

## Rhizosphere Microbial Diversity in Legume Cropping Systems

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**Abstract** Rhizospheric microorganisms associated with leguminous crops constitute a vital component in maintaining the stability of agroecosystems and promoting healthy plant growth; their diversity and functions directly influence soil nutrient cycling, nitrogen fixation efficiency, and crop stress tolerance. This study presents a comprehensive review of rhizospheric microbial diversity in leguminous crops, systematically analyzing the characteristics of the rhizosphere microenvironment, the composition of microbial communities, and their ecological functions. Particular emphasis is placed on exploring the roles of various microorganisms-including bacteria, fungi, and archaea-in plant nutrient uptake, disease suppression, and the maintenance of soil health. Furthermore, the article summarizes the primary factors influencing rhizospheric microbial diversity-such as plant genotype, tillage systems, fertilization methods, and environmental conditions-and introduces the application of modern research technologies, including high-throughput sequencing, metagenomics, and bioinformatics, in the study of rhizosphere microecology. Additionally, using soybean cropping systems as a case study, the paper analyzes variations in microbial community structure under different cultivation patterns and discusses their significance for sustainable agricultural development. Finally, this study outlines the challenges currently facing this field of research and identifies future directions-such as synthetic microbiomes, precision agriculture, and microbial engineering-with the aim of providing a theoretical foundation for the green and efficient production of leguminous crops and the effective management of agroecosystems.

**Keywords** Leguminous crops; Rhizospheric microorganisms; Microbial diversity; Symbiotic nitrogen fixation; Sustainable agriculture

## 1 Introduction

The rhizosphere, the narrow soil zone influenced by plant roots, harbors an immense and still largely unexplored diversity of microorganisms that shape plant nutrition, health, and soil functioning (Chukwuneme and Babalola, 2025). In legume-based systems, this belowground biodiversity underpins key agroecosystem services, particularly biological nitrogen fixation and improved soil fertility, making legumes central to sustainable agriculture and food security (Schaedel et al., 2021). Understanding how legumes assemble and interact with their rhizosphere microbiomes is therefore critical for designing low-input, high-efficiency cropping systems.

Research has established that the rhizosphere microbiome is a central driver of nutrient cycling, carbon sequestration, and ecosystem functioning in terrestrial systems. Microbial communities associated with plant roots form a “second genome” whose collective genes far exceed those of the host plant and are crucial for growth promotion, stress tolerance, and disease suppression. For legumes, the best-known interaction is the symbiosis with rhizobia, but it is now clear that non-rhizobial members of the rhizosphere and nodule microbiome also contribute to nodule formation, plant fitness, and broader agroecosystem benefits (Yang et al., 2024). Given the pressures of climate change and the need to reduce synthetic fertilizer inputs, harnessing this microbial diversity has major significance for sustainable intensification of legume cropping systems.

Recent advances in high-throughput sequencing and multi-omics approaches have transformed understanding of rhizosphere microbiomes by enabling cultivation-independent analysis of taxonomic and functional diversity (Chukwuneme and Babalola, 2025). Large-scale comparative studies show that legumes assemble rhizosphere communities with lower overall diversity but with strong enrichment of nitrogen-cycling taxa and nitrogen-fixing genes relative to non-legumes, revealing a pronounced functional specialization for nitrogen acquisition. At the

same time, factors such as plant species, soil type, and land-use history jointly shape microbial community structure, with bulk soil serving as the main reservoir from which rhizosphere communities are selected. These insights highlight both the selectivity of legumes in recruiting beneficial microbes and the context dependence of microbiome composition across soils and management regimes.

Despite rapid progress, important knowledge gaps remain in how rhizosphere microbial diversity in legume systems can be systematically characterized, predicted, and managed at field and farming-system scales. Most work has focused on individual symbioses or single legume species, while the broader networks of beneficial, pathogenic, and even human-pathogenic microorganisms in the legume rhizosphere remain only partially resolved. The present review aims to synthesize current knowledge on the taxonomic and functional diversity of rhizosphere microbiomes in legume cropping systems, with particular attention to nitrogen fixation, plant health, and agroecosystem services. It also seeks to integrate emerging omics-based insights with ecological theory on community assembly, and to identify opportunities to manipulate rhizosphere communities-via breeding, inoculants, and cropping system design-to enhance the sustainability and resilience of legume-based agriculture.

