Feature Review Open Access

Natural Nitrogen Boosters: The Symbiotic Relationship Between Legumes and Rhizobia

Jiayi Wu, Shudan Yan
Institute of Life Science, Jiyang College of Zhejiang A&F University, Zhuji, 311800, China
Corresponding email: shudan.yan@jicat.org
Molecular Soil Biology, 2024, Vol.15, No.2 doi: 10.5376/msb.2024.15.0009
Received: 20 Feb., 2024
Accepted: 28 Mar., 2024
Published: 13 Apr., 2024

Preferred citation for this article:
Wu J.Y., and Yan S.D., 2024, Natural nitrogen boosters: the symbiotic relationship between legumes and rhizobia, Molecular Soil Biology, 15(2): 74-86 (doi: 10.5376/msb.2024.15.0009)

Abstract Studies have shown that the effectiveness of nitrogen fixation varies due to factors such as environmental stress, compatibility between legumes and rhizobia, and the presence of other soil microorganisms. Innovative diagnostic techniques, such as leaf perforation tests, have been developed to rapidly screen the SNF activity of rhizobia inoculants, providing a cost-effective, high-throughput method for increasing the yield of legume crops. The symbiotic relationship between legumes and rhizobia is a cornerstone of sustainable agriculture, especially in arid and nutrient-deficient soils. The symbiotic relationship between legumes and rhizobia is an important natural process that increases nitrogen availability in the soil and promotes sustainable agricultural practices. Understanding and improving the efficiency of this symbiotic relationship can significantly improve crop productivity and soil health, reduce reliance on fertilizers, and mitigate environmental impacts. The purpose of this study was to investigate the symbiotic relationship between legumes and rhizobia, focusing on the natural nitrogen increasing capacity of this interaction, and to further understand the mechanism, effectiveness and potential agricultural benefits of symbiotic nitrogen fixation (SNF) in legumes.
Keywords Symbiotic nitrogen fixation; Legumes; Rhizobia; Sustainable agriculture; Soil fertility

1 Introduction

Nitrogen is a critical nutrient for plant growth, playing a fundamental role in the synthesis of amino acids, proteins, and nucleic acids. It is a major component of chlorophyll, which is essential for photosynthesis, and is involved in energy transfer within the plant. Despite its abundance in the atmosphere, nitrogen is often a limiting factor in plant growth because atmospheric nitrogen (N_2) is not directly accessible to most plants. Therefore, plants rely on various forms of nitrogen available in the soil, such as ammonium (NH_4^+) and nitrate (NO_3^-), to meet their nutritional needs (Santi et al., 2013).

Biological nitrogen fixation (BNF) is a natural process by which certain microorganisms convert atmospheric nitrogen into a form that plants can assimilate. This process is primarily carried out by diazotrophic bacteria, including rhizobia, which form symbiotic relationships with leguminous plants. In this symbiosis, rhizobia infect the roots of legumes, leading to the formation of specialized structures called nodules. Within these nodules, rhizobia convert atmospheric nitrogen into ammonium, which the plant can then use for growth and development (Masson-Boivin and Sachs, 2018; Lepetit and Brouquisse, 2023). This mutualistic relationship not only benefits the host plant by providing a readily available source of nitrogen but also contributes significantly to the nitrogen economy of ecosystems (Lodwig et al., 2003).

The legume-rhizobia symbiosis is a cornerstone of sustainable agriculture due to its ability to naturally enrich soil nitrogen levels, reducing the need for synthetic nitrogen fertilizers. This symbiotic relationship enhances soil fertility and promotes sustainable crop production by improving plant growth and yield without the environmental drawbacks associated with chemical fertilizers. Additionally, the symbiosis is highly adaptive, with the ability to respond to various biotic and abiotic factors, ensuring efficient nitrogen fixation under different environmental conditions (Thompson and Lamp, 2021). Understanding the molecular mechanisms and ecological dynamics of this symbiosis can lead to innovative agricultural practices that leverage natural processes for enhanced productivity and sustainability (Masson-Boivin and Sachs, 2018).

The aim of this study was to systematically investigate the symbiotic relationship between legumes and rhizobia, focusing on the mechanism of nitrogen fixation and its impact on sustainable agriculture. By examining the regulatory pathways, environmental interactions, and evolutionary aspects of this symbiotic relationship, this study seeks to provide a comprehensive understanding of how legumes and rhizobia collaborate to improve nitrogen availability, and hopes to highlight the critical role of natural nitrogen boosters in promoting agricultural sustainability and environmental health.

2 Historical Background and Discovery

2.1 Early observations and scientific discoveries

The symbiotic relationship between legumes and rhizobia has been a subject of scientific curiosity for centuries. Early agricultural practices recognized the beneficial effects of legumes on soil fertility long before the underlying biological mechanisms were understood. The positive impact of legumes on soil health and crop yields was noted in ancient agricultural texts, highlighting the empirical knowledge of farmers who observed improved plant growth following legume cultivation (Lindström and Mousavi, 2019).

The scientific exploration of this symbiosis began in earnest in the late 19th and early 20th centuries. Researchers discovered that legumes formed unique root structures called nodules, which were later identified as the sites of nitrogen fixation. This discovery was pivotal, as it linked the presence of these nodules to the enhanced nitrogen content in the soil, providing a biological explanation for the observed agricultural benefits (Lindström and Mousavi, 2019). The identification of rhizobia as the bacterial partners responsible for nitrogen fixation within these nodules marked a significant milestone in microbiology and plant sciences (Masson-Boivin et al., 2009).

2.2 Milestones in the study of legume-rhizobia interactions

The study of legume-rhizobia interactions has progressed through several key milestones, each contributing to researchers' current understanding of this complex symbiosis. One of the early breakthroughs was the recognition of the specificity between legume species and their compatible rhizobial strains. This specificity is crucial for the successful establishment of the symbiotic relationship and effective nitrogen fixation (Clúa et al., 2018).

Advancements in molecular biology and genetics have further elucidated the mechanisms underlying this specificity. The discovery of Nod factors, signaling molecules produced by rhizobia, was a major milestone. These molecules are recognized by specific receptors on the legume roots, initiating the formation of nodules and the symbiotic process (Figure 1) (Costa et al., 2021). The identification and characterization of these signaling pathways have provided insights into how legumes discriminate between beneficial rhizobia and other soil microbes, including pathogens (Clúa et al., 2018; Wang et al., 2018).

Recent metagenomic studies have expanded researchers' understanding of the soil microbiome and its influence on legume-rhizobia interactions. These studies have revealed the complexity of microbial communities in the soil and their impact on plant health and symbiosis efficiency (Clúa et al., 2018). Additionally, research has shown that environmental factors, such as soil nitrate levels and herbivory, can significantly alter the dynamics of nitrogen fixation and resource allocation within the plant (Thompson and Lamp, 2021).

