guo YX, Chen LY, Liang RK, Gu MA, Xu GH, et al. Functional characterization of 14 Pht1 family genes in yeast and their expressions in response to nutrient starvation in soybean. PLoS One. 2012;7:e47726. DOI: 10.1371/journal.pone.0047726

[43] Tamura Y, Kobae Y, Mizuno T, Hata S. Identification and expression analysis of arbuscular mycorrhiza-inducible phosphate transporter genes of soybean. Bioscience, Biotechnology and Biochemistry. 2012;**76**:309-313. DOI: 10.1271/bbb.110684

[44] Kobae Y, Hata S. Dynamics of periarbuscular membranes visualized with a fluorescent phosphate transporter in arbuscular mycorrhizal roots of rice. Plant and Cell Physiology. 2010;**51**:341-353. DOI: 10.1093/pcp/pcq013

[45] Inoue Y, Kobae Y, Omoto E, Tanaka A, Banba M, Takai S, et al. The soybean mycorrhiza-inducible phosphate transporter gene, *GmPT7*, also shows localized expression at the tips of vein endings of senescent leaves. Plant and Cell Physiology. 2014;**55**:2102-2111. DOI: 10.1093/pcp/pcu138

[46] Kobae Y, Tamura Y, Takai S, Banba M, Hata S. Localized expression of arbuscular mycorrhiza-inducible ammonium transporters in soybean. Plant and Cell Physiology. 2010;**51**: 1411-1415. DOI: 10.1093/pcp/pcq099

[47] Sieh D, Watanabe M, Devers EA, Brueckner F, Hoefgen R, Krajinski F. The arbuscular mycorrhizal symbiosis

influences sulfur starvation responses of *Medicago truncatula*. New Phytologist. 2013;**197**:606-616. DOI: 10.1111/nph.12034

[48] Garcia K, Chasman D, Roy S, Ané J-M. Physiological responses and gene co-expression network of mycorrhizal roots under K deprivation. Plant Physiology. 2017;**173**:1811-1823. DOI: 10.1104/99.16.01959

[49] Garcia K, Ané J-M. Polymorphic responses of *Medicago truncatula* accessions to potassium deprivation. Plant Signaling & Behavior. 2017;**12**:e1307494. DOI: 10.1080/15592324.2017.1307494

[50] Busse M, Ellis J. Vesicular–arbuscular mycorrhizal (*Glomus fasciculatum*) influence on soybean drought tolerance in high phosphorus soil. Canadian Journal of Botany. 1985;**63**:2290-2294. DOI: 10.1139/b85-327

[51] Porcel R, Ruiz-Lozano JM. Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. Journal of Experimental Botany. 2004;**55**:1743-1750. DOI: 10.1093/jxb/erh188

[52] Ruiz-Lozano JM, Collados C, Barea JM, Azcón R. Arbuscular mycorrhizal symbiosis can alleviate drought-induced nodule senescence in soybean plants. New Phytologist. 2001;**151**:493-502

[53] Porcel R, Aroca R, Azcon R, Ruiz-Lozano JM. PIP aquaporin gene expression in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants in relation to drought stress tolerance. Plant Molecular Biology. 2006;**60**: 389-404. DOI: 10.1007/s11103-005-4210-y

[54] Liu Z, Li Y, Ma L, Wei H, Zhang J, He X, et al. Coordinated regulation of arbuscular mycorrhizal fungi

and soybean MAPK pathway genes improved mycorrhizal soybean drought tolerance. Molecular Plant-Microbe Interactions. 2015;**28**:408-419. DOI: 10.1094/MPMI-09-14-0251-R

[55] Sharifi M, Ghorbanli M, Ebrahimzadeh H. Improved growth of salinity-stressed soybean after inoculation with salt pre-treated mycorrhizal fungi. Journal of Plant Physiology. 2007;**164**:1144-1151. DOI: 10.1016/j.jplph.2006.06.016