## 2 Characteristics of the Rhizosphere Microenvironment in Legume Cropping Systems

### 2.1 Root exudates and rhizosphere formation

Legume roots release a wide array of primary and secondary metabolites (sugars, organic acids, amino acids, flavonoids, phenolics) that both feed and signal to rhizosphere microorganisms, thereby structuring the microbial community close to the root (Chen and Liu, 2024). Temporal shifts in exudate composition during plant development generate a “chemical succession” that selects for microbes with matching substrate preferences, creating predictable patterns of community assembly along the soil-root interface (Zhou et al., 2022).

Specific exudate components, especially flavonoids and related phenolic compounds, act as key signaling molecules guiding symbioses and broader rhizomicrobiome recruitment in legumes (Chen et al., 2022; Kumar et al., 2024). These compounds mediate chemotaxis and colonization by beneficial rhizobacteria and mycorrhizal fungi, and under nutrient limitations or other stresses can be modulated to favor microbes that enhance nutrient acquisition and stress tolerance (Gong et al., 2023). In diversified or intercropped systems, changes in legume rhizodeposition can further adjust metabolite profiles and microbial functions, strengthening beneficial interactions (Qiao et al., 2024).

### 2.2 Soil physicochemical properties in legume rhizospheres

Legume establishment and rhizosphere activity progressively modify soil physicochemical properties, often improving pH status, organic matter, and nutrient availability. For example, legume planting in saline or degraded soils has been associated with decreased salinity and pH, and increased soil organic carbon and nitrogen pools, promoting more diverse and functionally complex bacterial networks (Liu et al., 2021; Amaya-Gómez et al., 2025). Over years of perennial or woody legume growth, rhizosphere soils can show rising organic matter and available P and K, coupled with elevated enzyme activities (e.g., urease, phosphatase) that support nutrient turnover and microbial proliferation (Ren et al., 2021; Mu et al., 2024).

Soil pH emerges as a central driver of rhizosphere bacterial diversity, composition, and function, often outweighing vegetation type or other variables (Wan et al., 2020). In acidic cropping soils, lower pH ( $\leq 5.5$ ) is linked to reduced bacterial abundance and downregulated genes involved in C, N, P, and S cycling, which can constrain crop yield (Abd-Alla et al., 2023). Conversely, amendments such as lime, organic manure, or biochar can adjust pH and nutrient status, shifting bacterial communities toward taxa (e.g., Actinobacteria, Proteobacteria) associated with enhanced disease suppression and improved plant physiological status (Ren et al., 2021; Chen et al., 2022).

### 2.3 Symbiotic nitrogen fixation and nutrient cycling

Symbiotic nitrogen fixation (SNF) between legumes and rhizobia is a core process structuring rhizosphere function, converting atmospheric  $N_2$  into plant-available ammonia in nodules and enriching soil N pools (Neda, 2021). The effectiveness of SNF varies among rhizobial strains and is strongly influenced by soil conditions and

plant demand; highly efficient symbioses can install endosphere and rhizosphere microbiomes that promote nutrient uptake beyond simple nutrient supply, including accumulation of beneficial Actinobacteria in roots (Lagunas et al., 2023). Over time, fixed nitrogen is transferred to soil through rhizodeposition, senescing roots, and residues, supporting non-legume crops and stimulating broader microbial activities in diversified systems (Qiao et al., 2024).

Excessive mineral N fertilization can suppress SNF by interfering with nodulation signaling, rhizobial chemotaxis to roots, and nitrogenase activity, thereby weakening the mutualism and altering rhizosphere microbial relationships (Abd-Alla et al., 2023). In contrast, organic inputs such as compost and vermicompost generally enhance nodulation, nodule biomass, plant growth, and yield, while improving soil biological quality and nitrogen availability (Mu et al., 2024). Free-living nitrogen-fixing bacteria in the legume rhizosphere, stimulated by legume-derived exudates (including flavonoids and coumarins), further contribute to N inputs and interact functionally with symbiotic rhizobia under intercropping or rotation schemes (Chen et al., 2022; Qiao et al., 2024).

### 3 Composition and Diversity of Rhizosphere Microbial Communities

#### 3.1 Bacterial diversity in legume rhizospheres

Large-scale comparative analyses show that legume rhizospheres often exhibit lower bacterial  $\alpha$ -diversity than non-legumes but are strongly enriched in nitrogen-cycling taxa and nitrogen-fixing genes, suggesting a specialized, function-driven bacterial assembly (Qin et al., 2025). Typical legume rhizospheres are dominated by Proteobacteria and Bacteroidetes, with notable representation of Bradyrhizobiaceae, Rhizobiaceae, and other diazotrophs, reflecting the central role of biological nitrogen fixation (Pivato et al., 2021; Yang et al., 2024). In intercropping systems, legume presence can shift bacterial communities in associated non-legume rhizospheres toward copiotrophic and nitrogen-transforming assemblages, often without major changes in overall diversity indices (Pang et al., 2022).