The ongoing exploration of genetic and molecular mechanisms continues to uncover new aspects of this symbiosis. For instance, the horizontal transfer of symbiotic genes among rhizobial species has been identified as a factor contributing to the widespread distribution and evolutionary success of these bacteria (Masson-Boivin and Sachs, 2018). This horizontal gene transfer has allowed rhizobia to adapt to diverse legume hosts and environmental conditions, further enhancing their ecological and agricultural significance.

3 Biological Mechanism of Nitrogen Fixation

3.1 Structure and function of root nodules

Root nodules are specialized structures formed on the roots of leguminous plants as a result of symbiotic interactions with rhizobia. These nodules house the nitrogen-fixing bacteria, providing a microaerobic environment essential for the nitrogen fixation process. The formation of root nodules begins with the recognition and attachment of rhizobia to the root hairs of the host plant, followed by the infection process where rhizobia enter the root cells and induce nodule formation. Inside the nodules, rhizobia differentiate into bacteroids, which are capable of fixing atmospheric nitrogen into ammonia, a form that the plant can utilize for growth and development (Masson-Boivin and Sachs, 2018; Schwember et al., 2019).

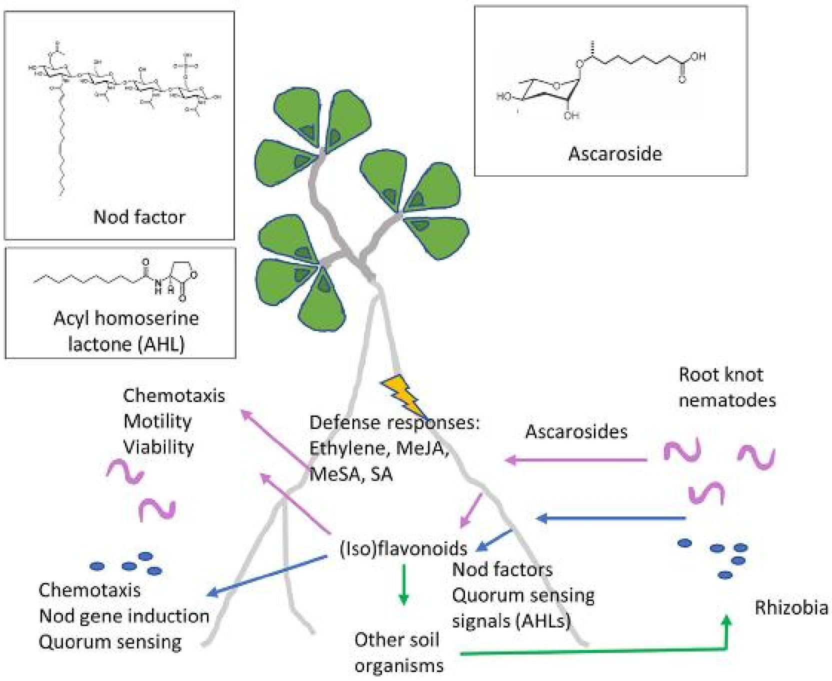

Figure 1 Examples of rhizosphere chemical signaling that could influence the interaction of roots with both rhizobia and nematodes (Adopted from Costa et al., 2021)

Image caption: Root-knot nematodes(RKN) produce ascarosides in the rhizosphere. Rhizobia produce Nod factors, quorum-sensing signals, and other signals perceived by the host. Both rhizobiaand nematodes can induce the production and exudation of (iso)flavonoids that can have functions in chemotaxis of rhizobia toward the root or attraction orrepulsion of nematodes to and from the root. They can also inhibit nematode motility, further enhance or repress Nod gene expression in rhizobia, and alterproduction of rhizobial quorum-sensing signaling. Ascarosides as well as Nod factors, quorum-sensing signals, and surface polysaccharides of rhizobia canalso modulate defense responses, which may have indirect effects on the tolerance of further infection with rhizobia or RKN. These can include induction ofethylene, methyl jasmonate (MeJA), methyl salicylate (MeSA), and salicylic acid (SA). Isoflavonoids can also influence other organisms in the rhizosphere thatcould indirectly interact with RKN or rhizobia or the host. For example, isoflavonoids can be inhibitors of fungal pathogens that may form secondary infectionsin roots infected by RKN (Adopted from Costa et al., 2021)

3.2 Biochemical pathways involved in nitrogen fixation

The nitrogen fixation process in legume nodules involves several biochemical pathways that facilitate the conversion of atmospheric nitrogen (N_2) into ammonia (NH_3). One of the key pathways is the carbonic anhydrase (CA)-phosphoenolpyruvate carboxylase (PEPC)-malate dehydrogenase (MDH) pathway, which plays a crucial role in regulating the nitrogen fixation process. This pathway helps in maintaining the balance of carbon and nitrogen metabolism within the nodules, ensuring efficient nitrogen fixation. Additionally, the uptake hydrogenase activity in certain bacteroid strains recycles hydrogen produced during nitrogen fixation, thereby enhancing the overall efficiency of the process by recouping ATP used for hydrogen production (Ciccolella et al., 2010; Schwember et al., 2019).

3.3 Role of nitrogenase enzyme complex

The nitrogenase enzyme complex is central to the nitrogen fixation process. This enzyme complex, found in the bacteroids within the root nodules, catalyzes the reduction of atmospheric nitrogen (N_2) to ammonia (NH_3). The nitrogenase complex is highly sensitive to oxygen, which necessitates the microaerobic conditions provided by the root nodules. The enzyme complex consists of two main components: the dinitrogenase reductase and the dinitrogenase. The dinitrogenase reductase transfers electrons to the dinitrogenase, which then reduces N_2 to NH_3 in an ATP-dependent reaction. The efficiency and regulation of the nitrogenase enzyme complex are critical for the effectiveness of the nitrogen fixation process, and understanding these mechanisms is key to improving the symbiotic relationship between legumes and rhizobia (Masson-Boivin and Sachs, 2018; Lindström and Mousavi, 2019).

By understanding these biological mechanisms, researchers can better appreciate the intricate symbiotic relationship between legumes and rhizobia, which has significant implications for sustainable agriculture and food production.

