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Discussion on the Symbiotic Relationship between Legume Crops and Soil Microorganisms and Their Ecological Benefits

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Abstract Leguminous crops form a unique symbiotic system with soil microorganisms (especially nitrogen-fixing rhizobia and arbuscular mycorrhizal fungi), which plays an important role in agricultural ecosystems. This study reviews the biological basis of leguminous crop-microorganism symbiosis and its significance to soil microecology and agricultural sustainable development, introduces the mechanism of symbiotic nitrogen fixation, discusses how leguminous crops promote soil microecological balance by increasing soil organic matter, improving soil enzyme activity and improving soil physical and chemical properties, and explains the supporting role of microbial symbiosis on nutrient absorption and stress resistance of leguminous plants, such as improving nitrogen fixation efficiency, enhancing plant drought and salt tolerance, inducing plant immunity and biological control potential. Furthermore, from the perspective of ecological benefits, the contribution of leguminous-microorganism symbiosis in reducing fertilizer dependence and greenhouse gas emissions, restoring degraded soil functions and enhancing the stability of agricultural ecosystems is analyzed, and illustrated by cases such as soybean-rhizobium, peanut inoculants and leguminous rotation. This study hopes to provide a theoretical basis and practical reference for the role of leguminous crops and soil microbial symbiosis in sustainable agriculture.

Keywords Legume crops; Soil microorganisms; Symbiotic nitrogen fixation; Ecological benefits; Sustainable agriculture

1 Introduction

Symbiotic nitrogen fixation between legumes and soil microorganisms is an important source of nitrogen supply in agricultural ecosystems and has the potential to replace some chemical nitrogen fertilizers. It is estimated that the symbiosis between legumes and rhizobia can fix a large amount of nitrogen for terrestrial ecosystems every year, thereby reducing dependence on chemical fertilizers. However, environmental problems caused by long-term excessive application of chemical fertilizers are becoming increasingly prominent, such as increased greenhouse gas emissions, eutrophication of water bodies, and soil degradation. Therefore, making full use of the mutualistic symbiotic relationship between legumes and soil microorganisms to achieve the biological cycle of nutrients in the soil is of great practical significance for the development of sustainable agriculture. In addition, legumes are rich in protein and bioactive substances, and they also play a key role in ensuring food security and nutrition. Their symbiotic nitrogen fixation ability enables them to survive in poor environments and improve soil fertility (Fahde et al., 2023).

Legumes have outstanding value in ecological agriculture. On the one hand, legumes provide nitrogen sources for subsequent crops through symbiotic nitrogen fixation, reduce the application of chemical fertilizers, and realize the green cycle of agricultural production (Mahama et al., 2020). For example, the introduction of leguminous crops into the crop rotation system of corn and other food crops can increase crop yields and nitrogen use efficiency, and reduce nitrogen fertilizer input and nitrogen loss (Yu et al., 2021). On the other hand, leguminous crops are often used as green manure and cover crops to improve soil, increase soil organic matter and nutrient content, promote soil microbial diversity, and thus improve the biological cycle function of farmland ecosystems. Leguminous crops have well-developed root systems and extensive symbiosis with mycorrhizal fungi, and also play a positive role in improving soil structure, preventing soil erosion, and restoring degraded soils. Therefore, planting and making good use of leguminous crops in the ecological agricultural model has an important role in promoting the realization of weight loss and efficiency, soil fertility, and sustainable agricultural development.

This study will systematically explore the symbiotic relationship between leguminous crops and soil microorganisms (mainly nitrogen-fixing rhizobia and arbuscular mycorrhizal fungi) and the ecological benefits they bring, and explain the role of leguminous crops in improving soil microecology, such as increasing soil organic matter, enzyme activity, and improving soil physical and chemical properties. Then, the supporting role of soil microorganisms on nutrient absorption and stress resistance of legumes is analyzed, including nitrogen fixation to improve nitrogen utilization, high drought and salt tolerance, and induction of plant immunity and biological control potential. On this basis, from the perspective of ecological and environmental benefits, the role of legume-microorganism symbiosis in reducing fertilizer use and greenhouse gas emissions, repairing degraded soil functions, and enhancing the stability of agricultural ecosystems is discussed. The above viewpoints are confirmed through specific case studies (such as soybean-rhizobium long-term positioning experiments, peanut microbial inoculation yield increase experiments, and the role of legumes in crop rotation). This study summarizes the role of symbiotic relationships in sustainable agriculture and proposes directions for future research and practice to promote its better application in agriculture.

2 Symbiotic Basis between Legumes and Soil Microbes

2.1 Classification and symbiotic mechanism of rhizobia

Nitrogen-fixing rhizobia are a type of soil bacteria that can infect the roots of legumes and form nodules. They mainly belong to the α- and β-subgroups of the Proteobacteria, including multiple taxa such as *Rhizobium*, *Bradyrhizobium*, and *Bacillus thuringiensis* (now Ensifer/Sintoferria). Different legumes tend to form symbiotic relationships with specific types of rhizobia. For example, soybeans usually coexist with *Bradyrhizobium* strains, while alfalfa prefers Rhizobia strains. The process of symbiotic nitrogen fixation between rhizobia and legumes is very complex, involving strict species or strain matching and a series of exquisite "molecular dialogues". At the beginning of the symbiosis, the roots of legumes secrete flavonoids to attract rhizobia in the soil to swim toward the roots. Matching rhizobia regulate the nodulation (nod) gene through NodD, synthesize and release specific "nodulation factor" (Nod factor), which is a modified oligosaccharide signal molecule. After the receptor kinase complex on the epidermal cells of the plant roots (such as NFR1/NFR5 of *Lotus japonicus*, LYK3/NFP of *Medicago truncatula*, etc.) senses the nodulation factor, it activates the downstream symbiotic signaling pathway, induces the root hair to bend and be invaded by bacteria. Subsequently, the root hair cell wall is partially degraded to form an infection line for the rhizobia to enter. The bacteria elongate and divide continuously along the infection line, and finally invade the cortical cells. At the same time, the cortical cells divide again to form the initial flowering meristem and develop into nodules. In the nodule, the rhizobia are wrapped by the plant cell membrane to become bacteroids, and begin to efficiently fix atmospheric nitrogen and reduce it to ammonium nitrogen for plant use. The entire symbiotic process requires strict coordination between the two parties: the plant provides a carbon source and a shelter environment, the microorganisms provide nitrogen, and through complex signal exchanges to ensure the establishment and maintenance of a mutually beneficial symbiotic relationship.

2.2 Role of mycorrhizal fungi in synergistic interaction

In addition to rhizobia, many legumes also establish symbiosis (i.e., mycorrhizal symbiosis) with arbuscular mycorrhizal fungi (AM fungi) in the soil, which is of great significance to the nutrient acquisition and environmental adaptability of plants. Interestingly, there is a high degree of commonality in the signal transduction pathway between mycorrhizal symbiosis and nodule symbiosis: the receptors and downstream signals of mycorrhizal factors (Myc factors) secreted by AM fungi that plants recognize partially overlap with the pathways for recognizing nodulation factors, which is considered to be the result of legumes using evolutionary commonalities to establish two symbiotic relationships. Studies have shown that arbuscular mycorrhizal symbiosis appeared in terrestrial plants more than 400 million years ago, and legumes later "borrowed" the signal mechanism of this ancient symbiosis to develop a new symbiosis with rhizobia. Therefore, there is synergy and a common signal network between AM fungi symbiosis and nodule symbiosis.

In actual function, mycorrhizal fungi significantly expand the effective absorption area of legume roots through their hyphae networks, helping to absorb nutrients (such as phosphorus) and water that are difficult to move in the soil. At the same time, AM bacteria can also change the rhizosphere microbial community. Studies have found that the hyphae of arbuscular mycorrhizal fungi can serve as a "bridge" to help rhizobia spread in the soil and reach the roots of legumes faster, thereby improving nodulation efficiency. For example, a microcosm experiment on peanuts showed that in the presence of an endophytic fungus (*Phomopsis liquidambaris*) hyphae, the enrichment of rhizobia and the number of nodules in the peanut rhizosphere increased significantly; the fungal hyphae provided a "highway" for rhizobia, making it easier for them to migrate to the roots and induce the formation of more nodules. The study also found that rhizobia exhibited special chemotaxis and proliferation behaviors when moving along the hyphae, and hyphal secretions may promote this process (Figure 1) (Zhang et al., 2020). It can be seen that the presence of AM bacteria can assist rhizobia in colonization and nodulation at the physical and chemical levels. Other studies have shown that the simultaneous inoculation of rhizobia and mycorrhizal fungi has a synergistic effect on the growth of legumes: for example, in alfalfa, the double inoculation treatment significantly increased the plant's nitrogen and phosphorus absorption and biomass accumulation, which was more conducive to nutrient cycling than the single inoculation treatment. This shows that the interaction between arbuscular mycorrhizal fungi and rhizobia can form a more functional microbial consortium in the rhizosphere of legumes, helping plants to fully obtain a variety of nutrients.

More profoundly, recent molecular evolutionary studies have revealed that legume-rhizobium symbiosis and AM bacteria symbiosis may have a synergistic relationship in evolution. Some key genes (such as SymRK receptor kinase, etc.) were found to be involved in regulating both symbioses. In their study of the evolutionary mechanism of the legume symbiotic system, Wang and Zhang (2021) proposed that it was precisely because early terrestrial plants already had the ability of mycorrhizal symbiosis that legumes evolved a new symbiosis with nitrogen-fixing rhizobia on this basis, and the two symbioses influenced each other and improved synergistically during the evolution process. This view is supported by symbiotic biogenomics research: the presence of mycorrhizal symbiosis affects the response of legumes to rhizobia and the nodulation characteristics, and legumes without AM bacteria are often more difficult to nodulate efficiently. Therefore, the legume crop symbiotic system can be regarded as a complex tripartite interaction network including plants, fungi, and bacteria, and each component promotes each other, ultimately improving the nutrient utilization and environmental adaptability of plants.