Cropping mode and fertilization regimes substantially modulate bacterial community richness, structure, and potential function in legume-involving systems. In hullless barley-pea mixed cropping, increasing nitrogen and phosphorus inputs caused a hump-shaped response in bacterial  $\alpha$ -diversity, with mixed cropping supporting higher diversity than monocropping and enriching *Allorhizobium*-*Neorhizobium*-*Pararhizobium*-*Rhizobium* relative to cereal monoculture (Fu et al., 2023; Guo et al., 2024). Sugarcane-peanut and cereal-legume intercrops similarly increased bacterial richness and the diversity of nitrogen-fixing bacteria in rhizosphere and bulk soils compared with monocultures, aligning with improved crop performance and altered soil pH and phosphorus availability (Pang et al., 2022; Yang et al., 2023). These findings indicate that legume integration and moderate nutrient inputs can promote diverse, functionally advantageous bacterial consortia in the rhizosphere.

#### 3.2 Fungal diversity and mycorrhizal associations

Arbuscular mycorrhizal fungi (AMF) are key fungal components of legume rhizospheres, enhancing phosphorus acquisition and stress tolerance while interacting with rhizobia-dependent nitrogen fixation (Alimi et al., 2021; Pires et al., 2021). Surveys of indigenous South African legumes revealed diverse AMF communities dominated by *Glomus* and *Acaulospora*, with species richness and spore density varying markedly among hosts and being strongly structured by soil properties such as texture and nutrient status (Alimi et al., 2025). Morphological assessments across leguminous and non-leguminous crops further identified *Acaulospora*, *Funneliformis*, *Gigaspora*, *Glomus*, and *Rhizophagus* as common AMF genera, with legume hosts often supporting higher spore counts and colonization frequencies, underlining their importance as AMF reservoirs (Pires et al., 2021).

Intercropping and integrated crop-livestock systems that include legumes can enhance AMF diversity, colonization, and inoculum potential in subsequent legume phases. In maize-soybean intercropping, AMF  $\alpha$ -diversity in soybean rhizosphere soil increased relative to monoculture at comparable nitrogen levels, and *Glomus*-related taxa were dominant in both soil and roots, with their abundance responding to nitrogen inputs and crop identity (Figure 1) (Zhang et al., 2020; Alimi et al., 2021). In systems where grasses are intercropped with cowpea or pigeon pea, AMF spore density, colonization, and species richness in legume-associated rhizospheres

rise, and these mycorrhizal improvements correlate positively with soybean productivity in following crops (Pires et al., 2021; Guo et al., 2024). Together, these studies show that legumes and diversified management can foster rich AMF assemblages that contribute to nutrient use efficiency and yield stability.

