Molecular Soil Biology 2024, Vol.15, No.3, 129-139
http://bioscipublisher.com/index.php/msb

Feature Review **Open Access**

Boosting Soil Health: The Role of Rhizobium in Legume Nitrogen Fixation

Wenzhong Huang
Tropical Plant Research Center, Hainan Intitute of Tropical Agricultural Resources, Sanya, 572025, Hainan, China
Corresponding email: wenzhong.huang@hitar.org

Molecular Soil Biology, 2024, Vol.15, No.3 doi: 10.5376/msb.2024.15.0014
Received: 08 Apr., 2024
Accepted: 18 May, 2024
Published: 7 Jun., 2024

Preferred citation for this article:
Huang W.Z., 2024, Boosting soil health: the role of rhizobium in legume nitrogen fixation, Molecular Soil Biology, 15(3): 129-139 (doi: 10.5376/msb.2024.15.0014)

Abstract Soil health is a critical component of sustainable agriculture, influencing crop productivity and environmental balance. Nitrogen fixation, a process central to plant growth, is significantly enhanced through the symbiotic relationship between legumes and *Rhizobium* bacteria. This study explores the detailed mechanisms of *Rhizobium* infection in legume roots, the formation of root nodules, and the biochemical pathways involved in nitrogen fixation. The ecological and agricultural benefits of this symbiosis are profound, including enhanced soil nitrogen levels, reduced reliance on synthetic fertilizers, improved soil structure, and greater microbial diversity, all contributing to sustainable agricultural practices. The diversity of *Rhizobium* strains and their specific interactions with different legume species, as well as their adaptations to various environmental conditions, are discussed. The study also addresses the factors influencing *Rhizobium* efficiency, including soil conditions, agricultural practices, and genetic factors. Advances in *Rhizobium* inoculant technology and their application in agriculture are reviewed, along with the challenges and limitations faced in widespread adoption. Finally, future perspectives and research directions are proposed, emphasizing the potential for genetic engineering, integration with other soil health practices, and expanding the use of *Rhizobium* beyond legumes. The study concludes by highlighting the pivotal role of *Rhizobium* in promoting soil health and sustainable agriculture, and calls for continued research and development in *Rhizobium*-based solutions.

Keywords *Rhizobium*; Nitrogen fixation; Soil health; Sustainable agriculture; Legume symbiosis

1 Introduction

Soil health is a critical component of sustainable agriculture, influencing crop productivity, environmental quality, and the resilience of agricultural systems. Healthy soils support plant growth by providing essential nutrients, water, and a habitat for a diverse range of organisms. However, modern agricultural practices, including the excessive use of chemical fertilizers and pesticides, have led to soil degradation, reduced fertility, and increased vulnerability to climate change (Mabrouk et al., 2018). Sustainable farming practices, such as crop rotation, organic amendments, and the use of biofertilizers, are essential to restore and maintain soil health (Abd-Alla et al., 2023).

Nitrogen is a vital nutrient for plant growth, playing a key role in the synthesis of proteins, nucleic acids, and chlorophyll. While atmospheric nitrogen (N_2) is abundant, it is not directly accessible to most plants. Biological nitrogen fixation (BNF) is a natural process where certain bacteria convert atmospheric nitrogen into ammonia, a form that plants can readily absorb and utilize (Lindström and Mousavi, 2019). This process is particularly important for legumes, which form symbiotic relationships with nitrogen-fixing bacteria, such as *Rhizobium*, to meet their nitrogen requirements (Dall'Agnol et al., 2013; Allito et al., 2020). By enhancing nitrogen availability, BNF supports plant growth, improves soil fertility, and reduces the need for synthetic nitrogen fertilizers (Santi et al., 2013).

The symbiotic relationship between *Rhizobium* bacteria and leguminous plants is a cornerstone of sustainable agriculture. *Rhizobia* infect the roots of legume plants, leading to the formation of root nodules where nitrogen fixation occurs (Thompson and Lamp, 2021). This symbiosis not only provides the host plant with a direct source of nitrogen but also enriches the soil with nitrogen, benefiting subsequent crops in rotation systems (Yang et al., 2021). Effective *Rhizobium*-legume symbiosis can significantly enhance crop yields, improve soil health, and reduce the environmental impact of agriculture by decreasing the reliance on chemical fertilizers (Yates et al., 2021b). Moreover, certain *Rhizobium* strains have been shown to tolerate abiotic stresses, such as extreme temperatures and soil acidity, making them valuable for improving crop resilience in challenging environments (Yates et al., 2021a).

This study aims to provide a comprehensive review of the role of *Rhizobium* in legume nitrogen fixation and its impact on soil health and sustainable agriculture. It will explore the mechanisms underlying *Rhizobium*-legume symbiosis, the benefits of this symbiotic relationship for soil fertility and crop productivity, and the potential of *Rhizobium* as a biofertilizer to reduce the need for synthetic nitrogen inputs. Additionally, the study will discuss the challenges and opportunities in optimizing *Rhizobium*-legume interactions to enhance agricultural sustainability and resilience in the face of climate change. By synthesizing current research findings, this study seeks to highlight the importance of *Rhizobium* in promoting soil health and sustainable agricultural practices.

2 The *Rhizobium*-Legume Symbiosis

2.1 Detailed mechanism of *Rhizobium* infection in legume roots

The infection process of *Rhizobium* in legume roots is a highly coordinated sequence of events that begins with the recognition of rhizobial Nod factors by the plant (Wang et al., 2018a). This recognition triggers a cascade of responses, including the growth of root hairs and the formation of infection threads through which the bacteria enter the root cells. (Chakraborty et al., 2022) The rhizobia then induce cell division in the root cortex, leading to the formation of nodule primordia (Figure 1) (Yang et al., 2021). The bacteria are eventually enclosed in intracellular compartments called symbiosomes, where they differentiate into bacteroids capable of nitrogen fixation.