2.3 Recognition and regulation of symbiotic signal molecules

The establishment of a symbiotic relationship between legumes and microorganisms requires a fine balance between the exchange of signal molecules and the regulation of the plant's own defense response. In the rhizobium-legume symbiosis, the most critical signal molecule is the aforementioned nodulation factor (Nod factor). After the plant recognizes the Nod factor through the NFR1/NFR5 receptor located on the surface of the root hair, it activates the downstream cascade reaction including calcium ion oscillation and symbiosis-related gene expression, triggering the nodulation process. At the same time, the plant needs to suppress its own immune system to allow the invasion and colonization of symbiotic bacteria, otherwise the rhizobia may be regarded as pathogens and blocked by the plant's defense response. Studies have shown that legumes have functionally differentiated the pathways for identifying symbiotic microorganisms and pathogens: on the one hand, plants trigger symbiotic signals through a unique Nod factor receptor pathway, thereby bypassing the strong immune response induced by typical pathogen-associated molecular patterns (MAMPs); on the other hand, plants still use some immune mechanisms to monitor the symbiotic process to ensure that only the correct strains can successfully nodulate without harming the health of the host.

For example, the plant microbial receptor kinase EPR3 can bind to the surface polysaccharide signal on the surface of rhizobia to distinguish whether it is a suitable symbiotic strain. If an "unqualified" signal is detected, a defense response is triggered to limit its infection. For another example, plants produce reactive oxygen and antimicrobial substances to inhibit excessive bacterial growth, but symbiotic rhizobia have evolved tolerance mechanisms that can moderately resist plant defenses to allow themselves to colonize smoothly. In the later stages of nodulation, plants limit the number of nodules through the "autonomous regulation of nodulation (AON)" mechanism: the signal produced by the already formed nodules is transmitted through the aboveground part to inhibit the occurrence of new nodules, so as to avoid the formation of too many nodules and waste host resources. Environmental signals such as nitrate nitrogen also regulate nodulation through this pathway: when the external nitrogen is sufficient, nitrate can act as a signal to upregulate negative regulatory factors in plants (such as NIN-like protein transcription factors, etc.), thereby inhibiting nodule formation and nitrogen fixation to save plant energy. Luo and Xie (2019) summarized the mechanism by which nitrate inhibits nodule symbiosis in two ways: local and systemic, and emphasized the key role of NLP transcription factors and AON pathways.

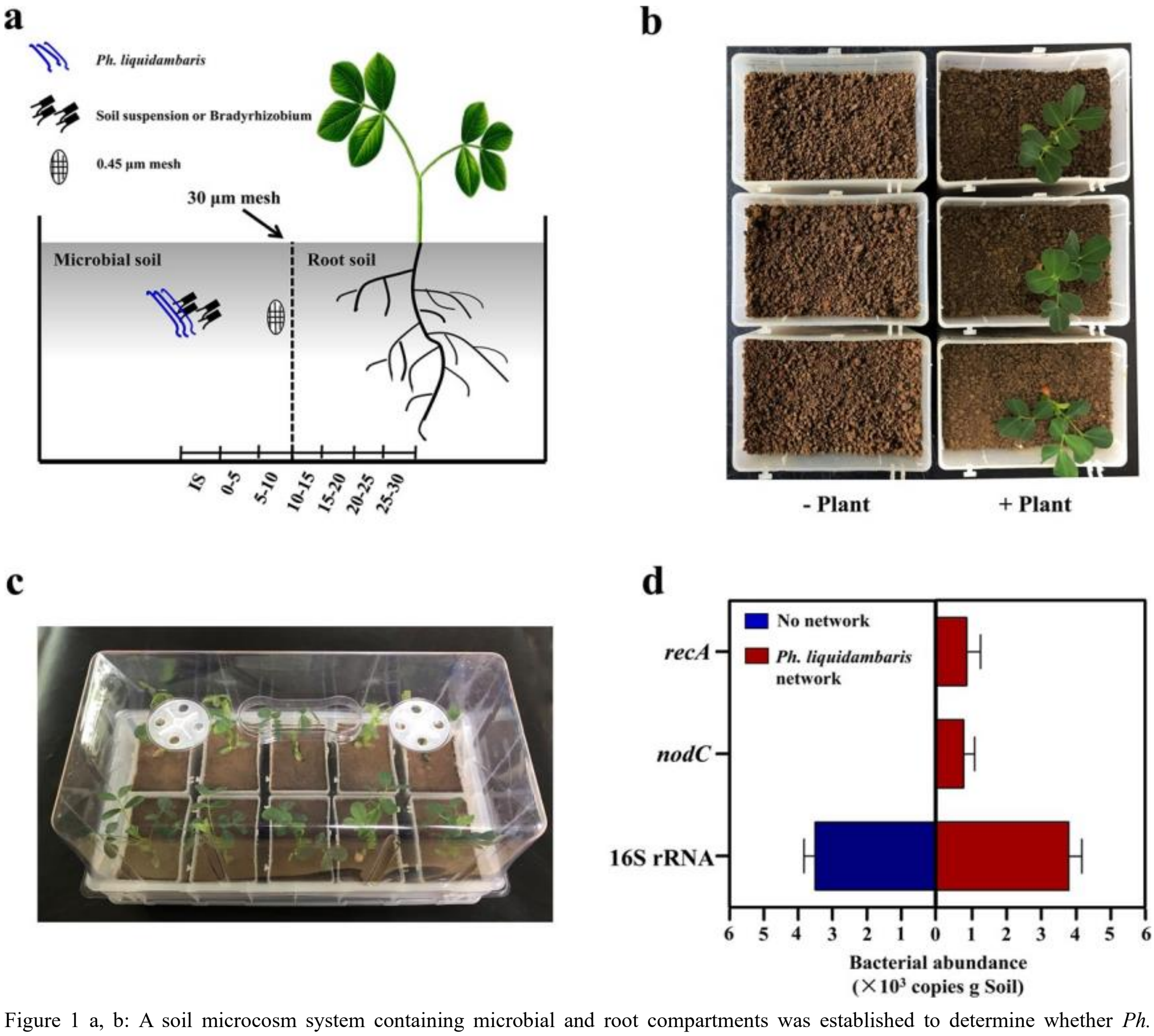

Figure 1 a, b: A soil microcosm system containing microbial and root compartments was established to determine whether *Ph. liquidambaris* networks transfer rhizobia to the rhizosphere of peanut. The microbial and root compartments were separated by a sterile 30 μm mesh. Roots were confined to the root soil, but fungi and rhizobia in the microbial soil were able to cross the mesh and enter root soil. The treatments with peanut cultivation contained individual seedling planted in root compartment. Sterile 0.45 μm mesh in soil microcosm was used to observe *Ph. Liquidambaris-Bradyrhizobium* interaction. c: Microcosms were placed in transparent boxes with closed lids to minimize water evaporation and avoid microbial contamination. d: 16S rRNA, nodC, and recA copies in the rhizosphere soil of peanut. At 15 dai, rhizosphere soil of peanut was collected to determine the copy numbers of 16S rRNA, *nodC*, and recA when the microbial compartment was inoculated with soil suspension with or without *Ph. liquidambaris*. Data and error bars are the mean ± SE (n = 4). CFUs colony-forming units; dai days after inoculation; IS inoculation site (Adopted from Zhang et al., 2020)

Plants also need to maintain a certain degree of immune vigilance during the symbiotic process. Once the behavior of symbiotic bacteria deviates from mutualism (for example, they no longer fix nitrogen and consume host nutrients), plants will use immune mechanisms to punish or even eliminate the "cheating" bacteria. This phenomenon is called "host checks and balances on symbionts" in symbiotic ecology. It can be seen that legumes actually face a "dual task": they must accept symbiotic microorganisms, but they must not let the symbiotic relationship destroy their own defense balance. In recent years, studies on plant hormones and signaling pathways have revealed that a variety of plant hormones (such as ethylene, jasmonic acid, etc.) are involved in the coordination of symbiosis and immunity. Some factors that positively regulate nodulation are also negative immune regulators, such as Medicago's PUB1 ubiquitin ligase, which not only inhibits excessive infection signals but also promotes moderate nodulation. Some immune-related factors have a negative impact on symbiosis, such as the plant disease resistance gene E3 ubiquitin ligase, which limits rhizobia infection. If it is knocked out, the number of nodules can be increased. Through these complex molecular regulations, legumes achieve a dynamic balance between symbiotic signals and immune responses. Under abiotic stress conditions (such as high salt osmotic stress), this balance is more likely to be broken: stress signals often enhance plant defense pathways, thereby inhibiting the nodulation symbiosis process. Recent studies have revealed that certain GSK3-like protein kinases are induced in large quantities in soybeans under high salt conditions, and after upregulation, they inhibit the symbiotic signaling pathway, resulting in a decrease in nodule number and nitrogen fixation capacity.

3 Legumes in Enhancing Soil Microecology

3.1 Increasing organic matter content in soil

Leguminous crops generally have the effect of improving soil fertility, the most direct manifestation of which is in increasing soil organic matter content. Leguminous plant residues (such as roots, stems and leaves) are rich in nitrogen and carbon, and can significantly increase soil organic matter and nutrient levels after litter decomposition. Especially when leguminous crops are turned into soil as green manure, their organic carbon input is large and decomposes quickly, which can increase soil organic carbon storage in a relatively short period of time. Studies have shown that long-term application of leguminous green manure can increase soil organic matter content year by year. For example, in the long-term green manure experiment of irrigated desert soil, soil organic matter increased significantly compared with the control after applying leguminous green manure for more than 10 years, with an average annual increase of more than 5%. For example, planting alfalfa, peas, peanuts and other green manures in the soil of southern orchards can increase soil organic matter content by 5%~27% after one year. The research review of Xu et al. (2021) also pointed out that intercropping leguminous green manure between orchard rows significantly increased soil organic carbon content and improved soil quality. This is consistent with observations in other regions: planting legume cover crops not only increases organic matter and total nitrogen in the soil, but also helps to improve the yield and quality of subsequent crops.