[56] Spagnoletti FN, Balestrasse K, Lavado RS, Giacometti R. Arbuscular mycorrhiza detoxifying response against arsenic and pathogenic fungus in soybean. Ecotoxicology and Environmental Safety. 2016;**133**:47-56. DOI: 10.1016/j.ecoenv.2016.06.012

[57] Zhang S, Zhou J, Wang G, Wang X, Liao H. The role of mycorrhizal symbiosis in aluminum and phosphorus interactions in relation to aluminum tolerance in soybean. Applied Microbiology and Biotechnology. 2015;**99**:10225-10235. DOI: 10.1007/s00253-015-6913-6

[58] Wrather A, Shannon G, Balardin R, Carregal L, Escobar R, Gupta G, et al. Effect of diseases on soybean yield in the top eight producing countries in 2006. Plant Health Progress. 2010;**10**:1094. DOI: 10.1094/PHP-2010-0125-01-RS

[59] Tylka GL, Marett CC. Distribution of the soybean cyst nematode, *Heterodera glycines*, in the United States and Canada: 1954 to 2014. Plant Health Progress. 2014;**15**(85). DOI: 10.1094/PHP-BR-14-0006

[60] Schouteden N, De Waele D, Panis B, Vos CM. Arbuscular mycorrhizal fungi for the biocontrol of plant-parasitic nematodes: A review of the mechanisms involved. Frontiers in Microbiology. 2015;**6**:1280. DOI: 10.3389/fmicb.2015.01280

[61] Tylka G, Hussey R, Roncadori R. Interactions of vesicular-arbuscular mycorrhizal fungi, phosphorus, and *Heterodera glycines* on soybean. Journal of Nematology. 1991;**23**:122

[62] Babikova Z, Gilbert L, Bruce TJA, Birkett M, Caulfield JC, Woodcock C, et al. Underground signals carried through common mycelial networks warn neighbouring plants of aphid attack. Ecology Letters. 2013;**16**:835-843. DOI: 10.1111/ele.12115

[63] Cannon SB, Ilut D, Farmer AD, Maki SL, May GD, Singer SR, et al. Polyploidy did not predate the evolution of nodulation in all legumes. PLoS One. 2010;**5**:e11630. DOI: 10.1371/journal.pone.0011630

[64] Yokota K, Hayashi M. Function and evolution of nodulation genes in legumes. Cellular and Molecular Life Sciences. 2011;**68**:1341-1351. DOI: 10.1007/s00018-011-0651-4

[65] Lindström K, Murwira M, Willems A, Altier N. The biodiversity of beneficial microbe-host mutualism: The case of rhizobia. Research in Microbiology. 2010;**161**:453-463. DOI: 10.1016/j.resmic.2010.05.005

[66] Maillet F, Poinsot V, Andre O, Puech-Pages V, Haouy A, Gueunier M, et al. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature. 2011;**469**:58-U1501. DOI: 10.1038/nature09622

[67] Clarke VC, Loughlin PC, Day DA, Smith PMC. Transport processes of the legume symbiosome membrane. Frontiers in Plant Science. 2014;**5**. DOI: 10.3389/fpls.2014.00699

[68] Downie JA. Legume nodulation. Current Biology. 2014;**24**:R184-R190. DOI: 10.1016/j.cub.2014.01.028

[69] Oldroyd GED, Murray JD, Poole PS, Downie JA. The rules of

engagement in the legume-rhizobial symbiosis. Annual Review of Genetics. 2011;**45**:119-144. DOI: 10.1146/annurev-genet-110410-132549

[70] Fortin MG, Morrison NA, Verma DP. Nodulin-26, a peribacteroid membrane nodulin is expressed independently of the development of the peribacteroid compartment. Nucleic Acids Research. 1987;**15**:813-824. DOI: 10.1093/nar/15.2.813

[71] Oldroyd GED. Speak, friend, and enter: Signaling systems that promote beneficial symbiotic associations in plants. Nature Reviews Microbiology. 2013;**11**:252-263. DOI: 10.1038/nrmicro2990

[72] Udvardi M, Poole P. Transport and metabolism in legume-rhizobia symbioses. Annual Review of Plant Biology. 2013;**64**:781-805. DOI: 10.1146/annurev-arplant-050312-120235