4 Molecular and Genetic Basis of Symbiosis

4.1 Genetic signals and molecular interactions between legumes and rhizobia

The symbiotic relationship between legumes and rhizobia is a complex process that involves a series of genetic signals and molecular interactions. Legumes release flavonoids into the soil, which are recognized by rhizobia, triggering the production of Nod factors. These Nod factors are essential signaling molecules that initiate the symbiotic process by inducing root hair curling and the formation of infection threads, which allow rhizobia to enter the plant root cells (Wang et al., 2018). The specificity of this interaction is governed by a variety of host and bacterial genes, ensuring compatibility between the symbiotic partners (Wang et al., 2018). Additionally, systemic signaling mechanisms within the plant adjust the symbiotic relationship based on the plant's nutritional status, ensuring that nodule formation and function are tightly regulated to meet the plant's nitrogen demands (Figure 2) (Lepetit and Brouquisse, 2023).

4.2 Nodulation genes and signaling pathways

Nodulation in legumes is controlled by a complex network of genes and signaling pathways. Nearly 200 genes have been identified as essential for symbiotic nitrogen fixation (SNF) in legumes, including those involved in the control of microbial infection, nodule development, and nodule function. Key genes such as *NIN* (Nodule Inception) and *NSP1*/*NSP2* (Nodulation Signaling Pathway) play crucial roles in the early stages of nodule formation by regulating the expression of other nodulation-related genes (Roy et al., 2020). The Nod factor-independent symbiotic signaling pathway, observed in some legume species like *Aeschynomene indica*, represents an alternative mechanism where nodulation occurs without the typical Nod factors, highlighting the diversity of nodulation strategies among legumes (Bonaldi et al., 2010).

4.3 Recent advancements in genetic engineering to enhance symbiosis

Recent advancements in genetic engineering have opened new avenues for enhancing the symbiotic relationship between legumes and rhizobia. Techniques such as CRISPR/Cas9 and other genome editing tools have been employed to modify key genes involved in nodulation and nitrogen fixation, aiming to improve the efficiency and effectiveness of these processes (Roy et al., 2020). For instance, the development of Agrobacterium rhizogenes-mediated transformation protocols for non-model legumes like *Aeschynomene indica* has facilitated the study and manipulation of symbiotic genes in these species (Bonaldi et al., 2010). Additionally, the horizontal transfer of symbiosis islands, which contain essential symbiotic genes, has been shown to rapidly evolve new, competitive strains of rhizobia, although these strains may sometimes be suboptimal in nitrogen fixation (Nandasena et al., 2007). These advancements hold promise for developing legume varieties with enhanced symbiotic capabilities, potentially leading to increased agricultural productivity and sustainability.

5 Diversity of Rhizobia and Host Specificity

5.1 Different genera and species of rhizobia

Rhizobia, the symbiotic bacteria responsible for nitrogen fixation in legumes, belong to several genera within the Alphaproteobacteria and Betaproteobacteria classes. Key genera include *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Ensifer* (formerly *Sinorhizobium*), *Azorhizobium*, and *Burkholderia* (Gage, 2004; Remigi et al., 2016). Each genus encompasses multiple species, which exhibit varying degrees of host specificity and symbiotic efficiency. For instance, *Bradyrhizobium* species are the exclusive symbionts for legumes in the Caesalpinioideae sub-family, while *Mesorhizobium* and *Burkholderia* are prevalent in the Fynbos region of South Africa (Lemaire et al., 2015; Andrews and Andrews, 2016).

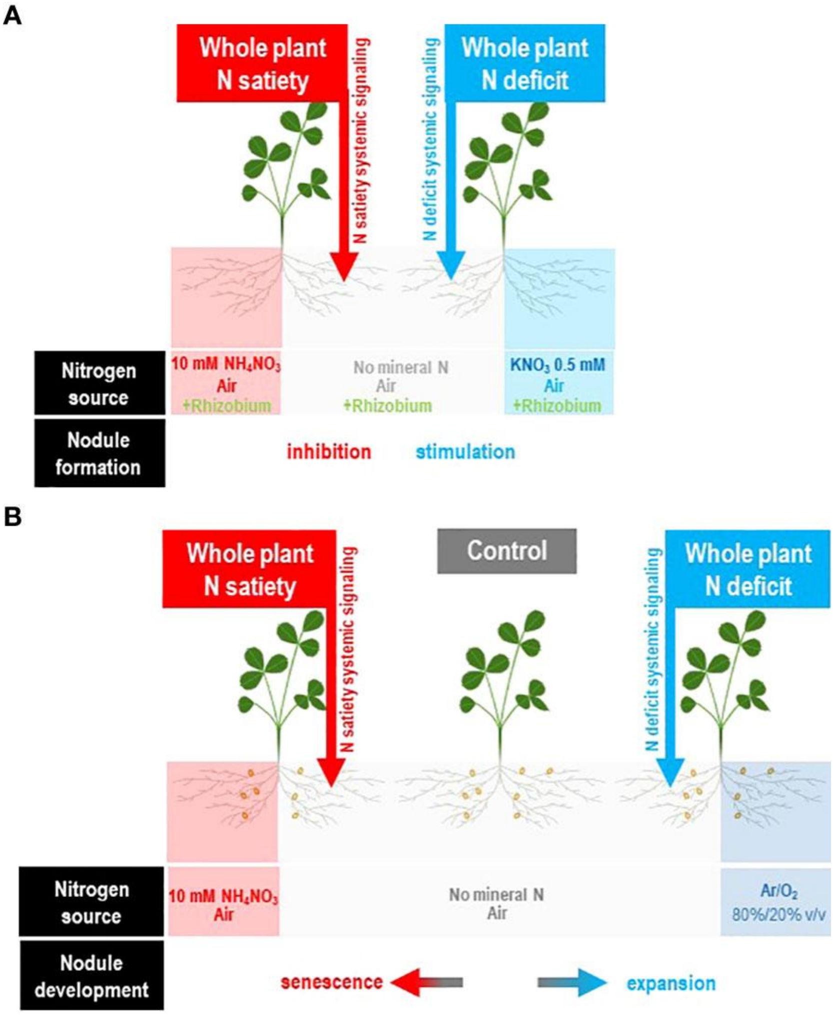

Figure 2 Split-root systems to characterize the control of nitrogen-fixing symbiosis by systemic N signaling of the plant N demand (Adopted from Lepetit and Brouquisse, 2023)

Image caption: (A) Split root systems used to study the control of nodule formation by plant N demand. Non symbiotic plants are used. Contrasted mineral N supply applied to half-root systems may either fully satisfy the plant N demand (N satiety) or results in plant N limitation (N deficit). The response to inoculation by rhizobium in untreated roots strongly relies on the level of N supply of the plant revealing the strong systemic control of nodule formation by systemic signaling of the plant N demand. (B) Split root systems used to study the control of mature nodules by the plant N demand. Symbiotic plants supplied with air as a unique N source through the function of mature N_2 fixing nodules are used. N treatment applied to a half-root system may either fully satisfy the plant N demand (N satiety) or result in plant N limitation (N deficit). Plant N satiety resulted in nodule senescence whereas plant N limitation stimulates the expansion of mature nodules. The development of the nodules in untreated roots is strongly dependent on the level of supply of the whole plant, revealing the control by the systemic signaling of the whole plant N demand (Adopted from Lepetit and Brouquisse, 2023)