Legume crops fix nitrogen in the air as organic nitrogen through symbiotic nitrogen fixation, introduce it into the soil, and make an important contribution to the accumulation of soil organic matter. Compared with the simple application of nitrogen fertilizers, the organic nitrogen produced by biological nitrogen fixation is more conducive to the formation of soil humus and reduces nitrogen leaching. The field experiment of Mahama et al. (2020) showed that planting winter legume cover crops in a no-till sorghum system can reduce the amount of nitrogen fertilizer in the later season, while increasing the content of soil organic nitrogen and organic carbon, thereby reducing nitrogen fertilizer demand and soil N_2O emissions. This shows that the application of legume green manure crops has achieved the replacement of part of inorganic nitrogen with biological organic nitrogen, which not only maintains crop yields, but also increases soil organic matter, achieving the dual benefits of fertilizing soil fertility and reducing emissions. Using legume crops to increase soil organic matter is one of the important measures for fertilizing soil in traditional agriculture. For example, in many areas of China, farmers have always had the habit of planting leguminous green manures such as astragalus and vetch to maintain soil fertility and soil fertility. Modern research has more systematically confirmed the scientific nature and long-term benefits of this practice. It can be foreseen that in future sustainable agriculture, making full use of leguminous green manure and returning leguminous crop straw to the field will play a greater role in restoring soil carbon pools and improving soil health.

3.2 Promoting soil enzyme activity and aggregate structure

Another prominent role of leguminous plants in promoting soil microecological functions is to enhance soil enzyme activity and improve soil aggregate structure. Leguminous crop root secretions and residues provide rich carbon and nitrogen sources for soil microorganisms, activate soil microbial metabolism, and thus enhance the activity of a variety of soil enzymes. Studies have shown that in the rhizosphere of leguminous plants, the activity of key enzymes such as carbohydrate activating enzymes, ureases, and phosphatases is usually higher than that of non-leguminous plants, which is conducive to the recycling of nutrients. For example, Chang Dana et al. found that compared with the control without green manure, planting astragalus green manure can increase soil dissolved organic carbon by 29%, dissolved organic nitrogen by 257%, and significantly increase soil urease and sucrase activities. In the legume/grass intercropping system, the activities of multiple hydrolases in the rhizosphere of legumes are more uniform and higher than those of gramineous plants. This may be related to the continuous secretion of soluble organic materials and the supply of microbial activity by the legume roots. Yang et al. (2020) also found through high-throughput sequencing and functional prediction that inoculation of rhizobia and AM fungi can increase the abundance of nitrogen-fixing bacteria and phosphate-solubilizing bacteria in the rhizosphere of alfalfa, and the abundance of enzyme genes related to soil nitrogen cycle increases, which is beneficial to plant nitrogen and phosphorus nutrition acquisition. Therefore, the legume crop symbiotic system can stimulate the activity of soil enzymes and promote the mineralization and circulation of organic nutrients by enriching the rhizosphere nutrient supply and microbial community.

The improvement of soil structure by legumes is reflected in promoting aggregate formation and improving aggregate stability. The large amount of polysaccharide mucilage and hyphae network produced by leguminous roots and their symbiotic microorganisms are natural "soil cementing agents" that can cement soil particles into larger aggregates. In particular, the "glomerular protein" (i.e., mycorrhizal mycocolloid, GRSP) secreted by arbuscular mycorrhizal fungi is called the "adhesive" of soil structure. It can significantly increase the content of water-stable aggregates and improve the aggregate structure and water holding capacity of the soil through hyphae entanglement and cementation. The orchard experiment of Yan et al. (2024) showed that planting leguminous green manure significantly increased the proportion of large aggregates in citrus orchard soil, improved aggregate stability and organic carbon content. Similarly, the introduction of leguminous crops into farmland soil can also help form a more loose and porous soil structure, improve soil ventilation and water retention. This is not only beneficial to the growth of crop roots, but also provides a more suitable habitat for soil microorganisms. It is worth mentioning that mycorrhizal fungi play a unique role in improving soil structure. Al-Arjani et al. (2020) pointed out that inoculation of AM fungi increased soil water holding capacity and stabilized soil structure by promoting the formation of large aggregates. Therefore, in the legume-microorganism symbiotic system, plants, bacteria and fungi work together to promote the development of soil biological structure: plants provide organic matter, rhizobia contribute polysaccharide mucus, and mycorrhizal fungi provide mycelial networks and adhesive substances. Under the synergy of the three, soil aggregates are generated in large quantities and become more stable. This is of great significance for preventing soil erosion and maintaining soil health. In general, legume crops enhance the ecological function and environmental resistance of soil by increasing soil enzyme activity and improving aggregate structure.

3.3 Improving soil physicochemical properties

The planting and management of legume crops can also cause a series of positive changes in soil physical and chemical properties. First, in terms of soil pH, the nitrogen fixation of legumes can partially neutralize soil acidity. Generally, the application of ammonium nitrogen fertilizer will release H^+ in the nitrification process, leading to soil acidification. The nitrogen provided by symbiotic nitrogen fixation is slowly released in the form of organic nitrogen and ammonium nitrogen, which is relatively mild and not easy to significantly reduce soil pH. Studies have observed that in fields where leguminous green manures are planted for consecutive years, the downward trend of soil pH is buffered, and the degree of acidification is lighter than that of fields where only chemical fertilizers are applied. This may be because leguminous plants absorb and utilize part of the nitrate in the soil, and the nitrogen fixation process consumes H+, which regulates the soil. In addition, some leguminous forage grasses (such as alfalfa) have the effect of improving acidic soil. Their deep roots can bring alkaline nutrients from the deep layer to the surface, and secrete organic anions to chelate with aluminum ions, reducing aluminum toxicity, thereby improving the suitability of acidic soil.

Secondly, leguminous plants also significantly improve the physical and chemical properties of soil such as aeration and water permeability by improving soil structure and increasing organic matter. The pore network formed by the deep roots of leguminous plants and mycorrhizal hyphae in the soil is conducive to soil aeration and water infiltration. This is particularly important for heavy or compacted soils. Studies have shown that in orchard soils with long-term monoculture and heavy fertilization, the introduction of leguminous green manure intercropping can reduce soil bulk density, increase porosity, and significantly improve ventilation conditions. The experiment of Zhang et al. (2021) also showed that intercropping leguminous green manure in kiwifruit orchards can enhance soil permeability and water holding capacity, improve soil aggregate structure and water content, and thus create a good growth environment for fruit tree roots. At the same time, the microclimate of soil temperature and humidity is also regulated by leguminous mulching: green manure mulching can reduce the daily difference in surface temperature, reduce water evaporation, and achieve the effect of "cooling and conserving moisture in summer, and keeping warm and preventing freezing in winter". These changes also have a positive impact on soil microbial flora. Microbial diversity and activity will increase under more stable and suitable physical and chemical conditions, thus forming a virtuous circle. Leguminous crops can also reduce the risk of soil salinization. Introducing leguminous forage (such as alfalfa) in some saline-alkali lands can adapt to and improve saline-alkali soils through their deep root system and apoplast barrier mechanism. Leguminosae absorb a large amount of cationic nitrogen (ammonium) and calcium and magnesium, which helps to reduce the proportion of sodium ions in the soil and regulates the balance of soil salt ions. Although leguminous plants themselves are not tolerant to extreme salinity, the combined use of salt-tolerant growth-promoting bacteria (such as inoculation of salt-tolerant rhizobia or salt-tolerant PGPR) can improve the saline soil environment and increase plant survival rate to a certain extent.

4 Microbial Support for Nutrient Uptake and Stress Tolerance

4.1 Nitrogen fixation and nitrogen use efficiency

The most significant effect of the symbiosis between soil microorganisms and legumes is to provide nitrogen nutrients to host plants, significantly improving the nitrogen utilization efficiency of legumes. Rhizobia reduce the inert N_2 in the atmosphere to ammonium ions in the symbiotic nodules, providing tens to hundreds of kilograms of nitrogen per hectare per year for legumes, which is equivalent to a certain amount of nitrogen fertilizer input. For major legume crops such as soybeans, the nitrogen provided by biological nitrogen fixation during the growth period can account for more than 50% of their total nitrogen demand. Some studies have estimated that this proportion can reach 60%~70% for soybeans. This means that through the rhizobium symbiotic system, legumes have greatly improved their self-sufficiency in nitrogen sources, thereby reducing their dependence on exogenous nitrogen fertilizers.

Under the action of symbiotic nitrogen fixation, legumes often show higher nitrogen utilization efficiency (NUE). Compared with the application of chemical fertilizers, legumes have less loss and more complete conversion of symbiotic fixed nitrogen. Studies have shown that when leguminous crops are grown without nitrogen fertilizer, their N_2O greenhouse gas emissions are significantly lower than those of non-leguminous crops that rely on nitrogen fertilizer. Model simulations also show that replacing chemical fertilizers with leguminous crops will not reduce yields, but can effectively reduce field N_2O emissions. Therefore, from an agronomic and environmental perspective, the nitrogen provided by rhizobium symbiotic nitrogen fixation is more "efficient" (Savvas et al., 2017; Goyal et al., 2021). On the one hand, it supplies the needs of plants in real time, avoiding the waste of excessive fertilizer application and leaching; on the other hand, the nitrogen fixation process is accompanied by carbohydrate consumption, which helps more carbon to be fixed in the soil to form humus and improve soil fertility. Long-term positioning test data support the conclusion that symbiotic nitrogen fixation improves NUE: in soybean fields without nitrogen fertilizer but inoculated with excellent rhizobia, soybean yield and nitrogen uptake can be maintained at a high level, comparable to the treatment of small amounts of nitrogen application, while inorganic nitrogen residues and N_2O emissions in the soil are lower. This potential of "replacing fertilizer with solids" is exactly what sustainable agriculture needs. With the development of efficient rhizobia strains and the application of inoculation technology, the nitrogen fixation effect of leguminous crops has been further enhanced in many systems. For example, under the condition of reducing nitrogen fertilizer by 20% to 40%, leguminous crops such as peanuts can still maintain stable yields or even slightly increase. The field test results of Ding et al. (2024) showed that under the treatment of conventional fertilization with 40% nitrogen reduction and rhizobia inoculation, the peanut yield reached the highest, which was 5.3% higher than the control without fertilizer reduction and inoculation, indicating that part of the nitrogen was supplemented by nitrogen-fixing bacteria and the utilization efficiency was higher. It can be seen that microbial nitrogen fixation has a significant supporting effect on the nitrogen nutrition of leguminous crops and improves the nitrogen utilization efficiency of the crop system.