[73] Collier R, Tegeder M. Soybean ureide transporters play a critical role in nodule development, function and nitrogen export. Plant Journal. 2012;**72**:355-367. DOI: 10.1111/j.1365-313X.2012.05086.x

[74] Provorov NA, Tikhonovich IA. Genetic resources for improving nitrogen fixation in legume-rhizobia symbiosis. Genetic Resources and Crop Evolution. 2003;**50**:89-99. DOI: 10.1023/a:1022957429160

[75] Gebril S, Seger M, Villanueva FM, Ortega JL, Bagga S, Sengupta-Gopalan C. Transgenic alfalfa (*Medicago sativa*) with increased sucrose phosphate synthase activity shows enhanced growth when grown under N_2-fixing conditions. Planta. 2015;**242**:1009-1024. DOI: 10.1007/s00425-015-2342-0

[76] Udvardi MK, Day DA. Metabolite transport across symbiotic membranes of legume nodules. Annual Review of Plant Physiology and Plant Molecular Biology. 1997;**48**:493-523. DOI: 10.1146/annurev.arplant.48.1.493

[77] Sugiyama A, Saida Y, Yoshimizu M, Takanashi K, Sosso D, Frommer WB, et al. Molecular characterization of LjSWEET3, a sugar transporter in nodules of *Lotus japonicus*. Plant and Cell Physiology. 2017;**58**:298-306. DOI: 10.1093/pcp/pcw190

[78] Yan J, Han XZ, Ji ZJ, Li Y, Wang ET, Xie ZH, et al. Abundance and diversity of soybean-nodulating rhizobia in black soil are impacted by land use and crop management. Applied and Environmental Microbiology. 2014;**80**:5394-5402. DOI: 10.1128/AEM.01135-14

[79] Kafle A, Garcia K, Wang X, Pfeffer PE, Strahan GD, Bucking H. Nutrient demand and fungal access to resources control the carbon allocation to the symbiotic partners in tripartite interactions of *Medicago truncatula*. Plant, Cell & Environment. 2018. DOI: 10.1111/pce.13359

[80] Harris D, Pacovsky R, Paul E. Carbon economy of soybean–rhizobium–glomus associations. New Phytologist. 1985;**101**:427-440. DOI: 10.1111/j.1469-8137.1985.tb02849.x

[81] Larimer AL, Clay K, Bever JD. Synergism and context dependency of interactions between arbuscular mycorrhizal fungi and rhizobia with a prairie legume. Ecology. 2014;**95**:1045-1054. DOI: 10.1890/13-0025.1

[82] Vadez V, Beck DP, Lasso JH, Drevon JJ. Utilization of the acetylene reduction assay to screen for tolerance of symbiotic N_2 fixation to limiting P nutrition in common bean. Physiologia Plantarum. 1997;**99**:227-232. DOI: 10.1111/j.1399-3054.1997.tb05406.x

[83] Mortimer PE, Perez-Fernandez MA, Valentine AJ. The role of arbuscular mycorrhizal colonization in the carbon

and nutrient economy of the tripartite symbiosis with nodulated *Phaseolus vulgaris*. Soil Biology & Biochemistry. 2008;**40**:1019-1027. DOI: 10.1016/j.soilbio.2007.11.014

[84] Chen BD, Li XL, Tao HQ, Christie P, Wong MH. The role of arbuscular mycorrhiza in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc. Chemosphere. 2003;**50**:839-846. DOI: 10.1016/s0045-6535(02)00228-x

[85] Ibiang YB, Mitsumoto H, Sakamoto K. Bradyrhizobia and arbuscular mycorrhizal fungi modulate manganese, iron, phosphorus, and polyphenols in soybean (*Glycine max* (L.) Merr.) under excess zinc. Environmental and Experimental Botany. 2017;**137**:1-13. DOI: 10.1016/j.envexpbot.2017.01.011