5.2 Host range and specificity factors

The host range and specificity of rhizobia are influenced by both genetic and environmental factors. Some legumes exhibit promiscuity, nodulating with a wide range of rhizobial species, while others are highly specific. For example, Mimosa species in Brazil show specificity towards *Burkholderia*, whereas in Mexico, they prefer *Rhizobium*/*Ensifer*. Similarly, *Cicer arietinum* (chickpea) specifically nodulates with *Mesorhizobium* species (Andrews and Andrews, 2016). Environmental factors such as soil acidity and site elevation also play a role in determining rhizobial diversity and host specificity (Lemaire et al., 2015). Additionally, the lateral transfer of symbiosis genes within rhizobial genera allows for adaptation to specific soil conditions, further influencing host specificity (Nandasena et al., 2007; Remigi et al., 2016).

5.3 Co-evolution of legumes and rhizobia

The co-evolution of legumes and rhizobia is a dynamic process driven by genetic exchanges and environmental pressures. The evolution of nitrogen-fixing symbiosis involves the horizontal transfer of key symbiotic genes, enabling soil bacteria to become effective legume symbionts (Nakagawa et al., 2011; Remigi et al., 2016). This genetic exchange is facilitated by the presence of symbiosis islands, which can be transferred between different rhizobial strains, leading to the rapid evolution of new, competitive strains (Nandasena et al., 2007). The interaction between legumes and rhizobia also involves the recognition of rhizobial Nod factors by plant receptors, which has evolved from plant defense mechanisms against pathogens (Nakagawa et al., 2011). This intricate co-evolutionary relationship has allowed rhizobia to achieve ecological success and has significantly impacted global nitrogen cycles (Masson-Boivin and Sachs, 2018).

The diversity and specificity of rhizobia are shaped by a combination of genetic, environmental, and evolutionary factors. Understanding these interactions is crucial for optimizing legume-rhizobia symbioses for agricultural and ecological benefits.

6 Environmental Factors Influencing Symbiosis

6.1 Soil pH, temperature, and nutrient availability

The symbiotic relationship between legumes and rhizobia is significantly influenced by soil pH, temperature, and nutrient availability. Soil pH can affect the survival and efficiency of rhizobia, with extreme pH levels (either too acidic or too alkaline) being detrimental to the symbiotic process. Temperature also plays a crucial role, as both high and low temperatures can inhibit the growth and nitrogen-fixing ability of rhizobia. Nutrient availability, particularly nitrogen, is another critical factor. Excessive nitrogen in the soil can reduce the plant's reliance on symbiotic nitrogen fixation, thereby diminishing the benefits of the legume-rhizobia relationship (Ficano et al., 2021; Lepetit and Brouquisse, 2023).

6.2 Impact of abiotic stress on nitrogen fixation

Abiotic stresses such as drought, salinity, and heavy metal contamination can severely impact the efficiency of nitrogen fixation in legume-rhizobia symbiosis. Drought stress, for instance, can reduce the water availability necessary for the metabolic activities of both the plant and the rhizobia, leading to decreased nitrogen fixation. Salinity stress can disrupt the osmotic balance and ion homeostasis, adversely affecting the symbiotic relationship. Heavy metals in the soil can be toxic to rhizobia, inhibiting their growth and nitrogen-fixing capabilities. Despite these challenges, some rhizobial strains have shown tolerance to such stresses, maintaining their symbiotic functions under adverse conditions (Nandasena et al., 2007; Lindström and Mousavi, 2019).

6.3 Strategies to mitigate environmental challenges

To mitigate the environmental challenges affecting legume-rhizobia symbiosis, several strategies can be employed. One approach is the selection and use of stress-tolerant rhizobial strains that can withstand adverse conditions such as drought, salinity, and heavy metal contamination. Another strategy involves the application of biofertilizers that enhance the symbiotic efficiency and reduce the need for synthetic nitrogen fertilizers, thereby promoting sustainable agriculture. Additionally, improving soil management practices, such as maintaining optimal pH levels and ensuring adequate but not excessive nutrient supply, can support a healthy symbiotic relationship. Research into the molecular mechanisms underlying stress tolerance in rhizobia and their interaction with legumes can also provide insights for developing more resilient symbiotic systems (Goyal et al., 2021; Abd-Alla et al., 2023; Lepetit and Brouquisse, 2023).

7 Agricultural Practices to Enhance Symbiosis

7.1 Inoculation techniques and commercial rhizobial inoculants

Inoculation techniques and the use of commercial rhizobial inoculants are critical for enhancing the symbiotic relationship between legumes and rhizobia. Effective inoculation ensures that legumes are colonized by efficient nitrogen-fixing rhizobia, which can significantly improve nitrogen fixation and plant growth. Studies have shown that combined inoculation with arbuscular mycorrhizal fungi (AMF) and rhizobia can enhance nodulation and nitrogen fixation efficiency in legumes such as common beans (*Phaseolus vulgaris* L.). Additionally, the use of commercial inoculants like *Rhizobium tropici* CIAT899 has been demonstrated to improve phosphorus use efficiency, which is crucial for optimal nitrogen fixation (Tajini et al., 2012). However, the effectiveness of these inoculants can be influenced by various factors, including soil conditions and the presence of other microbial communities (Nandasena et al., 2007).

7.2 Crop rotation and intercropping with legumes

Crop rotation and intercropping with legumes are sustainable agricultural practices that can enhance nitrogen fixation and improve soil health. Intercropping legumes with cereals, for example, has been shown to increase the proportion of nitrogen derived from biological nitrogen fixation (BNF) and improve the overall nitrogen use efficiency in agroecosystems (Rodriguez et al., 2020). A meta-analysis of field-scale studies revealed that intercropping grain legumes with cereals not only enhances nitrogen fixation but also increases soil nitrogen acquisition by cereals, thereby reducing the need for external nitrogen inputs. This practice can lead to more resilient cropping systems and contribute to the sustainability of agriculture by diversifying cropping systems and reducing greenhouse gas emissions (Rodriguez et al., 2020).