Mycorrhizal fungal symbiosis can also improve the absorption and utilization of soil nitrogen by leguminous plants. AM bacteria expand the absorption range of ammonium nitrogen and nitrate nitrogen by the root system, and can activate the expression of nitrate transporters and ammonium transporters in plants, thereby improving the utilization rate of residual nitrogen in the soil by leguminous plants to a certain extent. Studies have found that legumes inoculated with AM bacteria in nitrogen-deficient soils absorb more total nitrogen than those that are not inoculated. Mycorrhizal symbiosis can also change the rhizosphere microbial community, promote the reproduction of organic nitrogen mineralizing bacteria, associative nitrogen-fixing bacteria, etc., and provide plants with more available nitrogen sources. Therefore, the dual symbiosis of rhizobia and mycorrhizal fungi can complement each other and play a synergistic role in meeting the nitrogen needs of legumes.

4.2 Improved adaptation to drought and salinity

Leguminous crops often have limited growth under abiotic stress conditions such as drought and salinity, and symbiosis with beneficial microorganisms can significantly enhance their stress resistance. In this regard, mycorrhizal fungi and rhizosphere growth-promoting bacteria (PGPR) have played an important role. Under drought stress, arbuscular mycorrhizal fungi can improve the drought resistance of legumes in various ways. AM fungal symbiosis can promote the improvement of root morphology and function of host plants, such as increasing root length and root hair density to absorb water more effectively; mycorrhizal hyphae can bring additional water to plants from deep soil or compact pores to maintain a high moisture condition in the plant body. AM fungal infection can induce the host to improve water use efficiency, reduce stomatal aperture and thus reduce transpiration water loss. More importantly, mycorrhizal symbiosis can trigger a series of physiological and biochemical changes in plants, making them better tolerant to drought: including upregulating antioxidant enzyme activity (such as superoxide dismutase SOD, peroxidase POD, etc.), accumulating osmotic regulating substances (such as proline, soluble sugars), and regulating plant hormone balance (such as increasing abscisic acid to close stomata). Hashem et al. (2019) studied chickpea and showed that plants inoculated with AM fungi combined with biochar showed higher leaf moisture, chlorophyll content and antioxidant capacity under drought, and thus the yield was significantly higher than that of the uninoculated control. Al-Arjani et al. (2020) reported that AM fungal symbiosis promotes antioxidant defense and gene expression adjustment of the desert plant Ephedra under drought stress, thereby maintaining its growth. On forage grasses such as alfalfa, it was similarly observed that inoculation with mycorrhizae can reduce the inhibition of nodule development and nitrogen fixation by drought and improve nitrogen fixation efficiency under drought conditions. These studies consistently show that mycorrhizal fungi can significantly improve the stability of legume crops under dry farming environments by improving the physiological adaptability of the host, enabling legumes to maintain good growth and nutrient acquisition under water stress conditions.

For saline-alkali stress, beneficial microorganisms can also play a buffering role. High salt conditions can interfere with the symbiotic process of legumes, such as inhibiting nodule formation and reducing nitrogen fixation activity. However, salt-tolerant rhizosphere growth-promoting bacteria and some salt-tolerant rhizobia strains can help plants reduce salt damage. Their mechanisms of action include: secreting mucopolysaccharides to fix salt outside the cell, reducing the concentration of free Na^+ in the soil solution, thereby reducing the amount of salt ions absorbed by the plant; producing growth hormone (IAA) to promote plant root development to avoid surface salt; synthesizing ACC deaminase to reduce plant ethylene levels and alleviate premature aging caused by salt stress; and inducing the plant's antioxidant system and osmotic regulation. Sridhar et al. (2025) isolated a salt-tolerant Bacillus flexus, which was inoculated into sesame to significantly improve the survival and growth of plants under 100 mM~200 mM NaCl salt stress. The chlorophyll, soluble sugar and proline contents of sesame leaves treated with the inoculated bacteria increased significantly, while membrane damage indicators such as malondialdehyde decreased, and the activities of antioxidant enzymes such as superoxide dismutase and catalase were significantly enhanced, indicating that oxidative damage in the plant body was reduced and cell membrane stability was improved. Finally, the treated sesame plants maintained a good growth state and yield under high salt conditions. Similarly, many studies have confirmed that inoculation with suitable PGPR strains can improve the salt tolerance of legume crops. For example, after inoculation of peas and alfalfa with salt-tolerant Pseudomonas and *Bacillus brevis*, the electrolyte leakage and peroxide levels of the plants decreased, indicating that salt stress damage was alleviated. The screening and application of salt-tolerant rhizobium strains have also made progress. It has been reported that a Chinese rhizobium strain that can effectively nodulate and fix nitrogen in a relatively high-salt environment has been screened out. Inoculating it into legumes can improve nodulation and growth on saline-alkali soils to a certain extent. These results are of positive significance for improving saline soils and developing salt-tolerant legume crops using symbiotic systems.

4.3 Activation of plant immunity and biocontrol potential

In addition to direct nutrient supply, the interaction between beneficial microorganisms and legumes can also indirectly improve the resistance of legumes to diseases and pests by inducing the plant's own immune defense mechanism, which is the so-called "induced systemic resistance" (ISR) effect. Many rhizosphere growth-promoting bacteria and endophytic nitrogen-fixing bacteria have been found to have the effect of activating plant immunity. They can induce plants to produce broad-spectrum disease resistance without causing diseases and are considered to be potential biological control forces.

In legumes, some rhizobia can induce plant resistance by themselves. For example, during the invasion of symbiotic rhizobia, although plants reduce some immune responses to facilitate symbiosis, they also initiate low-level activation of certain defense pathways. This "mild" immunity is believed to increase the vigilance of plants against other pathogens. Studies have shown that after inoculation with rhizobia, the salicylic acid and jasmonic acid signaling pathways in legumes will change, which will accelerate the response of plants to subsequent pathogen infections. For example, Diaz-Valle et al. (2019) reported that inoculating beans with Rhizobium etli, which is effective in nitrogen fixation, not only increased their growth, but also induced the expression of defense-related genes in plants, showing stronger resistance to insect pests. This shows that symbiotic nitrogen-fixing bacteria can also act as "immune inducers."

More extensive research is on PGPR. After many fluorescent Pseudomonas, *Bacillus subtilis*, etc. colonize the rhizosphere of legumes, they will secrete some volatile organic compounds or secondary metabolites to stimulate plants to initiate ISR. The results show that the activity of resistance-related enzymes (such as phenylalanine ammonia lyase and peroxidase) in plant leaves increases, and defense substances such as lignin accumulate, which can limit the expansion of pathogens more quickly when encountering them. Experiments on crops such as peas and alfalfa have shown that the incidence of powdery mildew and damping-off disease decreases after the application of growth-promoting agents, which is exactly the ISR at work. Microbial-induced resistance is usually broad-spectrum and can have a certain inhibitory effect on multiple diseases and pests at the same time, unlike disease-resistant varieties that are specific. Therefore, using beneficial microorganisms as biopesticides or biocontrol agents is one of the green measures to reduce dependence on chemical pesticides.

Another potential of the legume-microorganism symbiotic system in biological control is the "barrier" effect of directly inhibiting soil-borne pathogens. Some members of the legume symbiotic microbial community have the ability to antagonize pathogenic microorganisms. For example, Bacillus colonized in the rhizosphere can produce antibiotics such as lichenin to inhibit the reproduction of fungal pathogens; Pseudomonas can compete for iron ions, carbon sources, etc., and take away the survival resources of pathogens. These effects can reduce the number of pathogens in the rhizosphere of legumes, thereby reducing the chance of infection. The luxuriant root system and large amount of mucus produced by legumes due to symbiosis also physically form a rhizosphere microenvironment dominated by beneficial bacteria, which is not conducive to the colonization of pathogens. Xu et al. (2021) found that the number of beneficial actinomycetes and actinomycetes in the soil of orchards planted with leguminous green manure increased, while the number of soil-borne pathogenic fungi such as Fusarium and Fusarium decreased, thereby reducing the incidence of root diseases in fruit trees. It can be seen that the legume symbiotic system can inhibit and prevent diseases by changing the structure of the rhizosphere microbial community.

5 Ecological Benefits of Legume-Microbe Symbiosis

5.1 Reducing chemical fertilizer use and greenhouse gas emissions

Vigorously developing and utilizing the legume-microbe symbiotic system can produce significant ecological and environmental benefits, the primary manifestation of which is to reduce the application of fertilizers and achieve a dual reduction in agricultural non-point source pollution and greenhouse gas emissions. As mentioned above, legumes can obtain a large part of their own nitrogen needs through symbiotic nitrogen fixation, thereby reducing nitrogen fertilizer input. Large-scale planting of legumes is regarded as a "biological nitrogen fertilizer" strategy that can reduce dependence on fertilizers at the regional and national scales. According to an analysis by the Acta Ecologica Sinica, under the scenario of optimizing the planting structure of major grain crops in China, an increase in the planting proportion of legumes will significantly reduce agricultural N_2O emissions, making an outstanding contribution to greenhouse gas emission reduction. The study by Mahama et al. (2020) specifically quantified this effect: compared with the conventional full nitrogen application, the planting system that introduced legume cover crops and reduced nitrogen fertilizers accordingly reduced soil N_2O emissions by 25% to 50%. The main mechanisms of leguminous crops in reducing greenhouse gases include: first, by replacing part of the chemical fertilizers, fossil energy consumption and indirect N_2O emissions in the process of fertilizer production and use are reduced; second, the nitrogen release of symbiotic nitrogen fixation is more stable, avoiding the peak phenomenon of excessive inorganic nitrogen in the soil being converted into N_2O. Therefore, promoting the symbiotic system of leguminous crops is conducive to controlling agricultural greenhouse gas emissions.