The research of Yang et al. (2021) illustrates the stages of nodule organogenesis in *Medicago truncatula*, highlighting the role of various gene regulatory networks. The process begins with the priming of root cells, especially cortical cells, facilitated by the SHR–SCR module. Key transcriptional complexes activate cell divisions in response to symbiotic signals, leading to the initiation of nodule formation. This is followed by outgrowth, where extensive cell divisions result in the formation of the nodule primordium. Finally, the nodule matures, developing a vascular system and an infection zone, primarily derived from cortical cells. This organized process is crucial for effective symbiosis in legumes.

2.2 Formation and structure of root nodules

Root nodule formation is a complex process that involves several stages. Initially, rhizobia attach to the root hairs and produce Nod factors, which are recognized by the plant, leading to root hair curling and the formation of infection threads (Lindström et al., 2022). These threads guide the bacteria into the root cortex, where they induce cell division and form nodule primordia. The developing nodule then differentiates into a mature structure housing the nitrogen-fixing bacteroids within symbiosomes (Oldroyd et al., 2011). The nodule structure is specialized to facilitate efficient nitrogen fixation, with a well-organized vascular system to transport nutrients and fixed nitrogen between the plant and the bacteria (Andrews and Andrews, 2016).

2.3 Biochemical pathways involved in nitrogen fixation

The biochemical pathways involved in nitrogen fixation are intricate and tightly regulated. Within the symbiosomes, bacteroids convert atmospheric nitrogen (N_2) into ammonia (NH_3) using the nitrogenase enzyme complex (Lepetit and Brouquisse, 2023). This process is energy-intensive and requires a continuous supply of ATP and reducing power, which are provided by the plant in the form of dicarboxylates and other metabolites (Schulte et al., 2021). The plant also regulates oxygen levels within the nodule to maintain the microaerobic conditions necessary for nitrogenase activity. Key metabolic pathways, such as the carbonic anhydrase-phosphoenolpyruvate carboxylase-malate dehydrogenase (CA-PEPC-MDH) pathway, play crucial roles in maintaining the redox balance and facilitating the efficient exchange of nutrients between the plant and the bacteroids (Schwember et al., 2022).

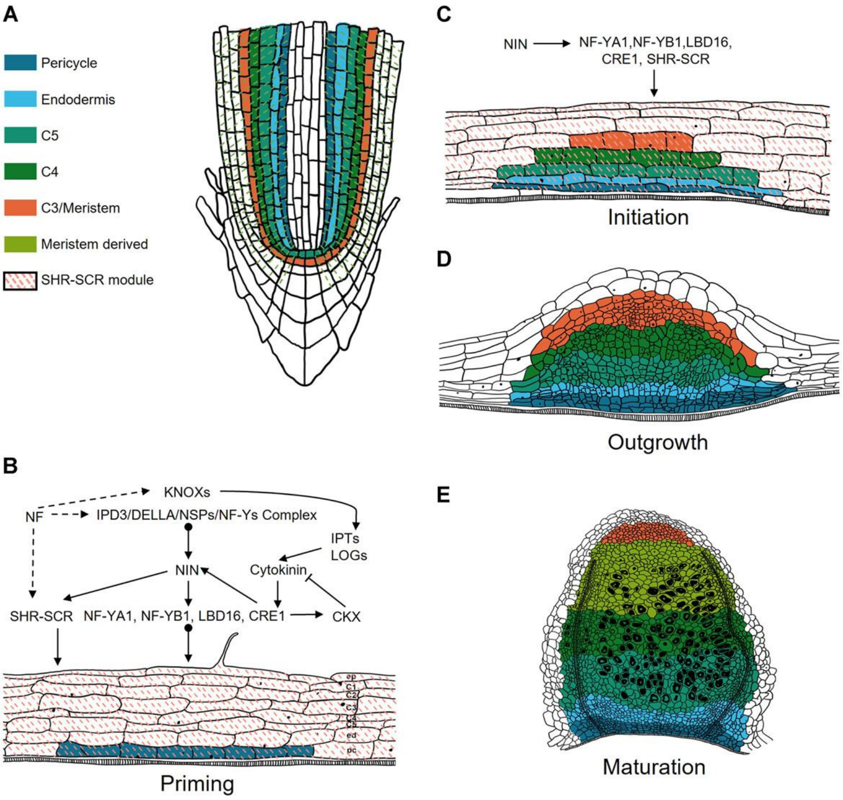

Figure 1 Stages and regulatory networks of indeterminate nodule organogenesis in *Medicago truncatula* (Adopted from Yang et al., 2021)

Image caption: (A) The structure of the *M. truncatula* root tip. The SHORT ROOT – SCARECROW (SHR–SCR) module is constitutively present in the pericycle, cortex, and epidermis, where it predisposes legume root cells, especially cortical cells, to divide. (B) Priming. The earliest anticlinal cell divisions are induced by *NIN* and downstream genes, which are activated by a transcriptional complex consisting of IPD3, DELLA, NSPs, and NF-Ys. These divisions are observed in pericycle cells in the epidermis in response to infection. The SHR–SCR module is strongly upregulated by symbiosis signals and *NIN*. *IPT* and LOG cytokinin (CK) biosynthesis genes are upregulated by the induction of *KNOX* genes. CK levels are sufficient to promote *NIN* expression via the CK receptor CRE1 and establish a negative feedback loop with CK oxidase. (C) Initiation. The inner cortex (C4/C5) cells undergo anticlinal and periclinal divisions, followed by anticlinal divisions in the middle cortex (C3). The expression of NIN and downstream genes in the nodule primordium and nodule apical meristem (NAM) is critical for these cell divisions. (D) Outgrowth. The epidermis cells fall off, and numerous cell divisions in the cortex result in the formation of a nodule primordium on the root, with an activated NAM that persistently divides and differentiates to enlarge the nodule. (E) Maturation. The organ forms a peripheral nodule vascular bundle (NVB) system and an infection zone containing bacteroids. Although cell divisions begin in the pericycle and endodermis, most cells in mature nodules are derived from cortical cells (Adopted from Yang et al., 2021)