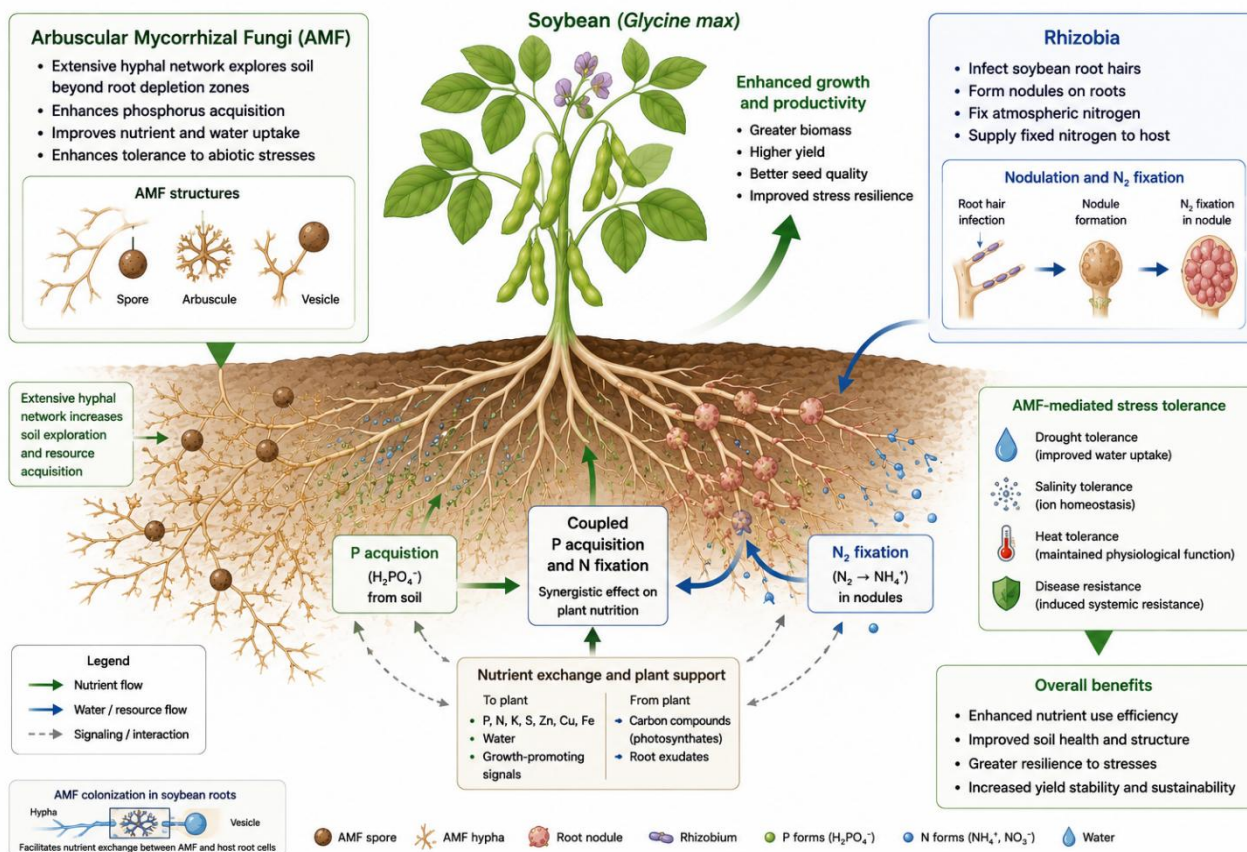


Figure 1 Illustrates the symbiotic interactions among arbuscular mycorrhizal fungi (AMF), legume roots, and rhizobia in the rhizosphere. AMF hyphae enhance phosphorus acquisition and stress tolerance, while rhizobia contribute to biological nitrogen fixation, together improving nutrient uptake and plant growth

### 3.3 Archaea, viruses, and other microorganisms

Beyond bacteria and fungi, legume rhizospheres host diverse archaea, phages, and other viruses whose ecological roles are only beginning to be elucidated. Conceptual and empirical work on rhizosphere “zoos” highlights that archaea, viruses, and other eukaryotes coexist with bacteria and fungi, contributing to nutrient turnover, organic matter decomposition, and plant health outcomes. Archaea, although less intensively characterized in legumes, are recognized as components of rhizosphere communities that may participate in nitrogen and carbon cycling, while protists and nematodes further shape microbial food webs and nutrient flows (Qin et al., 2025).

Recent advances in viromics demonstrate that soil and rhizosphere viral communities are taxonomically and functionally diverse, exhibiting strong spatial and temporal dynamics and exerting top-down control on bacterial hosts. Phages in rhizospheres regulate pathogen densities and suppress or exacerbate disease depending on whether they target pathogens or pathogen-suppressing bacteria, thereby influencing soil suppressiveness and plant health (Yang et al., 2023). Crop management and rotation can “prime” rhizosphere viral assemblages, altering DNA and RNA virus diversity and activity near roots and driving bacterial community succession through Kill-the-Winner dynamics (Braga et al., 2020; Muscatt et al., 2022). These findings, together with evidence that phage pressure can modify bacterial diversity and nitrogen availability, emphasize that viruses and associated microbial predators are integral but underappreciated drivers of rhizosphere community assembly and function in legume cropping systems (Wang et al., 2024).

## 4 Factors Influencing Rhizosphere Microbial Diversity

### 4.1 Plant genotype and species differences

Plant genetic variation shapes rhizosphere microbiome assembly by altering host filtering strength and the interface between rhizosphere and internal compartments. In *Medicago truncatula*, soil origin mainly structured rhizosphere communities, but plant genotype exerted strong effects in the root endosphere, indicating that different genotypes act as stronger or weaker microbial filters and influence which taxa progress inward (Brown et al., 2020). In soybean, soil type again dominated community composition, yet host genotype subtly “tuned” rhizosphere assembly and microbe-microbe interaction networks, demonstrating cooperative control by plant genetics and soil microbiome pool.