More broadly, the use of leguminous-microorganism symbiosis can also help reduce the dependence on fertilizers for other elements in agriculture. For example, mycorrhizal fungal symbiosis can improve the absorption efficiency of leguminous plants for nutrients such as phosphorus and potassium, thereby reducing the application of phosphorus and potassium fertilizers. This is meaningful for reducing the consumption of phosphate resources and preventing eutrophication of water bodies. Similarly, the large amount of organic matter and nutrients provided by leguminous green manure can replace part of the input of organic fertilizers and trace element fertilizers. Yu et al. (2021) pointed out that through the rotation or intercropping of Gramineae and Leguminosae, the nutrient cycle of farmland can be optimized, and the application and loss of chemical fertilizers can be significantly reduced while ensuring yield. In my country's efforts to reduce the use of chemical fertilizers and pesticides, developing leguminous crops as green manure is one of the important measures. At present, the promotion of green manures such as astragalus and sweet clover in southern rice fields and northern orchards has achieved good results, supplementing hundreds of thousands of tons of nitrogen nutrients to farmland every year and reducing the corresponding amount of chemical fertilizer application. It can be seen that the legume-microorganism symbiotic system provides a practical way to reduce agricultural inputs and build an environmentally friendly agriculture.

5.2 Restoration of degraded soil functions

Soil degradation (such as organic matter depletion, acidification, salinization, etc.) is an important issue threatening agricultural sustainability. The use of legume-microorganism symbiosis can restore the ecological function of degraded soil to a certain extent. First of all, for nutrient-deficient degraded land, planting leguminous plants and accumulating soil nitrogen through symbiotic nitrogen fixation is one of the effective means to restore soil fertility. In ecological restoration projects, leguminous shrubs or forages with strong nitrogen fixation ability are often planted first to improve barren soil. For example, planting alfalfa, clover, etc. in abandoned mining areas and desert margins will significantly increase the total nitrogen and organic matter content of the soil after a few years, and the productivity and stability of the plant community will also increase. Bakhoum et al. (2018) pointed out that legumes can even survive in poor sandy or nutrient-deficient environments, thanks to the nutrient support provided by their rhizobia and mycorrhizal fungi symbionts, which makes legumes a key species in the ecosystem and can be used for vegetation restoration in fragile habitats.

Secondly, legumes and their symbiotic microorganisms can improve the biodiversity and food web structure of degraded soils. Degraded soils often have impaired microbial community functions, and the roots of legumes continuously transport organic carbon and nitrogen, which is conducive to the reconstruction of soil microbial flora. In particular, after rhizobia and mycorrhizal fungi colonize in degraded soils, they not only benefit from legumes, but also promote the reproduction of other beneficial microorganisms through joint action, gradually restoring the balance of soil microecology. Chen et al. (2020) believe that the reconstruction of soil microbial communities is the key to ecological restoration of degraded land, and planting symbiotic nitrogen-fixing plants can help to rebuild this microbial community as soon as possible. Leguminosae are important hosts for many indigenous mycorrhizal fungi. Their introduction can restore the soil mycorrhizal network, thereby promoting the settlement of other plants and improving vegetation diversity. Therefore, in the management of degraded grasslands, sandy lands and saline-alkali lands, leguminous plants are often mixed with other plants to play the role of "soil improvement" and "microbial restoration". Experiments have compared the differences between annual and perennial leguminous forage in terms of overproduction and diversity effects on degraded grasslands, and found that the introduction of leguminous plants can significantly improve grassland productivity and biodiversity, and enable degraded grassland vegetation to recover faster.

Thirdly, for specific degraded types of soils such as salinization and acidification, the leguminous plant symbiotic microbial system also has a certain corrective effect. As mentioned above, rhizobium symbiosis can partially neutralize soil acid and increase pH; the combination of salt-tolerant growth-promoting bacteria and leguminous plants can reduce the effective concentration of soil salt (Wu and Yan, 2024).

5.3 Enhancing agroecosystem stability

A stable and efficient agricultural ecosystem requires diverse nutrient cycle pathways and robust biological networks. The introduction of legume crop-microorganism symbiosis increases the biological pathways and food web complexity of farmland nutrient cycles, thereby helping to improve the stability and resilience of the entire agricultural ecosystem. First, as a part of the nitrogen cycle, legume symbiotic nitrogen fixation complements fertilizer nitrogen, making farmland nitrogen supply more continuous and diversified. When fertilizer supply fluctuates or decreases, symbiotic nitrogen fixation can partially fill the gap and ensure crop growth. This "dual-track" nitrogen supply mechanism improves the buffering capacity of the production system against external dependence. Studies have shown that in years with low nitrogen fertilizer supply, the crop yield fluctuations of plots with legumes in rotation are small, while the yield of plots with pure nitrogen-consuming crops in rotation declines significantly. This shows that legume symbiotic nitrogen fixation provides a stabilizer for the system. In addition, the nitrogen fixed by the symbiotic system is gradually released in an organic form, reducing the accumulation of inorganic nitrogen and the risk of sudden nitrogen loss caused by heavy rain erosion, which is also conducive to the stability of nutrient supply.

Secondly, legumes play a role in enriching biodiversity in crop rotation and intercropping. Yu et al. (2021) concluded that intercropping/rotation between Gramineae and Legumes can reduce competition between crops and inhibit pests and diseases through temporal and spatial niche dislocation and root system interactions, thereby improving the stability of the entire farmland ecosystem. On the one hand, the addition of legumes breaks the cycle host of pests and diseases and reduces the obstacles of continuous cropping; on the other hand, due to its nitrogen fixation and promotion of microorganisms, it improves the growth environment of subsequent crops. This positive feedback improves the self-regulation ability of the system and reduces the interannual and spatial variation of yield. Many long-term experiments have shown that multi-crop rotations containing legumes have more stable yields, slower soil fertility decline, and stronger resistance to extreme climate than single-crop systems. For example, in North American experiments, two-year corn-soybean rotations have lower N_2O emissions and lower yield variation coefficients than continuous corn planting systems, and are considered a model that both reduces emissions and stabilizes yields.

Symbiotic microorganisms give legumes a certain degree of stress resistance and disease resistance, which will also enhance the overall resilience of agricultural ecosystems. When drought, high temperature or pest and disease epidemics occur, legume crops with microbial symbiosis protection may show stronger adaptability and avoid being wiped out, thus playing a role in stabilizing yields in multi-crop systems. For example, in mixed grasslands, grasslands containing symbiotic nitrogen-fixing legumes have a smaller decline in yield in drought years than pure grass grasslands and recover faster. For example, in the rice-legume green manure rotation, when the supply of chemical fertilizer is insufficient in a certain year, the nitrogen-fixing nutrients provided by the previous crop of legume green manure ensure that the rice yield does not decrease. This reflects the contribution of the symbiotic system to the stability of the system.

6 Case Studies: Field Applications of Symbiotic Relationships

6.1 Long-term trials on soybean-rhizobium systems

The symbiotic nitrogen fixation system of soybean (soybean) and rhizobium is one of the most extensively studied and applied legume symbiotic models. In the Northeast Black Soil and other regions, long-term positioning experiments have been used to evaluate the role of soybean-rhizobium symbiosis in sustainable production. A typical experiment compared the nutrient absorption and yield changes of soybean continuous cropping under different fertilization treatments. The results showed that: soybeans without nitrogen fertilizer but inoculated with rhizobia every season, their nitrogen absorption and yield can be maintained at a high level, and the nitrogen absorption is 30.5% to 100% higher than that of the non-inoculated treatment; the number of soybean nodules inoculated with rhizobia also increased significantly (an average increase of 9.3% to 53.8%), and the nitrogenase activity remained stable. These data show that in the soybean continuous cropping system, rhizobium symbiotic nitrogen fixation effectively compensates for the lack of exogenous nitrogen and supports the long-term stability of soybean yield. More interestingly, microbiome analysis found that continuous soybean cultivation without nitrogen fertilizer will gradually shape a unique rhizosphere microbial community: some functional bacteria related to nitrogen fixation (such as *Bradyrhizobium*, *Azospirillum*, etc.) are enriched, while the abundance of such beneficial bacteria is lower under excessive nitrogen fertilizer treatment (Figure 2) (Wei et al., 2023; Huang, 2024). The rhizosphere bacterial diversity of soybean plots without nitrogen application for a long time is higher, and the community structure tends to be more beneficial functional types such as nitrogen fixation and phosphorus solubilization, which is considered to be one of the reasons why soybeans can still maintain yield under low nutrient input. However, the long-term treatment without phosphorus fertilizer has observed a decrease in the abundance of rhizosphere microorganisms and hindered community development, indicating that the symbiotic system also requires other nutrient combinations.

6.2 Peanut-microbial inoculant synergy for yield enhancement

Peanut is an important leguminous oil crop, and its symbiotic nitrogen fixation also occupies a place in agricultural production. In recent years, research on improving peanut yield and resource utilization by inoculating high-efficiency bacterial agents has made considerable progress. Ding et al. (2024) reported a field plot experiment to investigate the effects of rhizobium seed dressing on peanut growth and benefits under different nitrogen reduction fertilization levels. The experiment set up six nitrogen application levels, including conventional fertilization (100% nitrogen) and nitrogen reduction of 20%, 40%, 60%, 80%, and 100%. Peanuts with rhizobia seed dressing were planted at each level, and conventional fertilization without seed dressing was used as a control. The results showed that inoculation with rhizobia significantly increased the number of nodules and growth of peanut plants, and this gain was more obvious under nitrogen reduction treatment. In particular, in the treatment of 40% nitrogen reduction (nitrogen application rate was only 60% of the conventional one) and rhizobia inoculation, the growth indicators of peanuts were the best: the number of nodules reached the maximum at the flowering needle stage, which was 2.3 more per plant than the control (no nitrogen reduction and no inoculation), and agronomic traits such as plant height, lateral branch length, and number of effective branches were all improved. Finally, the peanut yield of this treatment reached 5 216 kg/hm^2, an increase of 5.3% over the control, and the net income increased by 2 526 yuan/hm^2, with significant benefits. On the contrary, the peanut yield under the pure nitrogen reduction treatment without inoculation decreased significantly with the increase of nitrogen reduction, and a significant reduction occurred when the nitrogen reduction was more than 80%. This shows that the appropriate nitrogen reduction combined with inoculation can achieve the "stable yield and increased efficiency" of peanuts, which not only reduces the amount of chemical fertilizers but also maintains the yield and income.