7.3 Management practices to optimize nitrogen fixation

Optimizing nitrogen fixation in legumes involves a combination of management practices that address both biotic and abiotic factors. One key aspect is the regulation of soil nitrogen levels, as excessive nitrate can inhibit nitrogen fixation by rhizobia. Research has shown that moderate soil nitrate levels, irrespective of herbivory, result in minimal fixed nitrogen allocation, whereas non-supplemented soils can enhance aboveground nitrogen allocation following herbivore damage (Thompson and Lamp, 2021). Additionally, systemic signaling mechanisms within the plant play a crucial role in adjusting nodule formation and function based on the plant's nitrogen demand (Lepetit and Brouquisse, 2023). Understanding these regulatory circuits can help in developing strategies to optimize nitrogen fixation under varying environmental conditions. Furthermore, the compatibility between legumes and rhizobia is essential for successful symbiosis. Plants must recognize and select efficient rhizobial partners from a diverse soil microbiome, which can be influenced by metagenomic studies and advances in understanding plant-microbe interactions (Clúa et al., 2018).

By integrating these agricultural practices, farmers can enhance the symbiotic relationship between legumes and rhizobia, leading to improved nitrogen fixation, better soil health, and more sustainable agricultural systems.

8 Case Studies and Practical Applications

8.1 Successful examples of legume-rhizobia symbiosis in different agricultural systems

The symbiotic relationship between legumes and rhizobia has been extensively studied and applied in various agricultural systems, demonstrating significant benefits in terms of nitrogen fixation and plant growth. For instance, *Medicago sativa* (alfalfa) has shown increased aboveground allocation of biologically fixed nitrogen in response to herbivory, particularly in soils without additional nitrate supplementation. This indicates that herbivory can enhance the function of legume-rhizobia symbioses, leading to better nitrogen fixation and plant growth in certain conditions (Thompson and Lamp, 2021).

Another successful example is the symbiosis between lima bean (*Phaseolus lunatus*) and rhizobia, which not only promotes plant growth but also enhances plant defense against herbivores. This dual benefit underscores the importance of rhizobia in improving both the productivity and resilience of leguminous plants in agricultural systems (Thamer et al., 2011).

In Western Australia, the introduction and inoculation of *Biserrula pelecinus* with *Mesorhizobium ciceri* bv. *biserrulae* have led to the rapid evolution of diverse competitive strains of mesorhizobia. Despite some strains being suboptimal in nitrogen fixation, the overall success of this symbiosis highlights the adaptability and potential of legume-rhizobia interactions in different environmental contexts (Nandasena et al., 2007).

8.2 Economic and ecological benefits in specific regions

The economic and ecological benefits of legume-rhizobia symbiosis are particularly evident in regions where sustainable agriculture practices are prioritized. For example, the use of legume crops that engage in symbiotic nitrogen fixation can significantly reduce the need for chemical nitrogen fertilizers, which are associated with greenhouse gas emissions and nitrogen pollution. This makes legume production systems more efficient and environmentally friendly, contributing to climate preservation and sustainable agriculture (Goyal et al., 2021).

In temperate grasslands, the biological nitrogen fixation by legume-rhizobia symbiosis is a crucial source of soil nitrogen. Studies on *Trifolium fragiferum*, a crop wild relative legume species, have shown that inoculation with native rhizobia can prevent nitrogen deficiency and enhance plant growth. This not only supports plant health but also improves soil fertility, which is vital for the sustainability of agricultural ecosystems in these regions (Figure 3) (Jēkabsone et al., 2022).

Furthermore, the compatibility between legumes and rhizobia plays a significant role in the success of nitrogen-fixing symbiosis. Understanding the molecular mechanisms by which legumes recognize and select their symbiotic partners can lead to biotechnological advances that improve the efficiency of this symbiosis in agronomic systems. This has the potential to enhance crop yields and reduce dependency on synthetic fertilizers, providing both economic and ecological benefits (Clúa et al., 2018).

8.3 Case study: enhancing soybean production in tropical soils

Soybean (*Glycine max*) is a crucial crop for many tropical regions, where it serves as a significant source of protein and oil. However, the productivity of soybean in these areas is often limited by poor soil fertility, particularly nitrogen deficiency. The symbiotic relationship between soybean and rhizobia, nitrogen-fixing bacteria, offers a sustainable solution to this problem by enhancing nitrogen availability in the soil.

In tropical soils, environmental factors such as high temperatures, drought, and soil acidity further complicate the establishment and effectiveness of rhizobial inoculants. Research in Brazil has shown that these conditions can severely limit rhizobial growth and survival, thereby reducing nodulation and nitrogen fixation. Selecting stress-tolerant rhizobial strains and implementing appropriate soil management practices, such as liming to reduce soil acidity, have been effective strategies to overcome these challenges and improve soybean yields (Hungriaa and Vargasb, 2000).

The genetic diversity and ecological adaptability of rhizobia also play a crucial role in their symbiotic efficiency. Studies have highlighted the importance of understanding the genetic and environmental factors that influence the localization and dominance of soybean-nodulating rhizobia. For instance, different rhizobial species and strains exhibit varying levels of nitrogen fixation efficiency and plant growth-promoting functions, which can be leveraged to optimize soybean production in specific tropical environments (Rodríguez-Navarro et al., 2011; Nakei et al., 2022).

Moreover, the interaction between rhizobia and other soil microbes can significantly influence the symbiotic efficiency. Research has shown that certain soil bacteria, such as Bacillus species, can either promote or inhibit the growth of rhizobia, thereby affecting nodulation and nitrogen fixation. For example, *Bacillus cereus* has been found to promote the growth of *Sinorhizobium* while suppressing *Bradyrhizobium*, highlighting the need for a holistic approach to managing soil microbial communities to enhance soybean production (Han et al., 2020).

9 Challenges and Future Directions

9.1 Limitations and challenges in harnessing legume-rhizobia symbiosis

One of the primary challenges in harnessing the legume-rhizobia symbiosis is the "rhizobial competition problem," where applied rhizobial inoculants often fail to compete effectively with native soil rhizobia, leading to suboptimal nitrogen fixation and reduced crop yields (Mendoza-Suárez et al., 2021; Quides and Atamian, 2021). This competition is exacerbated by the genetic diversity and adaptability of native rhizobia, which can outcompete inoculant strains for nodule occupancy. Additionally, the persistence of inoculant strains in the field is often limited due to the transfer of symbiotic genes to native populations, further reducing their effectiveness over time (Mendoza-Suárez et al., 2021).