[86] Sakamoto K, Ogiwara N, Kaji T. Involvement of autoregulation in the interaction between rhizobial nodulation and AM fungal colonization in soybean roots. 2013;**49**:1141-1152. DOI: 10.1007/s00374-013-0804-8

[87] Kassaw T, Bridges W Jr, Frugoli J. Multiple autoregulation of nodulation (AON) signals identified through split root analysis of *Medicago truncatula sunn* and *rdn1* mutants. Plants. 2015;**4**: 209-224. DOI: 10.3390/plants4020209

[88] Meghvansi MK, Prasad K, Harwani D, Mahna SK. Response of soybean cultivars toward inoculation with three arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* in the alluvial soil. European Journal of Soil Biology. 2008;**44**:316-323. DOI: 10.1016/j.ejsobi.2008.03.003

[89] Farid M, Navabi A. N-2 fixation ability of different dry bean genotypes. Canadian Journal of Plant Science. 2015;**95**:1243-1257. DOI: 10.4141/cjps-2015-084

[90] Snellgrove RC, Splittstoesser WE, Stribley DP, Tinker PB. The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. New Phytologist. 1982;**92**:75-87. DOI: 10.1111/j.1469-8137.1982.tb03364.x

[91] Kleinert A, Venter M, Kossmann J, Valentine A. The reallocation of carbon in P deficient lupins affects biological nitrogen fixation. Journal of Plant Physiology. 2014;**171**:1619-1624. DOI: 10.1016/j.jplph.2014.07.017

[92] Fellbaum CR, Mensah JA, Cloos AJ, Strahan GD, Pfeffer PE, Kiers ET, et al. Fungal nutrient allocation in common mycelia networks is regulated by the carbon source strength of individual host plants. New Phytologist. 2014;**203**:645-656. DOI: 10.1111/nph.12827

[93] Fellbaum CR, Gachomo EW, Beesetty Y, Choudhari S, Strahan GD, Pfeffer PE, et al. Carbon availability triggers fungal nitrogen uptake and transport in the arbuscular mycorrhizal symbiosis. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**:2666-2671. DOI: 10.1073/pnas.1118650109

[94] Konvalinková T, Jansa J. Lights off for arbuscular mycorrhiza: On its symbiotic functioning under light deprivation. Frontiers in Plant Science. 2016;**7**:782. DOI: 10.3389/fpls.2016.00782

[95] Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, et al. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science. 2011;**333**:880-882. DOI: 10.1126/science.1208473

[96] Afkhami ME, Stinchcombe JR. Multiple mutualist effects on genomewide expression in the tripartite association between *Medicago*

truncatula, nitrogen-fixing bacteria and mycorrhizal fungi. Molecular Ecology. 2016;**25**:4946-4962. DOI: 10.1111/mec.13809

[97] Doidy J, Van Tuinen D, Lamotte O, Corneillat M, Alcaraz G, Wipf D. The *Medicago truncatula* sucrose transporter family: Characterization and implication of key members in carbon partitioning towards arbuscular mycorrhizal fungi. Molecular Plant. 2012;**5**:1346-1358. DOI: 10.1093/mp/sss079

[98] Chen LQ. SWEET sugar transporters for phloem transport and pathogen nutrition. New Phytologist. 2014;**201**:1150-1155. DOI: 10.1111/nph.12445

[99] Kryvoruchko IS, Sinharoy S, Torres-Jerez I, Sosso D, Pislariu CI, Guan D, et al. MtSWEET11, a nodule-specific sucrose transporter of *Medicago truncatula* root nodules. Plant Physiology. 2016;**171**:554-565. DOI: 10.1104/pp.15.01910

[100] Petrini O. Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS, editors. Microbial Ecology of Leaves. New York: Springer Verlag; 1991. pp. 179-197. DOI: 10.1007/978-1-4612-3168-4_9

[101] Bacon CW, White JFJ. Microbial Endophytes. New York: Marcel Dekker, Inc; 2000

[102] Impullitti AE, Malvick DK. Fungal endophyte diversity in soybean. Journal of Applied Microbiology. 2013;**114**:1500-1506. DOI: 10.1111/jam.12164