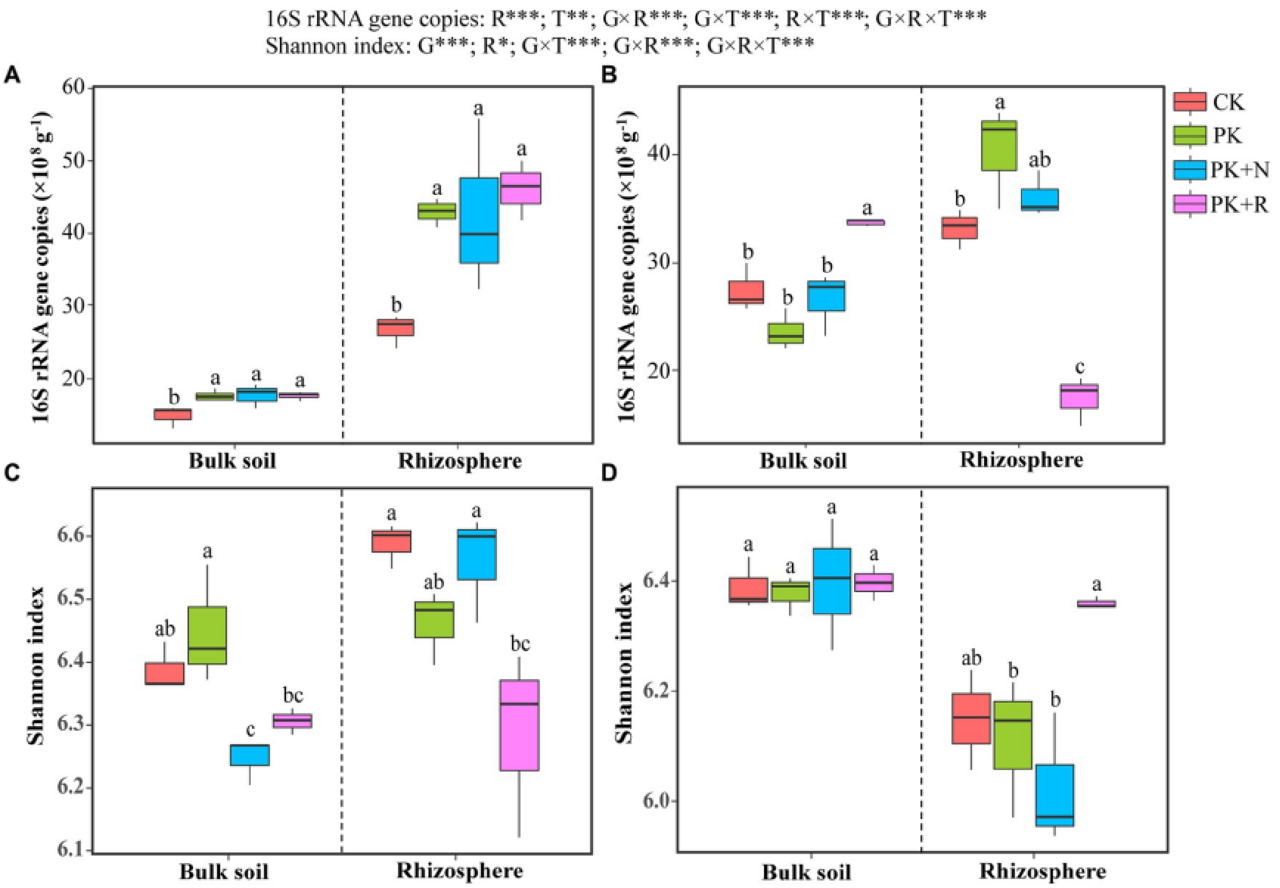

Figure 2 Bacterial abundance (A, B) and richness (C, D) in soybean field trials at the flowering–podding stage (A, C) and maturity stage (B, D). CK: non-inoculated control in soil; PK, superphosphorus and potassium chloride; PK + N, PK chemical fertilizers plus urea; PK + R, PK chemical fertilizers plus *Bradyrhizobium japonicum* 5821. Different letters above bars indicate significant differences (one-way ANOVA, $p < 0.05$, Duncan's multiple-range test) among different treatments at each growth stage. The overall effects of growth stage (G), rhizosphere effect (R), and treatment (T) on bacterial abundance and Shannon index were evaluated by three-way ANOVA, with the results shown at the top of the figure. *$0.01 < p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ (Adopted from Wei et al., 2023)

The successful case of this experiment shows that microbial agents (such as rhizobia agents) have great potential for field application. By scientifically selecting excellent strains and coordinating with fertilization systems, the symbiotic system of leguminous crops can achieve greater output under low input conditions. This is particularly important for modern agriculture that focuses on input-output ratio and environmental benefits. For example, in some peanut production areas, due to continuous planting, soil nutrients are unbalanced and nutrient utilization rate decreases. After the introduction of rhizobia agents, not only biological nitrogen sources are provided, but also the absorption rate of peanuts to residual nutrients (such as phosphorus) in fertilizers is improved, and the yield increases under the combined effect. The peanut case also reflects a key point: the symbiotic nitrogen fixation system does not operate in isolation, and it has a synergistic effect and critical balance with chemical nutrient input. Moderate nitrogen reduction can encourage plants to rely more on symbiotic nitrogen fixation, thereby giving full play to the role of rhizobia, but excessive nitrogen deficiency signals may inhibit plant growth and are not conducive to the start of symbiosis. In the experiment, the 40% nitrogen reduction treatment obviously found a "sweet spot", which is conducive to symbiotic nitrogen fixation and guarantees the basic nitrogen supply, achieving the complementarity of the two. This suggests that when promoting symbiotic bacteria agents, soil nutrient conditions and reasonable fertilization formulas must also be considered to achieve the best results.

6.3 Soil microbial community restructuring via legume-based rotation

The introduction of leguminous crops into the farmland rotation system not only improves the balance of nutrient income and expenditure, but also has a profound impact on the soil microbial community, playing a role in "reconstructing" the soil microecology. A research review by Yu et al. (2021) showed that in long-term monoculture systems, soil microbial diversity tends to decline and functions tend to be single. However, by rotating legumes with other crops, rhizosphere carbon and nitrogen inputs can be increased, microbial food sources can be enriched, and the originally unbalanced soil microbial community can be revitalized. In particular, in legume/grass rotations, the organic nitrogen and organic matter increased by legume nitrogen fixation in one season provide a "nutritional bedding" for the next season of gramineous crops. At the same time, the microbial groups involved in the carbon and nitrogen cycle in the soil have also changed significantly. Studies have found that in legume rotation systems, the richness of functional bacteria such as nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and cellulose-decomposing bacteria in the soil is often higher than that in monoculture systems, while the ratio of some pathogens and antagonistic bacteria is more balanced, which makes the soil food web more complex and stable. For example, an experiment compared the differences in soil microorganisms between corn-soybean rotation and continuous corn cropping. The results showed that beneficial bacteria such as actinomycetes and Trichoderma increased significantly in the rotation soil, the density of pathogenic fungi of corn damping-off decreased, and the diversity of corn rhizosphere microbial communities increased. This shows that legumes play a positive role in regulating soil microbial communities in crop rotation, which is conducive to forming an ecological environment with a relative balance between beneficial bacteria and potentially harmful bacteria, thereby reducing the risk of diseases and improving the production stability of the entire system.

Another case comes from the green manure system in southern rice fields. After long-term implementation of rice-legume green manure (such as astragalus) rotation, the community structure of facultative anaerobic nitrogen-fixing bacteria and denitrifying bacteria in the soil has changed, and the potential for N_2O production in the soil nitrogen cycle pathway has decreased. This means that legume green manure can also reduce nitrogen loss and greenhouse gas emissions in rice fields through microbial community regulation. Legume residues promote the development of some heterotrophic nitrogen-fixing bacteria, which can continue to fix nitrogen in the flooded and anoxic environment of rice fields, bringing additional value to the rice field ecosystem (Wang et al., 2021). At the same time, legume symbiotic bacteria do not completely disappear after a growth period. Studies have found that some rhizobia can survive in a free state in the soil, waiting to be re-infected when the next legume crop is sown. Therefore, in the legume rotation system, over time, the "seed pool" of symbiotic bacteria in the soil is continuously enriched, and the resources of native rhizobia increase, which also has a cumulative effect on the efficiency of symbiotic nitrogen fixation.

7 Technological Integration and Pathways for Green Development

7.1 Integration of legume-microbe systems with emerging agricultural technologies

To fully unleash the potential of legume-microorganism symbiosis system, it is necessary to combine it with new modern agricultural technologies to form synergistic effects. First, in terms of biotechnology, the optimization of microbial agents and inoculation technology is key. With the advancement of isolation and identification technology of nitrogen-fixing bacteria and growth-promoting bacteria, we can screen and obtain more efficient and more adaptable strains to the local environment to make inoculants. For example, commercial soybean rhizobium agents have been promoted in many countries around the world, and their application has significantly increased soybean yield and protein content. In the future, through genetic engineering, we may cultivate improved rhizobia strains with stronger nitrogenase activity, wider host range, and even plant growth stimulants. Some studies have attempted to knock out genes that negatively regulate symbiosis in rhizobia or introduce exogenous stress resistance genes, and the results show that the symbiotic adaptability of strains can be enhanced. At the same time, synthetic biology can also be used to design "modular" symbiotic systems, such as transferring essential genes for legume nodulation into certain non-legume crops to enable them to acquire nodulation-like abilities (Dong and Cao, 2019). Although it is still far from practical use, it has been listed as one of the potential revolutionary technologies to solve the global nitrogen fertilizer problem.