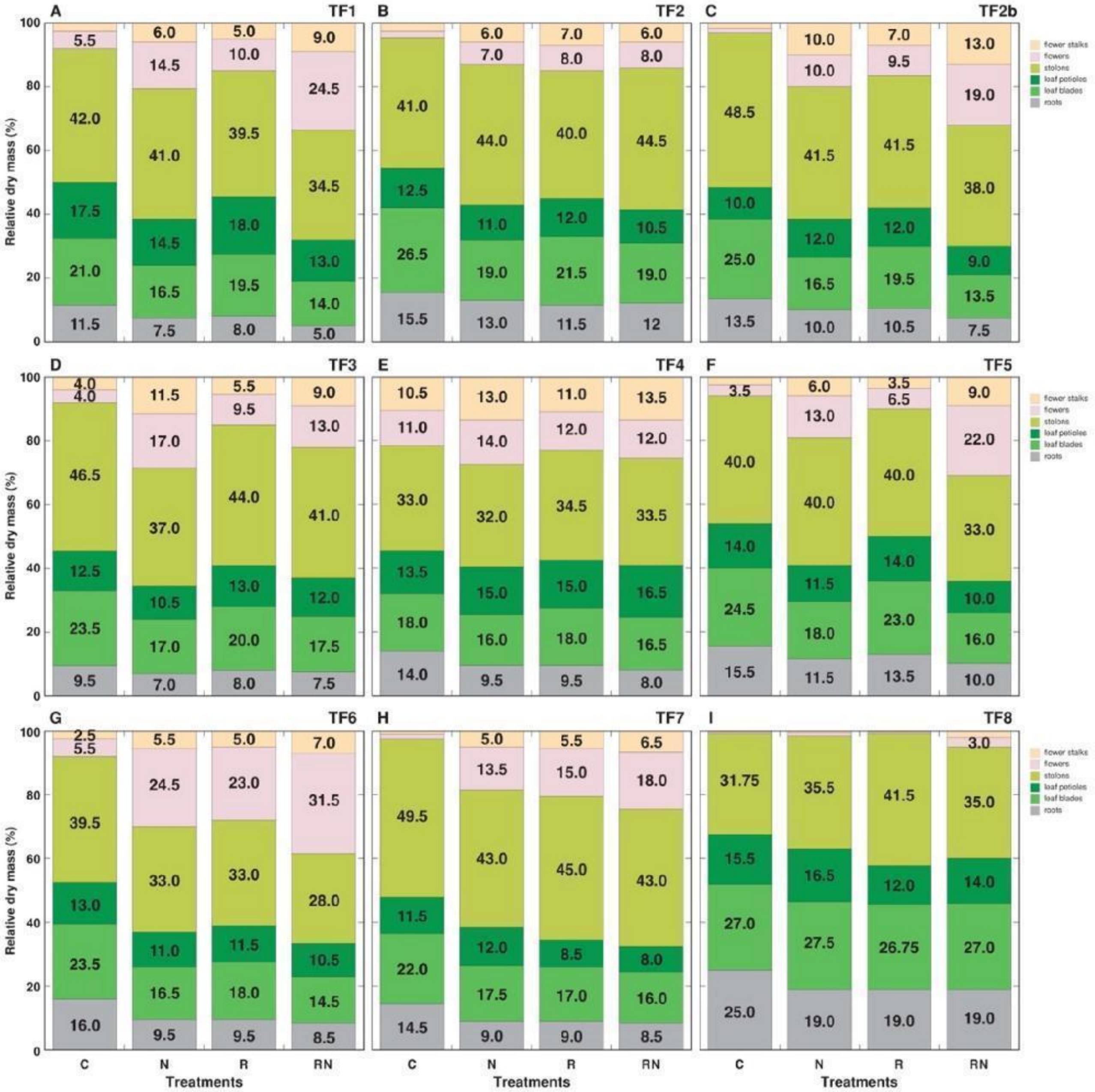

Figure 3 Biomass partitioning in *Trifolium fragiferum* plants of different accessions in control (C), N-fertilized (N), rhizobia-inoculated (R) and rhizobia inoculated + N-fertilized (RN) treatments (Adopted from Jēkabsone et al., 2022)

Image caption: Besides the increase in shoot biomass, another characteristic response of *T. fragiferum* plants to N fertilizer and rhizobial inoculation were changes in biomass partitioning. Accordingly, there was an increase in the proportion of biomass in flower stalks and inflorescences by the treatments, which was less pronounced for TF4, which already has high biomass of generative organs in control conditions, and for TF8 with the smallest biomass in generative organs (Adopted from Jēkabsone et al., 2022)

Another significant challenge is the specificity of the symbiotic relationship, where incompatibility between certain rhizobial strains and legume hosts can result in ineffective nodulation or nodules that do not fix nitrogen efficiently. This specificity is controlled by complex genetic and molecular mechanisms, making it difficult to engineer broad-spectrum solutions that work across different legume species and environmental conditions (Wang et al., 2012; Wang et al., 2018). Moreover, the integration of plant and bacterial metabolism required for effective nitrogen fixation is highly intricate, involving numerous transporters and metabolic pathways that must be finely tuned (Udvardi and Poole, 2013). Any disruption in these processes can lead to reduced efficiency of nitrogen fixation, posing a challenge for consistent agricultural application.

9.2 Future research directions

Future research should focus on developing strategies to overcome the rhizobial competition problem. One promising approach is microbiome engineering, which aims to create legume microbiomes that do not rely on exogenous rhizobia, thereby circumventing competition with native strains (Quides and Atamian, 2021). Additionally, selecting and breeding native rhizobial strains with high nitrogen fixation abilities and genetic adaptations to local environments could enhance the effectiveness of inoculants (Mendoza-Suárez et al., 2021).

Another important direction is the detailed study of the genetic and molecular mechanisms underlying symbiotic specificity. Understanding these mechanisms can lead to the development of genetically engineered rhizobial strains or legume cultivars that are compatible with a broader range of partners, thereby improving the efficiency of nitrogen fixation across different agricultural settings (Wang et al., 2012; Wang et al., 2018).

Research should also explore the metabolic integration between legumes and rhizobia, identifying key transporters and metabolic pathways that can be targeted to enhance nitrogen fixation (Udvardi and Poole, 2013). Advanced genomic and systems biology approaches can provide insights into these complex interactions, enabling the development of more effective symbiotic systems (diCenzo et al., 2018).

9.3 Potential of synthetic biology in improving nitrogen fixation

Synthetic biology offers exciting opportunities to enhance nitrogen fixation in legume-rhizobia symbiosis. By assembling synthetic plasmids containing key symbiotic loci, researchers can potentially convert non-symbiotic soil bacteria into effective nitrogen-fixing symbionts. This approach can be used to engineer rhizobial strains with improved infection capabilities and nitrogen fixation efficiency, tailored to specific legume hosts (Unay and Perret, 2019).

Moreover, synthetic biology can facilitate the design of novel symbiotic interactions, either by improving existing symbioses or by creating entirely new ones. For instance, synthetic biology tools can be used to manipulate the signaling pathways and molecular signals exchanged between legumes and rhizobia, enhancing the establishment and efficiency of the symbiotic relationship (diCenzo et al., 2018; Unay and Perret, 2019).