3 Ecological and Agricultural Benefits

3.1 Enhancement of soil nitrogen levels and fertility

Rhizobium bacteria play a crucial role in enhancing soil nitrogen levels through the process of biological nitrogen fixation. This symbiotic relationship between *Rhizobium* and leguminous plants allows for the conversion of atmospheric nitrogen into a form that plants can utilize, significantly improving soil fertility. For instance, studies have shown that inoculation with effective *Rhizobium* strains can lead to substantial increases in nitrogen fixation and nutrient uptake in crops like Vicia faba, resulting in improved soil nitrogen balance (Allito et al., 2020). Additionally, the presence of *Rhizobium* in the rhizosphere can enhance the availability of other essential nutrients, such as phosphorus, further contributing to soil fertility (Mabrouk et al., 2018).

3.2 Reduction in the need for synthetic nitrogen fertilizers

The use of *Rhizobium* in legume cultivation can significantly reduce the dependency on synthetic nitrogen fertilizers. This is particularly important given the environmental and economic costs associated with the production and application of these fertilizers. By enhancing biological nitrogen fixation, *Rhizobium* bacteria provide a sustainable alternative to chemical fertilizers. Research indicates that effective management of *Rhizobium*-legume symbiosis can lead to reduced nitrogen fertilizer use, thereby lowering greenhouse gas emissions and energy consumption associated with fertilizer production (Abd-Alla et al., 2023). This not only benefits the environment but also reduces the input costs for farmers (Kebede, 2021).

3.3 Improvement of soil structure and microbial diversity

Rhizobium bacteria contribute to the improvement of soil structure and the enhancement of microbial diversity. The formation of root nodules and the subsequent nitrogen fixation process can lead to better soil aggregation and stability. For example, the plantation of native legumes like *Albizzia julibrissin* has been shown to improve soil aggregate formation and enhance the microbial community structure in saline soils (Liu et al., 2021). This improved soil structure facilitates better water retention and root penetration, which are essential for healthy plant growth. Moreover, the presence of *Rhizobium* and other beneficial microbes in the soil can create a more diverse and resilient microbial ecosystem, promoting overall soil health (AbdElgawad et al., 2020).

3.4 Contribution to sustainable agriculture and environmental health

The integration of *Rhizobium*-legume symbiosis into agricultural practices is a key component of sustainable agriculture. By reducing the need for synthetic fertilizers and improving soil health, *Rhizobium* bacteria help create more sustainable farming systems. This approach not only enhances crop yields but also mitigates the environmental impact of agriculture. For instance, the use of biofertilizers like *Rhizobium* can lead to lower greenhouse gas emissions and reduced soil degradation, contributing to long-term environmental health (Yates et al., 2021b). Furthermore, the adoption of *Rhizobium*-based practices supports the development of eco-friendly and cost-effective agricultural systems, aligning with global efforts to promote sustainable food production.

The role of *Rhizobium* in legume nitrogen fixation offers numerous ecological and agricultural benefits, including enhanced soil nitrogen levels, reduced reliance on synthetic fertilizers, improved soil structure, and contributions to sustainable agriculture. These benefits underscore the importance of leveraging *Rhizobium*-legume symbiosis to boost soil health and promote environmentally sustainable farming practices.

4 Diversity of *Rhizobium* Strains

4.1 Taxonomy and classification of *Rhizobium* species

Rhizobium species are taxonomically diverse and are classified within the alpha and beta subclasses of the Proteobacteria. For instance, Mimosa pudica, a tropical invasive weed, has been found to associate with beta-rhizobia, including species within the *Burkholderia* and *Cupriavidus* genera. In New Caledonia, the majority of rhizobial strains isolated from *M. pudica* belonged to *Cupriavidus taiwanensis*, with a few strains identified as *Rhizobium mesoamericanum* (Klonowska et al., 2012). This diversity highlights the broad taxonomic range of rhizobia and their ability to adapt to different legume hosts and environmental conditions.

4.2 Specific interactions between different legume species and *Rhizobium* strains

The interaction between specific legume species and *Rhizobium* strains can significantly influence nitrogen fixation and plant health (Jēkabsone et al., 2022). For example, in *Vicia faba* (faba bean), inoculation with specific *Rhizobium* strains such as NSFBR-12 and NSFBR-15 resulted in the highest nitrogen fixation and nutrient uptake, demonstrating the importance of selecting effective rhizobial strains for enhancing legume productivity (Allito et al., 2020). Additionally, *Rhizobium leguminosarum* bv. *viciae* strain 33504-Alex1 not only promoted growth in faba beans but also induced systemic resistance against Bean yellow mosaic virus, showcasing the multifaceted benefits of specific *Rhizobium*-legume interactions (Figure 2) (Abdelkhalek et al., 2022).

The research of Abdelkhalek et al. (2022) illustrates the levels of various oxidative stress markers (SOD, CAT, APX, and PPO) in faba bean plants under different treatments, including control, Bean yellow mosaic virus (BYMV) infection, *Rhizobium* soil treatment, and foliar spray treatment. The results indicate that plants treated with *Rhizobium* before BYMV inoculation (G3) show a significant increase in enzyme activities (SOD, APX, PPO) compared to other groups, suggesting enhanced antioxidant defense. In contrast, the control group (G1) generally exhibits lower levels of these enzymes, highlighting the stress-mitigating effect of the *Rhizobium* treatment.