In terms of crop breeding, it is also necessary to pay attention to the improvement of traits related to the interaction between leguminous crops and symbiotic microorganisms. Traditional breeding focuses on yield, and the selection of symbiotic nitrogen fixation ability is insufficient. At present, breeding work on soybeans, alfalfa, etc. has begun to evaluate the nodulation and nitrogen fixation efficiency of varieties, and high nitrogen fixation efficiency is one of the breeding goals. In addition, stress-tolerant symbiotic varieties are also a direction, such as screening out leguminous varieties that can still effectively nodulate under low phosphorus, drought, and salinity conditions. In recent years, molecular breeding methods have helped us find many key symbiotic genes, which provides tools for targeted improvement. For example, gene editing can be used to knock out host genes that negatively regulate nodulation, or overexpress genes that regulate the positive pathway of symbiosis, thereby cultivating "more microbial-friendly" crops. Some experiments have successfully achieved cases of editing receptor kinase genes to increase the number of nodules in Medicago truncatula, providing a model for other legumes.

Modern agricultural technology also includes the integration of precision planting and information technology. We can use sensors to monitor nitrogen dynamics and plant nutrition in the soil, and combine model decision-making to reduce nitrogen fertilizer application during the period when symbiotic nitrogen fixation is strongest, so as to achieve the best match between fertilizer input and biological nitrogen fixation. UAV remote sensing technology can identify whether leguminous crops are nodulated normally, and feedback the symbiotic effect through plant growth and leaf color to guide field management. Agricultural big data and artificial intelligence can also be used to predict and optimize symbiotic systems. For example, according to meteorological forecasts of symbiotic nitrogen fixation potential, the sowing ratio or inoculation amount can be adjusted in advance to avoid unfavorable conditions. More cutting-edge rhizosphere microbiome can monitor soil microbial community succession through gene sequencing and analysis, so as to determine whether the leguminous symbiotic system is running healthily. Once the proportion of beneficial bacteria is found to decrease, the microbial agent can be supplemented in time or the planting plan can be adjusted. The introduction of these intelligent and precise tools will greatly improve the controllability and efficiency of legume-microorganism symbiosis in agricultural production, and give traditional "biological means" the wings of modern technology.

7.2 Synergy with ecological farming models

The legume crop-microorganism symbiotic system naturally fits the concept of ecological agriculture and has broad application space in organic agriculture, circular agriculture and other models. First of all, the use of chemical synthetic nitrogen fertilizers is prohibited in organic planting systems, and legume symbiotic nitrogen fixation becomes one of the main sources of nitrogen. Therefore, organic farms widely adopt legume green manure rotation and high-proportion legume forage forage planting to maintain the nutrient balance of farmland and grassland. For example, in organic rice production, the rotation system of one season of rice and one season of astragalus green manure not only meets the nitrogen demand of rice, but also suppresses weeds and pests, achieving stable production. Another example is the planting of alfalfa and clover in organic pastures to replace nitrogen fertilizer application, and the recycling of nitrogen is achieved by returning livestock manure to the fields. Facts have proved that these models based on legume nitrogen fixation can fully achieve productivity comparable to conventional agriculture while significantly reducing environmental load. Ecological agriculture also emphasizes reducing the use of pesticides, and the systemic resistance and soil health improvement effects induced by the legume symbiotic system can precisely reduce the risk of crop diseases and pests. Therefore, in ecological agricultural demonstration areas, we can often see intercropping of leguminous crops and other crops, and the application of symbiotic agents. These measures together build a more stable and low-cost farmland ecosystem.

The legume symbiotic system can also be combined with animal husbandry, forestry, etc. to form a comprehensive model. For example, in the "rice field crab farming" model, leguminous forage is planted on the ridge to fix nitrogen, which not only provides feed for field crabs but also fertilizes the soil, realizing a planting and breeding cycle. In the forestry and fruit industry, planting leguminous green manure between orchard rows has been proven to significantly improve soil and reduce pests and diseases, and is an important technology for orchard ecological management (Cheng et al., 2022). When afforestation is carried out on barren hills, leguminous shrubs and mycorrhizal fungi inoculation are often used to increase the survival rate of afforestation seedlings and the speed of soil improvement, which reflects the ecological benefits of combining agriculture and forestry and using fungi to help forestry. It can be foreseen that future ecological agriculture will more systematically integrate leguminous crops, symbiotic microorganisms and multiple industries to form a closed-loop agricultural ecosystem. For example, the circular agricultural model combining "grain-economic-feed-fertilizer": growing leguminous forage (feed) to raise livestock and poultry to produce fertilizer, and then using fertilizer to raise grain and economic crops; then using livestock waste to cultivate microbial agents for leguminous crops to inoculate, and so on, to maximize the potential of each link. Leguminous symbiotic nitrogen fixation provides a biological nitrogen source and soil fertilization link, which is one of the key supporting technologies. Therefore, we should strengthen the research on the role of symbiotic systems under different ecological agricultural models, and integrate them into organic, circular and other models according to local conditions to achieve synergistic efficiency.

7.3 Strategies for promoting regional agricultural sustainability

To promote the legume-microorganism symbiosis to serve the green transformation of agriculture on a larger scale, it is necessary to work together from multiple aspects such as policy, promotion, and science and education. First, at the policy level, governments at all levels can introduce incentives to encourage the planting of leguminous green manure and grain-bean rotation. For example, in the pilot program of the crop rotation and fallow system that has been implemented in my country, certain subsidies are given to the planting of green manure and leguminous crops. In the future, subsidies for nitrogen-fixing green manure can be further increased to motivate farmers to add leguminous crops to the staple food rotation. At the same time, a production and marketing guarantee system for leguminous crops should be established, such as developing a soybean revitalization plan to improve the income of soybean farmers and ensure the steady increase in the sown area of leguminous crops. With the expansion of the planting area of leguminous crops such as soybeans, the symbiotic nitrogen fixation effect in the soil will accumulate, the use of chemical fertilizers in the whole society is expected to achieve a turning point decline, and agricultural non-point source pollution will be alleviated.

Secondly, in the promotion of agricultural technology, it is necessary to strengthen the training and demonstration of microbial agents and scientific fertilization and weight loss technology. Many farmers do not have enough understanding of the nitrogen fixation potential of leguminous crops, and they are still accustomed to applying excessive nitrogen fertilizers to peanuts and soybeans, resulting in the inhibition of symbiotic nitrogen fixation. The methods of leguminous nitrogen reduction planting should be disseminated to farmers through field schools, technical manuals, etc., such as seed dressing with rhizobia agents, and appropriate control of inorganic nitrogen supply during the seedling stage, so as to maximize the utilization of symbiotic nitrogen fixation. At the same time, the "one spray, multiple effects" or "symbiotic package" technology should be promoted. For example, biological fertilizers can be sprayed on leguminous crops, which not only provide rhizobia but also contain growth-promoting bacteria and phosphate-solubilizing bacteria, so as to achieve multiple effects in one application and increase farmers' enthusiasm for adoption (El Attar et al., 2019; Kajić et al., 2020). The construction of demonstration bases is also very important. Typical areas can be selected to establish comparative fields for leguminous rotation, green manure utilization and microbial agent application to intuitively demonstrate their yield increase and environmental protection effects and change traditional concepts.

Finally, in terms of scientific research and education, it is necessary to cultivate interdisciplinary talents and strengthen basic research investment. Symbiotic nitrogen fixation involves fields such as microbiology, plant nutrition, and soil science, and compound talents are needed to promote technology integration and innovation. Colleges and universities and research institutes should strengthen the training of relevant professional talents, such as offering courses on biological nitrogen fixation and ecological agriculture to attract young scientists to join this field. At the same time, the country should continue to support basic research such as the mechanism of symbiotic nitrogen fixation and the development of new microbial agents. Only by making breakthroughs in in-depth mechanism research can we provide a steady stream of new ideas for application. For example, attempts to achieve root nodule symbiosis of rice and other cereal crops through synthetic biology, despite the difficulties, will bring disruptive changes once successful. Another example is the exploration of symbiotic strains in extreme environments, which can provide reserves for future agricultural challenges under climate change. Scientific research should also be closely integrated with practical problems, develop special symbiotic technologies for the needs of different industries (rice fields, orchards, grasslands, etc.), and establish a complete field monitoring and evaluation system. Through industry-university-research cooperation, laboratory results can be quickly transformed into new varieties, new bacterial agents, and new models that farmers can use, truly realizing the leadership of science and technology in the sustainable transformation of regional agriculture.

8 Concluding Remarks

The symbiotic relationship between legume crops and soil microorganisms (especially nitrogen-fixing rhizobia and mycorrhizal fungi) is a valuable asset given by nature to agricultural ecosystems. This study reviews the biological basis and ecological benefits of this symbiotic system. It can be seen that it not only provides nitrogen nutrients for legumes themselves, achieving efficient and low-consumption production; it also improves the soil environment by promoting the accumulation of soil organic matter, activating soil enzymes and optimizing soil structure; at the same time, it enhances the resistance of legumes to adversities such as drought and salinity, as well as pests and diseases, and reduces the risks of agricultural production. At the ecological level, the legume-microorganism symbiotic system helps to reduce the use of fertilizers and pesticides, reduce agricultural greenhouse gas emissions, restore the functions of degraded soils, and improve the diversity and stability of agricultural ecosystems. These effects make it an indispensable component of sustainable agriculture. It can be said that without the extensive participation of legumes and their symbiotic nitrogen fixation, there will be no complete nutrient cycle chain in the agricultural ecosystem; and by making full use of this symbiotic relationship, we can partially get rid of our dependence on fossil resources and move towards a greener and more efficient agricultural model.