In conclusion, while there are significant challenges in harnessing the legume-rhizobia symbiosis for agricultural benefit, future research and synthetic biology hold great promise for overcoming these obstacles and improving nitrogen fixation, thereby contributing to sustainable agriculture.

10 Concluding Remarks

The symbiotic relationship between legumes and rhizobia is a cornerstone of sustainable agriculture due to its ability to naturally fix atmospheric nitrogen, thereby reducing the need for synthetic fertilizers. This relationship is influenced by various biotic and abiotic factors, including soil nitrate levels and herbivory, which can alter the allocation of biologically fixed nitrogen in plants. The effectiveness of nitrogen fixation varies among different legume species and rhizobial strains, with some strains being more efficient than others. Additionally, the symbiosis not only promotes plant growth but also enhances plant defense mechanisms against herbivores. Despite the complexity of this interaction, significant progress has been made in understanding the molecular mechanisms and ecological dynamics that underpin this symbiosis.

The legume-rhizobia symbiosis plays a crucial role in sustainable agriculture by providing a natural source of nitrogen, which is essential for plant growth. This symbiotic relationship reduces the reliance on chemical nitrogen fertilizers, which are associated with greenhouse gas emissions and environmental pollution. The ability of legumes to fix nitrogen through their association with rhizobia makes them self-sufficient in nitrogen, contrasting sharply with cereal crops that require external nitrogen inputs. Furthermore, the symbiosis enhances soil fertility and promotes plant health, making it a valuable asset for sustainable farming practices. The potential for engineering this symbiotic capacity in non-legume plants could further revolutionize agricultural sustainability.

The future potential of natural nitrogen boosters, such as the legume-rhizobia symbiosis, is immense. Advances in understanding the genetic and molecular basis of this symbiosis could lead to the development of more efficient and resilient legume varieties. Additionally, exploring the use of non-rhizobial bacteria for nitrogen fixation in non-legume plants presents a promising avenue for enhancing soil fertility and plant growth in a broader range of crops. As we continue to face global challenges related to food security and environmental sustainability, harnessing the power of natural nitrogen boosters will be critical. Future research should focus on optimizing these symbiotic relationships and exploring their applications in diverse agricultural systems to maximize their benefits for sustainable agriculture.

Acknowledgments

The BioSci Publisher appreciate two anonymous peer reviewers for their comments on the manuscript of this study, which helps us to further improve the research content and structure.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Abd-Alla M., Al-Amri S., and El-Enany A., 2023, Enhancing *Rhizobium*- legume symbiosis and reducing nitrogen fertilizer use are potential options for mitigating climate change, Agriculture, 13(11): 2092.
https://doi.org/10.3390/agriculture13112092

Andrews M., and Andrews M., 2016, Specificity in legume-rhizobia symbioses, International Journal of Molecular Sciences, 18(4): 705.
https://doi.org/10.3390/ijms18040705

Bonaldi K., Gherbi H., Franche C., Bastien G., Fardoux J., Barker D., Giraud E., and Cartieaux F., 2010, The Nod factor-independent symbiotic signaling pathway: development of *Agrobacterium rhizogenes*-mediated transformation for the legume *Aeschynomene indica*, Molecular Plant-Microbe Interactions, 23(12): 1537-1544.
https://doi.org/10.1094/MPMI-06-10-0137

Ciccolella C., Raynard N., Mei J., Church D., and Ludwig R., 2010, Symbiotic legume nodules employ both rhizobial *Exo*- and *Endo*-hydrogenases to recycle hydrogen produced by nitrogen fixation, PLoS One, 5(8): e12094.
https://doi.org/10.1371/journal.pone.0012094

Clúa J., Roda C., Zanetti M., and Blanco F., 2018, Compatibility between legumes and rhizobia for the establishment of a successful nitrogen-fixing symbiosis, Genes, 9(3): 125.
https://doi.org/10.3390/genes9030125

Costa S., Ng J., and Mathesius U., 2021, Interaction of symbiotic rhizobia and parasitic root-knot nematodes in legume roots - from molecular regulation to field application, Molecular Plant-Microbe Interactions, 34(5): 470-490.
https://doi.org/10.1094/MPMI-12-20-0350-FI

diCenzo G., Zamani M., Checcucci A., Fondi M., Griffitts J., Finan T., and Mengoni A., 2018, Multidisciplinary approaches for studying rhizobium-legume symbioses, Canadian Journal of Microbiology, 65(1): 1-33.
https://doi.org/10.1139/cjm-2018-0377

Ficano N., Porder S., and McCulloch L., 2021, Tripartite legume-rhizobia-mycorrhizae relationship is influenced by light and soil nitrogen in Neotropical canopy gaps, Ecology, 102(11): e03489.
https://doi.org/10.1002/ecy.3489

Gage D., 2004, Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes, Microbiology and Molecular Biology Reviews, 68(2): 280-300.
https://doi.org/10.1128/MMBR.68.2.280-300.2004

Goyal R., Mattoo A., and Schmidt M., 2021, Rhizobial- host interactions and symbiotic nitrogen fixation in legume crops toward agriculture sustainability, Frontiers in Microbiology, 12: 669404.
https://doi.org/10.3389/fmicb.2021.669404

Han Q., Ma Q., Chen Y., Tian B., Xu L., Bai Y., Chen W., and Li X., 2020, Variation in rhizosphere microbial communities and its association with the symbiotic efficiency of rhizobia in soybean, The ISME Journal, 14: 1915-1928.
https://doi.org/10.1038/s41396-020-0648-9

Hungriaa M., and Vargasb M., 2000, Environmental factors affecting N_2 fixation in grain legumes in the tropics, with an emphasis on Brazil, Field Crops Research, 65(2-3): 151-164.
https://doi.org/10.1016/S0378-4290(99)00084-2

Jēkabsone A., Andersone-Ozola U., Karlsons A., Neiceniece L., Romanovs M., and Ievinsh G., 2022, Dependence on nitrogen availability and rhizobial symbiosis of different accessions of *Trifolium fragiferum*, a crop wild relative legume species, as related to physiological traits, Plants, 11(9): 1141.
https://doi.org/10.3390/plants11091141

Lemaire B., Dlodlo O., Chimphango S., Stirton C., Schrire B., Boatwright J., Honnay O., Smets E., Sprent J., James E., and Muasya A., 2015, Symbiotic diversity, specificity and distribution of rhizobia in native legumes of the Core Cape Subregion (South Africa), FEMS Microbiology Ecology, 91(2): 1-17.
https://doi.org/10.1093/femsec/fiu024