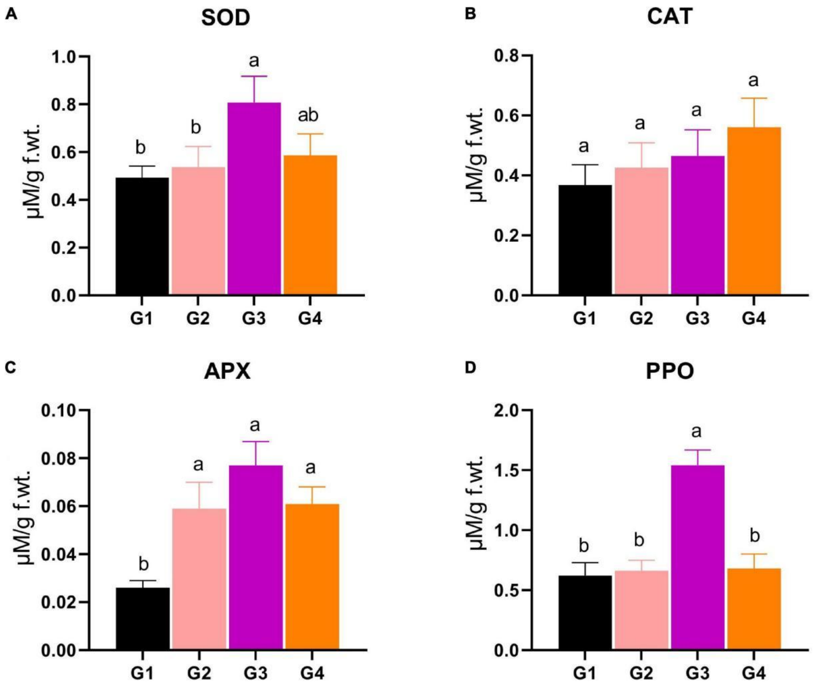

Figure 2 The histogram shows the evaluation of phenolic compounds and various oxidative stress markers in faba bean plants under the *Bean yellow mosaic virus* (BYMV) challenge (Adopted from Abdelkhalek et al., 2022)

Image caption: (A) total phenolic compounds, (B) free radical quenching activity (DPPH), (C) hydrogen peroxides (H_2O_2), and (D) malondialdehyde (MDA). All compounds were evaluated in the treatment groups: G1: control plants, G2: BYMV-infected plants, G3: plants of soil treated with a *Rhizobium* isolate 4 days before BYMV inoculation, G4: plants treated by foliar spraying of culture filtrate, 24 h before inoculation with BYMV. The columns reflect the mean of five biological replicates, while the bars represent the standard deviation (±SD). Columns with the same letter meaning do not differ significantly (Adopted from Abdelkhalek et al., 2022)

4.3 Adaptations of *Rhizobium* to various environmental conditions

Rhizobium strains exhibit remarkable adaptations to diverse environmental conditions, which can influence their effectiveness in nitrogen fixation (Wang et al., 2018b). For instance, *Cupriavidus taiwanensis* strains isolated from heavy metal-rich soils in New Caledonia showed various tolerances to metals like Ni, Zn, and Cr, suggesting their adaptation to these specific environments. Moreover, the co-inoculation of *Rhizobium* with plant growth-promoting rhizobacteria (PGPR) such as *Bacillus megaterium* has been shown to enhance nodulation and growth of common beans in low phosphorus soils, indicating that *Rhizobium* can work synergistically with other soil microbes to improve plant health under nutrient-limited conditions (Korir et al., 2017; Jach et al., 2022).

These findings underscore the importance of understanding the diversity, specific interactions, and environmental adaptations of *Rhizobium* strains to optimize their use in sustainable agriculture and soil health improvement.

5 Factors Influencing *Rhizobium* Efficiency

5.1 Soil pH, temperature, and moisture

The efficiency of *Rhizobium* in nitrogen fixation is significantly influenced by soil pH, temperature, and moisture. Optimal soil pH is crucial as it affects the availability of nutrients and the survival of *Rhizobium*. Acidic or highly alkaline soils can hinder the growth and activity of these bacteria, thereby reducing nitrogen fixation efficiency (Allito et al., 2020). Temperature also plays a vital role; extreme temperatures can lead to protein denaturation and nucleic acid damage in *Rhizobium*, affecting their ability to fix nitrogen (Owaresat et al., 2023). Soil moisture is another critical factor, as both drought and waterlogging can adversely impact *Rhizobium* activity and symbiotic efficiency.

5.2 Presence of other soil microorganisms

The presence of other soil microorganisms can either enhance or inhibit the efficiency of *Rhizobium*. Beneficial microorganisms, such as mycorrhizal fungi, can improve nutrient uptake and create a more favorable environment for *Rhizobium*. However, competition with native rhizobia and other soil bacteria can reduce the effectiveness of introduced *Rhizobium* strains. Native strains often outcompete inoculant strains for nodule occupancy, which can lead to lower nitrogen fixation rates if the native strains are less efficient (Mendoza-Suárez et al., 2021). Understanding the interactions between *Rhizobium* and other soil microorganisms is essential for improving symbiotic efficiency.

5.3 Agricultural practices (e.g., crop rotation, use of cover crops)

Agricultural practices such as crop rotation and the use of cover crops can significantly influence *Rhizobium* efficiency (Goyal et al., 2021). Crop rotation, especially with legumes, can enhance soil nitrogen levels and improve the overall health of the soil microbiome, creating a more conducive environment for *Rhizobium* (Fustec et al., 2011). The use of cover crops can also benefit *Rhizobium* by preventing soil erosion, improving soil structure, and maintaining soil moisture levels. These practices help sustain a healthy population of *Rhizobium* and other beneficial microorganisms, thereby enhancing nitrogen fixation efficiency.