[103] Bilal S, Khan AL, Shahzad R, Asaf S, Kang S-M, Lee I-J. Endophytic *Paecilomyces formosus* LHL10 augments *Glycine max* L. adaptation to Ni-contamination through affecting endogenous phytohormones and

oxidative stress. Frontiers in Plant Science. 2017;**8**:870. DOI: 10.3389/fpls.2017.00870

[104] Khan AL, Lee I-J. Endophytic *Penicillium funiculosum* LHL06 secretes gibberellin that reprograms Glycine max L. growth during copper stress. BMC Plant Biology. 2013;**13**:86. DOI: 10.1186/1471-2229-13-86

[105] Bajaj R, Hu WM, Huang YY, Chen SY, Prasad R, Varma A, et al. The beneficial root endophyte *Piriformospora indica* reduces of the soybean cyst nematode. Biological Control. 2015;**90**:193-199. DOI: 10.1016/j.biocontrol.2015.05.021

[106] Lopes KBD, Carpentieri-Pipolo V, Oro TH, Pagliosa ES, Degrassi G. Culturable endophytic bacterial communities associated with field-grown soybean. Journal of Applied Microbiology. 2016;**120**:740-755. DOI: 10.1111/jam.13046

[107] Subramanian P, Kim K, Krishnamoorthy R, Sundaram S, Sa TM. Endophytic bacteria improve nodule function and plant nitrogen in soybean on co-inoculation with *Bradyrhizobium japonicum* MN110. Plant Growth Regulation. 2015;**76**:327-332. DOI: 10.1007/s10725-014-9993-x

[108] Egamberdieva D, Jabborova D, Berg G. Synergistic interactions between *Bradyrhizobium japonicum* and the endophyte *Stenotrophomonas rhizophila* and their effects on growth, and nodulation of soybean under salt stress. Plant and Soil. 2016;**405**:35-45. DOI: 10.1007/s11104-015-2661-8

[109] De Santana AS, Tiburcio Cavalcante UM, De Sa Barreto Sampaio EV, Maia LC. Production, storage and costs of inoculum of arbuscular mycorrhizal fungi (AMF). Brazilian Journal of Botany. 2014;**37**:159-165. DOI: 10.1007/s40415-014-0056-3

[110] Douds DD Jr, Nagahashi G, Reider C, Hepperly PR. Inoculation with arbuscular mycorrhizal fungi increases the yield of potatoes in a high P soil. Biological Agriculture & Horticulture. 2007;**25**:67-78. DOI: 10.1080/01448765.2007.10823209

[111] Lojan P, Senes-Guerrero C, Pablo Suarez J, Kromann P, Schuessler A, Declerck S. Potato field-inoculation in Ecuador with *Rhizophagus irregularis*: No impact on growth performance and associated arbuscular mycorrhizal fungal communities. Symbiosis. 2017;**73**:45-56. DOI: 10.1007/s13199-016-0471-2

[112] Mensah JA, Koch AM, Antunes PM, Hart MM, Kiers ET, Bücking H. High functional diversity within arbuscular mycorrhizal fungal species is associated with differences in phosphate and nitrogen uptake and fungal phosphate metabolism. Mycorrhiza. 2015;**25**:533-546. DOI: 10.1007/s00572-015-0631-x

[113] Berruti A, Lumini E, Balestrini R, Bianciotto V. Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. Frontiers in Microbiology. 2016;**6**:1559. DOI: 10.3389/fmicb.2015.01559

[114] Li S, Cong Y, Liu Y, Wang T, Shuai Q, Chen N, et al. Optimization of *Agrobacterium*-mediated transformation in soybean. Frontiers in Plant Science. 2017;**8**:246. DOI: 10.3389/fpls.2017.00246

[115] Sun X, Hu Z, Chen R, Jiang Q, Song G, Zhang H, et al. Targeted mutagenesis in soybean using the CRISPR-Cas9 system. Scientific Reports. 2015;**5**:10342. DOI: 10.1038/srep10342