Lepetit M., and Brouquisse R., 2023, Control of the rhizobium- legume symbiosis by the plant nitrogen demand is tightly integrated at the whole plant level and requires inter-organ systemic signaling, Frontiers in Plant Science, 14: 1114840.
https://doi.org/10.3389/fpls.2023.1114840

Lindström K., and Mousavi S., 2019, Effectiveness of nitrogen fixation in rhizobia, Microbial Biotechnology, 13: 1314-1335.
https://doi.org/10.1111/1751-7915.13517

Lodwig E., Hosie A., Bourdès A., Findlay K., Allaway D., Karunakaran R., Downie J., and Poole P., 2003, Amino-acid cycling drives nitrogen fixation in the legume- *Rhizobium* symbiosis, Nature, 422: 722-726.
https://doi.org/10.1038/nature01527

Masson-Boivin C., and Sachs J., 2018, Symbiotic nitrogen fixation by rhizobia-the roots of a success story, Current Opinion in Plant Biology, 44: 7-15.
https://doi.org/10.1016/j.pbi.2017.12.001

Masson-Boivin C., Giraud E., Perret X., and Batut J., 2009, Establishing nitrogen-fixing symbiosis with legumes: how many rhizobium recipes? Trends in Microbiology, 17(10): 458-466.
https://doi.org/10.1016/j.tim.2009.07.004

Mendoza-Suárez M., Andersen S., Poole P., and Sánchez-Cañizares C., 2021, Competition, nodule occupancy, and persistence of inoculant strains: key factors in the *Rhizobium*- legume symbioses, Frontiers in Plant Science, 12: 690567.
https://doi.org/10.3389/fpls.2021.690567

Nakagawa T., Kaku H., Shimoda Y., Sugiyama A., Shimamura M., Takanashi K., Yazaki K., Aoki T., Shibuya N., and Kouchi H., 2011, From defense to symbiosis: limited alterations in the kinase domain of LysM receptor-like kinases are crucial for evolution of legume- *Rhizobium* symbiosis, The Plant Journal, 65(2): 169-180.
https://doi.org/10.1111/j.1365-313X.2010.04411.x

Nakei M., Venkataramana P., and Ndakidemi P., 2022, Soybean- nodulating rhizobia: ecology, characterization, diversity, and growth promoting functions, 6: 824444.
https://doi.org/10.3389/fsufs.2022.824444

Nandasena K., O'Hara G., Tiwari R., Sezmiş E., and Howieson J., 2007, In situ lateral transfer of symbiosis islands results in rapid evolution of diverse competitive strains of mesorhizobia suboptimal in symbiotic nitrogen fixation on the pasture legume *Biserrula pelecinus* L., Environmental Microbiology, 9(10): 2496-2511.
https://doi.org/10.1111/J.1462-2920.2007.01368.X

Quides K., and Atamian H., 2021, A microbiome engineering framework to evaluate rhizobial symbionts of legumes, Plant and Soil, 463: 631-642.
https://doi.org/10.1007/S11104-021-04892-2

Remigi P., Remigi P., Remigi P., Zhu J., Zhu J., Young J., Masson-Boivin C., and Masson-Boivin C., 2016, Symbiosis within symbiosis: evolving nitrogen-fixing legume symbionts, Trends in Microbiology, 24(1): 63-75.
https://doi.org/10.1016/j.tim.2015.10.007

Rodriguez C., Carlsson G., Englund J., Flöhr A., Pelzer E., Jeuffroy M., Makowski D., and Jensen E., 2020, Grain legume-cereal intercropping enhances the use of soil-derived and biologically fixed nitrogen in temperate agroecosystems, a meta-analysis, European Journal of Agronomy, 118: 126077.
https://doi.org/10.1016/j.eja.2020.126077

Rodríguez-Navarro D., Oliver M., Contreras A., and Ruiz-Sainz J., 2011, Soybean interactions with soil microbes, agronomical and molecular aspects, Agronomy for Sustainable Development, 31: 173-190.
https://doi.org/10.1051/agro/2010023

Roy S., Liu W., Nandety R., Crook A., Mysore K., Pislariu C., Frugoli J., Dickstein R., and Udvardi M., 2020, Celebrating 20 years of genetic discoveries in legume nodulation and symbiotic nitrogen fixation, The Plant Cell, 32(1): 15-41.
https://doi.org/10.1105/tpc.19.00279

Santi C., Bogusz D., and Franche C., 2013, Biological nitrogen fixation in non-legume plants, Annals of Botany, 111(5): 743-767.
https://doi.org/10.1093/aob/mct048

Schwember A., Schulze J., Pozo A., and Cabeza R., 2019, Regulation of symbiotic nitrogen fixation in legume root nodules, Plants, 8(9): 333.
https://doi.org/10.3390/plants8090333

Tajini F., Trabelsi M., and Drevon J., 2012, Combined inoculation with *Glomus intraradices* and *Rhizobium tropici* CIAT899 increases phosphorus use efficiency for symbiotic nitrogen fixation in common bean (*Phaseolus vulgaris* L.), Saudi Journal of Biological Sciences, 19(2): 157-163.
https://doi.org/10.1016/j.sjbs.2011.11.003

Thamer S., Schädler M., Bonte D., and Ballhorn D., 2011, Dual benefit from a belowground symbiosis: nitrogen fixing rhizobia promote growth and defense against a specialist herbivore in a cyanogenic plant, Plant and Soil, 341: 209-219.
https://doi.org/10.1007/s11104-010-0635-4

Thompson M., and Lamp W., 2021, Herbivory enhances legume-rhizobia symbioses function, increasing aboveground allocation of biologically fixed nitrogen, but only in soils without additional nitrate, Plant and Soil, 465: 301-316.
https://doi.org/10.1007/s11104-021-04999-6

Udvardi M., and Poole P., 2013, Transport and metabolism in legume-rhizobia symbioses, Annual Review of Plant Biology, 64: 781-805.
https://doi.org/10.1146/annurev-arplant-050312-120235

Unay J., and Perret X., 2019, Synthetic plasmids to challenge symbiotic nitrogen fixation between rhizobia and legumes, In: Reinhardt D., and Sharma A. (eds.), Methods in rhizosphere biology research, Springer, Singapore, pp.3-18.
https://doi.org/10.1007/978-981-13-5767-1_1

Wang D., Yang S., Tang F., and Zhu H., 2012, Symbiosis specificity in the legume - rhizobial mutualism, Cellular Microbiology, 14(3): 334-342.
https://doi.org/10.1111/j.1462-5822.2011.01736.x

Wang Q., Liu J., and Zhu H., 2018, Genetic and molecular mechanisms underlying symbiotic specificity in legume- rhizobium interactions, Frontiers in Plant Science, 9: 313.
https://doi.org/10.3389/fpls.2018.00313