5.4 Genetic factors in both *Rhizobium* and legume hosts

Genetic factors in both *Rhizobium* and their legume hosts play a crucial role in determining the efficiency of nitrogen fixation. The genetic compatibility between specific *Rhizobium* strains and legume varieties can significantly impact the success of the symbiotic relationship (Pankievicz et al., 2019). Advances in genomics have identified several quantitative trait loci (QTL) and candidate genes associated with symbiotic efficiency in both *Rhizobium* and legumes (Dwivedi et al., 2015). Breeding programs that focus on improving these genetic traits in both partners can lead to the development of high-yielding, nitrogen-efficient legume cultivars. Additionally, genetic engineering and the use of stress-tolerant *Rhizobium* strains can further enhance nitrogen fixation under adverse environmental conditions.

6 Advances in *Rhizobium* Inoculant Technology

6.1 Development and application of commercial *Rhizobium* inoculants

The development and application of commercial *Rhizobium* inoculants have significantly advanced over the years, driven by the need to enhance nitrogen fixation and improve crop yields. For instance, the use of peat carrier-based inoculants has been shown to significantly enhance nodulation, nitrogen fixation, and nutrient uptake in *Vicia faba* L. (Allito et al., 2020). Similarly, the formulation of inoculants based on autochthonous strains, such as *Rhizobium leguminosarum* bv. *phaseoli* LCS0306, has demonstrated superior performance in nitrogen fixation and grain yield in common beans, especially when combined with carriers like perlite and biochar (Table 1) (Pastor-Bueis et al., 2019). These advancements underscore the importance of selecting effective rhizobial strains and appropriate carriers to maximize the benefits of inoculation.

Table 1 Nodulation and nitrogen symbiotic fixation indicators for the combined analysis of 2017 and 2018 and inoculant treatments in field trial (Adopted from Pastor-Bueis et al., 2019)

Inoculation treatment	Number of nodules per plant	Nodule biomass (dry) (g per plant)	Aerial biomass (dry) (kg/ha)	Aerial biomass N (%)	Ndfa (%)
Negative control	36.8 ^{a}	0.647 ^{a}	3428 ^{a}	2.45^{a}	41.3 ^{ab}
Re CFN42^{T} (perlite)	37.7 ^{a}	1.002 ^{b}	5131 ^{bc}	2.50^{a}	43.3 ^{ab}
Rp ATCC 14482^{T} (perite)	29.0 ^{a}	1.154 ^{bc}	4911^{b}	2.53^{a}	46.6 ^{bc}
N ferilized non-inoculated control	34.0 ^{a}	0.512^{a}	5328 ^{bc}	2.35^{a}	39.2^{a}
RIp LCS0306 (perlite)	38.3 ^{a}	1.230C	5592 ^{C}	2.58^{a}	50.0 ^{C}

Note: The table contains the mean values for the following treatments: inoculation with the autochthonous strain *Rhizobium leguminosarum* bv. *phaseoli* LCS0306, the type strains of *Rhizobium eti* (Re CFN42^{T}) and *Rhizobium phaseoli* (Rp ATCC 14482^{T}), and the two non-inoculated controls (non-fertilized and N-fertilized). Data followed by the same letter did not significantly differ at $p<0.05$ in the LSD test (Adopted from Pastor-Bueis et al., 2019)

6.2 Methods for enhancing inoculant efficiency and effectiveness

Several methods have been explored to enhance the efficiency and effectiveness of *Rhizobium* inoculants. One approach involves the combined inoculation with arbuscular mycorrhizal fungi (AMF) and rhizobia, which has been shown to significantly increase nodulation, nitrogen, and phosphorus accumulation in common beans (Tajini et al., 2012). Another method includes the use of Fe_3O_4 nanoparticles in conjunction with *Rhizobium* inoculation, which has been found to improve nodulation, nitrogen fixation, and overall plant growth in common beans (Souza-Torres et al., 2021). Additionally, the selection of native rhizobia isolates that are well-adapted to local conditions can lead to higher symbiotic efficiency and better crop yields, as demonstrated in climbing beans in Kenya.

6.3 Case studies of successful inoculant use in different agricultural systems

Several case studies highlight the successful use of *Rhizobium inoculants* in various agricultural systems. In Egypt, the inoculation of faba beans with effective *Rhizobium* strains significantly improved seed yield and nitrogen uptake under low fertility sandy soil conditions, outperforming inorganic nitrogen fertilizers (Youseif et al., 2017). In another study, the inoculation of common beans with a combination of AMF and *Rhizobium tropici* CIAT899 resulted in enhanced phosphorus use efficiency and improved plant growth parameters. Furthermore, the use of native rhizobia isolates in climbing beans in Eastern Kenya led to significant improvements in nodulation, growth, and seed yields, demonstrating the potential of these isolates to enhance nitrogen fixation and bean production (Koskey et al., 2017). These case studies illustrate the diverse applications and benefits of *Rhizobium* inoculants in different agricultural contexts.

7 Challenges and Limitations

7.1 Environmental and soil constraints affecting *Rhizobium* performance

The performance of *Rhizobium* in legume nitrogen fixation is significantly influenced by environmental and soil conditions. Low pH and infertile soils present a major challenge, as they can hinder the formation of effective symbioses between legumes and rhizobia. For instance, research has shown that the symbiotic relationship between legumes and *Rhizobium leguminosarum* biovar *viciae* can be unreliable in low pH, dry soils, which are common in regions experiencing climate variability (Yates et al., 2021b). Additionally, soil phosphorus levels and pH can affect nutrient uptake and nitrogen fixation efficiency, as observed in Vicia faba inoculated with specific *Rhizobium* strains (Allito et al., 2020). The presence of competitive but inefficient resident rhizobia in the soil can also reduce the effectiveness of inoculants, as seen in common bean cultivation (Pastor-Bueis et al., 2019).