Looking ahead, achieving greater achievements of legume-microbe symbiosis in agriculture requires a close combination of research and practice. On the one hand, research work should move from the laboratory to the field, and continuously optimize technology for actual production problems. For example, according to the soil and crop conditions in different regions, select local excellent symbiotic strains and develop commercialized microbial agents, improve the operating procedures of seed dressing and seed soaking for legume crops, and improve the field effect and stability of microbial agents. For example, in planting areas with a high degree of mechanization, develop coated microbial agents suitable for mechanical sowing or slow-release nitrogen-fixing "biofertilizers" to facilitate large-scale promotion and application. On the other hand, field practice also raises new topics for scientific research. The bottlenecks encountered by farmers in promoting symbiotic nitrogen fixation technology (such as sometimes local ineffective bacteria in the soil interfere with the inoculation effect, or high temperature and drought cause the activity of microbial agents to decrease, etc.) need to be solved through scientific research. This benign interaction will accelerate the improvement of technology and truly achieve "good technology can be used and used well". At the same time, a long-term observation platform should be established to track and monitor the comprehensive benefits of the legume symbiotic system in different farmland ecosystems, providing a scientific basis for policy formulation and optimization. For example, through years of positioning experiments, the amount of nitrogen reduction and emission reduction can be quantified, providing data support for the government to promote legume rotation.

With the pursuit of sustainable agricultural development, the importance of the symbiotic relationship between legume crops and soil microorganisms will become more prominent. We have reason to expect that in the future ecological agricultural landscape, legume crops will flourish in wheat fields, rice fields or under orchards, and cooperate with invisible microorganisms to jointly build a self-sufficient and cyclical farmland ecosystem. Fertilizers and pesticides will fade out as a result, and will be replaced by effective management of biodiversity and ecological processes. All this requires the joint efforts of scientific research, technology and policies, as well as a change in the concept of agricultural producers. Judging from the current trend, countries are already taking action: the revitalization of legume green manure in many regions, the revival of crop rotation brought by soybeans and corn, and the rise of the microbial fertilizer industry are all positive signals. Looking to the future, we look forward to integrating traditional wisdom with modern technology through continuous innovation to maximize the potential of the legume-microorganism symbiotic system. While ensuring food security, we will reduce environmental costs and maintain ecological balance while increasing output. The "partnership" between legumes and microorganisms will make a "big contribution" to the sustainable development of agriculture. As long as we make good use of and carefully care for this symbiotic bond, the future of agriculture will surely be greener, healthier and more hopeful.

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Conflict of Interest Disclosure

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References

Al-Arjani A.B.F., Hashem A., amd Abd-Allah E.F., 2020, Arbuscular mycorrhizal fungi modulates dynamics tolerance expression to mitigate drought stress in *Ephedra foliata* Boiss, Saudi Journal of Biological Sciences, 27(2): 380-394. https://doi.org/10.1016/j.sjbs.2019.10.008

Bakhoum N., El-Borai A., Seleem M., and Darwesh O.M., 2018, Rhizobial inoculation enhances legume productivity and soil fertility in degraded sandy soils, Archives of Agronomy and Soil Science, 64(8): 1080-1091. https://doi.org/10.1080/03650340.2017.1405360

Chen W., Wang J., and Lin Y., 2020, Legume-induced microbial community shifts support restoration of degraded soils via nitrogen fixation and plant-microbe interactions, Applied Soil Ecology, 147: 103407. https://doi.org/10.1016/j.apsoil.2019.103407

Cheng H., Hu W., Zhou X., Dong R., Liu G., Li Q., and Zhang X., 2022, Fruit tree legume herb intercropping orchard system is an effective method to promote the sustainability of systems in a karst rocky desertification control area, Forests, 13(10): 1536. https://doi.org/10.3390/f13101536

Díaz-Valle A., López-Calleja A.C., and Álvarez-Venegas R., 2019, Enhancement of pathogen resistance in common bean plants by inoculation with *Rhizobium etli*, Frontiers in Plant Science, 10: 1317. https://doi.org/10.3389/fpls.2019.01317

Ding B., Feng M.S., Wang R., Chang L., Jiang Y., Xie J.X., and Wang L.B., 2024, Effects of different fertilizer application rates and rhizobia interaction on growth and efficiency of peanut, Guangdong Agricultural Sciences, 51(12): 131-141. https://doi.org/10.16768/j.issn.1004-874X.2024.12.012

Dong R., and Cao Y.R., 2019, Research progress on the immune regulation of symbiotic nitrogen fixation between legumes and rhizobia, Biotechnology Bulletin, 35(10): 25-33.
http://dx.doi.org/10.13560/j.cnki.biotech.bull.1985.2019-07160

El Attar I., Taha K., El Bekkay B., El Khadir M., Alami I., and Aurag J., 2019, Screening of stress tolerant bacterial strains possessing interesting multi-plant growth promoting traits isolated from root nodules of *Phaseolus vulgaris* L., Biocatalysis and Agricultural Biotechnology, 20: 101225.
https://doi.org/10.1016/j.bcab.2019.101225

Fahde S., Boughribil S., Sijilmassi B., and Amri A., 2023, Rhizobia: a promising source of plant growth-promoting molecules and their non-legume interactions: examining applications and mechanisms, Agriculture, 13(7): 1279.
https://doi.org/10.3390/agriculture13071279

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

Hashem A., Kumar A., Al-Dbass A.M., Alqarawi A.A., Al-Arjani A.B.F., Singh G., Farooq M., and Abd_Allah E.F., 2019, Arbuscular mycorrhizal fungi and biochar improves drought tolerance in chickpea, Saudi Journal of Biological Sciences, 26(3): 614-624.
https://doi.org/10.1016/j.sjbs.2018.11.005

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

Kajić S., Gradinščak A., Königshofer D., Fadljević I., Rajnović I., and Sikora S., 2020, Characterization of plant growth promoting rhizobacteria and their benefits on soybean growth, Legume Research, 48(1): 131-136.
https://doi.org/10.18805/lrf-819

Luo Z.P., and Xie F., 2019, Mechanism of nitrate regulating symbiotic nitrogen fixation between legumes and rhizobium, Biotechnology Bulletin, 35(10): 34-39.
https://doi.org/10.13560/j.cnki.biotech.bull.1985.2019-0780

Mahama G.Y., Prasad P.V.V., Roozeboom K.L., Nippert J.B., and Rice C.W., 2020, Reduction of nitrogen fertilizer requirements and nitrous oxide emissions using legume cover crops in a no-tillage sorghum production system, Sustainability, 12(11): 4403.
https://doi.org/10.3390/su12114403

Savvas D., Pappa V., Yfantopoulos D., Karkanis A., Travlos I., Bebeli P., Ntatsi G., and Bilalis D., 2017, Impact of organic practices on growth, yield, and greenhouse gas emissions by pea landraces, Acta Horticulturae, 1164: 77-84.
https://doi.org/10.17660/ACTAHORTIC.2017.1164.10

Sridhar D., Alheswairini S.S., Barasarathi J., El Enshasy H.A., Lalitha S., Mir S.H., Nithyapriya S., and Sayyed R., 2025, Halophilic rhizobacteria promote growth, physiology and salinity tolerance in *Sesamum indicum* L. grown under salt stress, Frontiers in Microbiology, 16: 1590854.
https://doi.org/10.3389/fmicb.2025.1590854

Wang E.T., and Zhang F.M., 2021, Evolution and regulation of nitrogen-fixing root nodule symbiosis in legumes, Journal of Integrative Plant Biology, 63(1): 25-40.
https://doi.org/10.1111/jipb.13051

Wang N., Zhao Y.H., Yu J.G., Xue L., Li H., and Yang L.Z., 2021, Roles of bulk and rhizosphere denitrifying bacteria in denitrification from paddy soils under straw return condition, Journal of Soils and Sediments, 21: 2179-2191.
https://doi.org/10.1007/s11368-021-02942-x

Wei W., Guan D., Ma M., Jiang X., Fan F., Meng F., Li L., Zhao B.S., Zhao Y.B., Cao F.M., Chen H.J., and Li J., 2023, Long-term fertilization coupled with rhizobium inoculation promotes soybean yield and alters soil bacterial community composition, Frontiers in Microbiology, 14: 1161983.
https://doi.org/10.3389/fmicb.2023.1161983

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.
https://doi.org/10.5376/msb.2024.15.0009

Xu Y., Shi C.D., and Mu Z.Z., 2021, Effects of green manure intercropping in kiwifruit orchard on soil quality and fruit quality, Hans Journal of Soil Science, 4: 162-165.
https://doi.org/10.12677/HJSS.2021.94020

Yan G.W., Dong W.B., Li Z.Y., Wei C.H., Tang H.Q., Deng Y.S., Zhang Y.T., and He T.G., 2024, Effects of green manure mulching on soil aggregates and organic carbon fractions in orchards, Chinese Journal of Applied Ecology, 35(12): 3427-3434.
https://doi.org/10.13287/j.1001-9332.202412.012

Yang P., Zhai Y.P., Zhao X., Wang S.M., Liu H.L., and Zhang X., 2020, Effect of interaction between arbuscular mycorrhizal fungi and Rhizobium on *Medicago sativa* rhizosphere soil bacterial community structure and PICRUSt functional prediction, Microbiology China, 11: 3868-3879.
https://doi.org/10.13344/j.microbiol.china.190978

Yu X., Xiao S.H., Li S.J., Yang W.T., and Huang G.Q., 2021, Review on crop yield and nitrogen utilization in cereal-legume intercropping system, Chinese Journal of Ecology, 40(8): 2601-2609.
https://doi.org/10.13292/j.1000-4890.202108.027

Zhang C., Li L., Tang Y., Peng Y., and Wu S., 2021, Effects of intercropping leguminous green manure on soil physical properties and fruit tree root growth in a kiwifruit orchard, Agricultural Water Management, 255: 107020.
https://doi.org/10.1016/j.agwat.2021.107020

Zhang W., Li X.G., Sun K., Tang M.J., Xu F.J., Zhang M., and Dai C., 2020, Mycelial network-mediated rhizobial dispersal enhances legume nodulation, The ISME Journal, 14: 1015-1029.
https://doi.org/10.1038/s41396-020-0587-5