7.2 Issues with inoculant formulation and application

The formulation and application of rhizobial inoculants face several challenges. One major issue is the survival and persistence of inoculant strains in the field. For example, the survival of *Rhizobium* strains in dry soil conditions is a significant concern, as dry sowing practices can reduce the effectiveness of inoculants (Yates et al., 2021a). Additionally, the competition between inoculant strains and native rhizobia for nodule occupancy can limit the success of inoculants. Native rhizobia often outcompete inoculant strains, leading to lower nodule occupancy and reduced nitrogen fixation (Mendoza-Suárez et al., 2021). The formulation of inoculants with appropriate carriers, such as peat or biochar, can enhance the survival and effectiveness of rhizobial strains, but this requires careful selection and testing (Souza-Torres et al., 2021).

7.3 Economic and practical barriers to widespread adoption

Economic and practical barriers also hinder the widespread adoption of rhizobial inoculants. The cost of developing and producing high-quality inoculants can be prohibitive, especially for small-scale farmers. Additionally, the application of inoculants requires specific knowledge and practices, which may not be readily available to all farmers. For instance, the need for precise application rates and methods, such as soil or foliar application, can complicate the use of inoculants (Abdelkhalek et al., 2022). Furthermore, the economic benefits of using rhizobial inoculants may not be immediately apparent, as the initial investment in inoculants and the required changes in farming practices can be substantial (Youseif et al., 2017). Despite these challenges, the potential for improved crop yields and reduced reliance on chemical fertilizers makes the adoption of rhizobial inoculants a promising strategy for sustainable agriculture (Yakubu et al., 2011; Castellano-Hinojosa et al., 2022).

8 Future Perspectives and Research Directions

8.1 Genetic engineering of *Rhizobium* for improved nitrogen fixation

Genetic engineering of *Rhizobium* strains holds significant promise for enhancing nitrogen fixation efficiency. By manipulating genes responsible for nodulation and nitrogenase activity, researchers can develop strains that perform better under various environmental conditions, including high nitrate concentrations which typically inhibit nodule formation and nitrogen fixation (Nguyen et al., 2019). Additionally, novel strains that exhibit superior nodulation and nitrogen fixation even under challenging conditions, such as low pH and high nitrate levels, have been identified, suggesting that genetic modifications could further optimize these traits (Yates et al., 2021a; 2021b).

8.2 Integration of *Rhizobium* inoculants with other soil health practices

Integrating *Rhizobium* inoculants with other soil health practices can amplify their benefits. For instance, co-inoculation with plant growth-promoting rhizobacteria (PGPR) has been shown to enhance nodulation, nitrogen fixation, and nutrient uptake in legumes grown in low phosphorus soils (Matse et al., 2020). Similarly, the combination of *Rhizobium* with Fe_3O_4 nanoparticles has demonstrated improved nodulation and nitrogen fixation in common beans, suggesting that such integrative approaches can significantly boost plant growth and soil health (Souza-Torres et al., 2021). Moreover, the use of *Rhizobium* in conjunction with arbuscular mycorrhizal fungi has been found to increase phosphorus use efficiency, further enhancing nitrogen fixation and overall plant health (Tajini et al., 2012).

8.3 Potential for using *Rhizobium* in non-legume crops

Exploring the potential of *Rhizobium* in non-legume crops could revolutionize agricultural practices by extending the benefits of biological nitrogen fixation beyond legumes. While traditionally associated with legumes, certain *Rhizobium* strains may have the potential to form symbiotic relationships with non-legume crops, thereby improving their nitrogen uptake and reducing the need for chemical fertilizers. This approach could be particularly beneficial in low-fertility soils where conventional nitrogen sources are less effective (Youseif et al., 2017).

8.4 Long-term sustainability and environmental impact assessments

Assessing the long-term sustainability and environmental impacts of using *Rhizobium* inoculants is crucial for their widespread adoption. Studies have shown that effective *Rhizobium* strains can significantly improve soil nitrogen balance and nutrient uptake, which can lead to reduced reliance on chemical fertilizers and enhanced soil health (Allito et al., 2020; Abdelkhalek et al., 2022). However, comprehensive environmental impact assessments are needed to evaluate the potential risks and benefits over extended periods. This includes monitoring the persistence and ecological interactions of introduced *Rhizobium* strains in various soil types and climatic conditions to ensure they do not disrupt native microbial communities or lead to unintended consequences.

9 Concluding Remarks

The role of *Rhizobium* in legume nitrogen fixation is pivotal for enhancing soil health and promoting sustainable agriculture. Various studies have demonstrated that specific *Rhizobium* strains significantly improve nodulation, nitrogen fixation, and nutrient uptake in leguminous plants, thereby enhancing soil nitrogen balance and overall soil fertility. The symbiotic relationship between *Rhizobium* and legumes not only reduces the need for synthetic nitrogen fertilizers but also mitigates environmental impacts such as greenhouse gas emissions and soil degradation. Additionally, *Rhizobium* strains have shown potential in improving plant growth under various abiotic stresses, making them valuable for modern agricultural practices.

Rhizobium bacteria play a critical role in promoting soil health by facilitating biological nitrogen fixation, which converts atmospheric nitrogen into a form that plants can utilize. This process is essential for the growth of leguminous plants and contributes to the enrichment of soil nutrients, particularly nitrogen and phosphorus. The symbiotic relationship between *Rhizobium* and legumes enhances soil fertility, reduces the dependency on chemical fertilizers, and supports sustainable agricultural practices. By improving nitrogen fixation efficiency and nutrient uptake, *Rhizobium* strains help maintain soil health and promote the growth of healthy crops, which is crucial for sustainable food production and environmental conservation.

Despite the significant advancements in understanding the *Rhizobium*-legume symbiosis, further research is needed to optimize the use of *Rhizobium* strains in agriculture. Future studies should focus on identifying and developing more efficient and resilient *Rhizobium* strains that can thrive under diverse environmental conditions and enhance nitrogen fixation and nutrient uptake. Additionally, research should explore the molecular mechanisms underlying the symbiotic relationship to improve the effectiveness of *Rhizobium* inoculants. There is also a need for field trials and practical applications to validate the benefits of *Rhizobium*-based solutions in different agricultural settings. By investing in research and development, we can harness the full potential of *Rhizobium* to promote sustainable agriculture and ensure food security.

Acknowledgments

Thanks to the two anonymous peer reviewers for their feedback on the manuscript.

Conflict of Interest Disclosure

The author affirms 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

AbdElgawad H., Abuelsoud W., Madany M., Selim S., Zinta G., Mousa A., and Hozzein W., 2020, Actinomycetes enrich soil rhizosphere and improve seed quality as well as productivity of legumes by boosting nitrogen availability and metabolism. Biomolecules, 10(12): 1675.
https://doi.org/10.3390/biom10121675

Abdelkhalek A., El-gendi H., Al-Askar A., Maresca V., Moawad H., Elsharkawy M., Younes H., and Behiry S., 2022, Enhancing systemic resistance in faba bean (*Vicia faba* L.) to *Bean yellow mosaic virus via* soil application and foliar spray of nitrogen-fixing *Rhizobium leguminosarum* bv. *viciae* strain 33504-Alex1, Frontiers in Plant Science, 13: 933498.
https://doi.org/10.3389/fpls.2022.933498

Allito B., Ewusi-Mensah N., and Logah V., 2020, Legume-rhizobium strain specificity enhances nutrition and nitrogen fixation in faba bean (*Vicia faba* L.), Agronomy, 10(6): 826.
https://doi.org/10.3390/agronomy10060826

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

Castellano-Hinojosa A., Mora C., and Strauss S., 2022, Native rhizobia improve plant growth, Fix N2, and reduce greenhouse emissions of sunnhemp more than commercial rhizobia inoculants in florida citrus orchards, Plants, 11(22): 3011.
https://doi.org/10.3390/plants11223011

Chakraborty S., Valdés-López O., Stonoha-Arther C., and Ané J., 2022, Transcription factors controlling the rhizobium-legume symbiosis: integrating infection, organogenesis, and the abiotic environment, Plant & Cell Physiology, 63(10): 1326-1343.
https://doi.org/10.1093/pcp/pcac063

Dall'Agnol R., Ribeiro R., Ormeño-Orrillo E., Rogel M., Delamuta J., Andrade D., Martínez-Romero E., and Hungria M., 2013, Rhizobium freirei sp. nov., a symbiont of *Phaseolus vulgaris* that is very effective at fixing nitrogen, International Journal of Systematic and Evolutionary Microbiology, 63(Pt 11): 4167-4173.
https://doi.org/10.1099/ijs.0.052928-0

Dwivedi S., Sahrawat K., Upadhyaya H., Mengoni A., Galardini M., Bazzicalupo M., Biondi E., Hungria M., Kaschuk G., Blair M., and Ortiz R., 2015, Advances in host plant and rhizobium genomics to enhance symbiotic nitrogen fixation in grain legumes, Advances in Agronomy, 129: 1-116.
https://doi.org/10.1016/BS.AGRON.2014.09.001

Fustec J., Lesuffleur F., Mahieu S., and Cliquet J., 2011, Nitrogen rhizodeposition of legumes. A review, Agronomy for Sustainable Development, 30: 57-66.
https://doi.org/10.1051/agro/2009003

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

Jach M., Sajnaga E., and Ziaja M., 2022, Utilization of legume-nodule bacterial symbiosis in phytoremediation of heavy metal-contaminated soils. Biology, 11(5): 676.
https://doi.org/10.3390/biology11050676

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

Kebede E., 2021, Contribution, utilization, and improvement of legumes-driven biological nitrogen fixation in agricultural systems, Front. Sustain. Food Syst., 5: 767998.
https://doi.org/10.3389/fsufs.2021.767998

Klonowska A., Chaintreuil C., Tisseyre P., Miché L., Melkonian R., Ducousso M., Laguerre G., Brunel B., and Moulin L., 2012, Biodiversity of Mimosa pudica rhizobial symbionts (Cupriavidus taiwanensis, Rhizobium mesoamericanum) in New Caledonia and their adaptation to heavy metal-rich soils, FEMS Microbiology Ecology, 81(3): 618-635.
https://doi.org/10.1111/j.1574-6941.2012.01393.x

Korir H., Mungai N., Thuita M., Hamba Y., and Masso C., 2017, Co-inoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil, Frontiers in Plant Science, 8: 141.
https://doi.org/10.3389/fpls.2017.00141

Koskey G., Mburu S., Njeru E., Kimiti J., Ombori O., and Maingi J., 2017, Potential of native rhizobia in enhancing nitrogen fixation and yields of climbing beans (*Phaseolus vulgaris* L.) in contrasting environments of eastern kenya, Frontiers in Plant Science, 8: 443.
https://doi.org/10.3389/fpls.2017.00443

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

Lindström K., Terefework Z., Suominen L., and Lortet G., 2022, Signalling and development of rhizobium: legume symbioses, Biology and Environment: Proceedings of the Royal Irish Academy, 102B: 61-64.
https://doi.org/10.3318/BIOE.2002.102.1.61

Liu X., Lu X., Zhao W., Yang S., Wang J., Xia H., Wei X., Zhang J., Chen L., and Chen Q., 2021, The rhizosphere effect of native legume *Albizzia julibrissin* on coastal saline soil nutrient availability, microbial modulation, and aggregate formation, The Science of the Total Environment, 806: 150705.
https://doi.org/10.1016/j.scitotenv.2021.150705

Mabrouk Y., Hemissi I., Salem I., Mejri S., Saidi M., and Belhadj O., 2018, Potential of rhizobia in improving nitrogen fixation and yields of legumes, Symbiosis, 107(73495): 1-16.
https://doi.org/10.5772/INTECHOPEN.73495

Matse D., Huang C., Huang Y., and Yen M., 2020, Effects of coinoculation of Rhizobium with plant growth promoting rhizobacteria on the nitrogen fixation and nutrient uptake of Trifolium repens in low phosphorus soil, Journal of Plant Nutrition, 43: 739-752.
https://doi.org/10.1080/01904167.2019.1702205

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

Nguyen H., Miwa H., Obirih-Opareh J., Suzaki T., Yasuda M., and Okazaki S., 2019, Novel rhizobia exhibit superior nodulation and biological nitrogen fixation even under high nitrate concentrations, FEMS Microbiology Ecology, 96(2): fiz184.
https://doi.org/10.1093/femsec/fiz184

Oldroyd G., Murray J., Poole P., and Downie J., 2011, The rules of engagement in the legume-rhizobial symbiosis, Annual Review of Genetics, 45: 119-144.
https://doi.org/10.1146/annurev-genet-110410-132549

Owaresat J., Siam M., Dey D., Jabed S., Badsha F., Islam M., and Kabir M., 2023, Factors impacting rhizobium-legume symbiotic nitrogen fixation with the physiological and genetic responses to overcome the adverse conditions: a review, Agricultural Reviews, 44(1): 22-30.
https://doi.org/10.18805/ag.rf-257

Pankievicz V., Irving T., Maia L., and Ané J., 2019, Are we there yet? The long walk towards the development of efficient symbiotic associations between nitrogen-fixing bacteria and non-leguminous crops, BMC Biology, 17: 99.
https://doi.org/10.1186/s12915-019-0710-0

Pastor-Bueis R., Sánchez-Cañizares C., James E., and González-Andrés F., 2019, Formulation of a highly effective inoculant for common bean based on an autochthonous elite strain of *Rhizobium leguminosaru*m bv. *phaseoli*, and genomic-based insights into its agronomic performance, Frontiers in Microbiology, 10: 2724.
https://doi.org/10.3389/fmicb.2019.02724

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

Schulte C., Borah K., Wheatley R., Terpolilli J., Saalbach G., Crang N., Groot D., Ratcliffe R., Kruger N., Papachristodoulou A., and Poole P., 2021, Metabolic control of nitrogen fixation in rhizobium-legume symbioses, Science Advances, 7: eabh2433.
https://doi.org/10.1126/sciadv.abh2433

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

Souza-Torres A., Govea-Alcaide E., Gómez-Padilla E., Masunaga S., Effenberger F., Rossi L., López-Sánchez R., and Jardim R., 2021, Fe_3O_4 nanoparticles and Rhizobium inoculation enhance nodulation, nitrogen fixation and growth of common bean plants grown in soil, Rhizosphere, 17: 100275.
https://doi.org/10.1016/j.rhisph.2020.100275

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

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

Wang Q., Liu J., and Zhu H., 2018a, 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

Wang X., Teng Y., Tu C., Luo Y., Greening C., Zhang N., Dai S., Ren W., Zhao L., and Li Z., 2018b, Coupling between nitrogen fixation and tetrachlorobiphenyl dechlorination in a rhizobium-legume symbiosis, Environmental Science & Technology, 52(4): 2217-2224.
https://doi.org/10.1021/acs.est.7b05667

Yakubu H., Kwari J., and Ngala A., 2011, N 2 Fixation by grain legume varieties as affected by rhizobia inoculation in the sandy loam soil of sudano-sahelian zone of north eastern Nigeria， Nigerian Journal of Basic and Applied Sciences, 18: 229-236.
https://doi.org/10.4314/NJBAS.V18I2.64325

Yang J., Lan L., Jin Y., Yu N., Wang D., and Wang E., 2021, Mechanisms underlying legume-rhizobium symbiose, Journal of Integrative Plant Biology, 64(2): 244-267.
https://doi.org/10.1111/jipb.13207

Yates R., Harrison R., Loi A., Steel E., Edwards T., Nutt B., Porqueddu C., Gresta F., and Howieson J., 2021a, Sourcing *Rhizobium leguminosarum* biovar *viciae* strains from Mediterranean centres of origin to optimize nitrogen fixation in forage legumes grown on acid soils, Grass and Forage Science, 76: 33-43.
https://doi.org/10.1111/GFS.12524

Yates R., Steel E., Poole C., Harrison R., Edwards T., Hackney B., Stagg G., and Howieson J., 2021b, Optimizing the growth of forage and grain legumes on low pH soils through the application of superior *Rhizobium leguminosarum* biovar *viciae* strains, Grass and Forage Science, 76: 44-56.
https://doi.org/10.1111/GFS.12523

Youseif S., El-Megeed F., and Saleh S., 2017, Improvement of faba bean yield using rhizobium/agrobacterium inoculant in low-fertility sandy soil, Agronomy, 7: 2.
https://doi.org/10.3390/AGRONOMY7010002