Species-level differences within legumes also generate distinct rhizosphere communities. Across five *Phaseolus* species, each recruited a characteristic bacterial assemblage, with notable contrasts in richness and dominant phyla; for example, *Phaseolus lunatus* showed the highest richness and an Acidobacteria-enriched rhizosphere, whereas Actinobacteria dominated several other species (Yang et al., 2023). More broadly, diversity in soil microbial community structure was greater among legume species than among grass species, and legumes generally supported higher bacterial diversity and enriched fungi, underscoring the strong niche differentiation imposed by legume species identity.

### 4.2 Agricultural management practices

Tillage and fertilization regimes alter soil structure, resources, and disturbance intensity, thereby reshaping rhizosphere-associated microbiomes in legume-based systems. In a long-term corn-soybean system, both tillage and fertility significantly shifted bacterial, fungal and oomycete communities, with no-till favoring ecological guilds such as arbuscular mycorrhizal fungi, mycoparasites, and nematophagous fungi, while conventional tillage promoted saprotrophs and plant pathogens. Fertilization further modified bacterial and fungal  $\beta$ -diversity and supported copiotrophic bacteria and *Fusarium* under conventional regimes, indicating that intensive inputs select for fast-growing competitors rather than mutualists (Srouf et al., 2020).

Cropping sequences involving legumes also drive rhizosphere diversity and N-cycling potential. In sorghum systems, precropping with cowpea or soybean, compared with maize or no precrop, significantly altered rhizosphere bacterial  $\alpha$ - and  $\beta$ -diversity and shifted key nitrogen-cycling genes (e.g., *amoC*, *narH*, *gltB*, *glnA*, *ureC*), with legume rotations enriching several N-transformation pathways (Enagbonma et al., 2025). In sugarcane rotations, soybean and peanut residues increased microbial biomass C, C mineralization, and nitrification capacity, although high-N soybean residues released more mineral N than low-N peanut residues, revealing crop- and residue-specific impacts on microbial functions linked to fertility (Paungfoo-Lonhienne et al., 2021).

### 4.3 Environmental and climatic factors

Environmental variables such as soil type, pH, and climate gradients strongly regulate rhizosphere microbial diversity and its functional consequences. Along an altitudinal and climatic gradient in mountain ecosystems, geographical and climatic factors directly and indirectly controlled rhizosphere bacterial and fungal diversity, with bacterial  $\alpha$ -diversity and particular dominant taxa exerting strong positive or negative effects on soil multifunctionality. The balance of these effects determined net multifunctionality, and higher richness at the phylum level generally led to gains in multiple soil functions, highlighting the sensitivity of rhizosphere communities to long-term climatic contexts (Yang et al., 2023).

Among soil properties, pH is a particularly powerful predictor of rhizosphere bacterial diversity, structure, and function. In acidic crop soils, communities in  $\text{pH} < 5.5$  versus  $> 5.5$  clustered into distinct groups, with higher pH associated with greater bacterial abundance and diversity and more active nutrient-cycling functions (C, N, P, S) (Wan et al., 2020). In more acidic soils, bacterial interaction networks suggested reduced competition but downregulated functional genes, implying constrained ecosystem services and potentially lower crop yields when pH is not managed (Wan et al., 2020).

## 5 Functional Roles of Rhizosphere Microbiota

### 5.1 Plant growth promotion and nutrient acquisition

Legume rhizospheres are enriched in PGPR and other beneficial microbes that promote growth via biological nitrogen fixation, phosphate solubilization, siderophore production, and phytohormone synthesis (Timofeeva et al., 2023). Rhizospheric diazotrophs and phosphate-solubilizing bacteria increase plant-available N and P, while co-inoculation strategies combining these groups often outperform single strains by simultaneously enhancing nutrient supply and root development (Zeng et al., 2022). In grain legumes, such PGPR also stimulate nodulation and strengthen rhizobium-legume symbioses, further boosting nitrogen inputs and yield (Swarnalakshmi et al., 2020).

Beyond direct nutrient mobilization, PGPR and mycorrhizal fungi improve nutrient use efficiency and root architecture, enabling legumes to exploit heterogeneous soil resources (Tahat et al., 2020; Timofeeva et al., 2023). Reviews on rhizosphere-plant interactions highlight that these microbes alter root physiology, exudation, and transporter activity, thereby increasing uptake of N, P, and micronutrients while supporting growth under nutrient deficiency (Hakim et al., 2021). Harnessing these functions through microbial fertilizers and seed inoculants is increasingly proposed as a means to reduce mineral N and P inputs without compromising productivity (De Andrade et al., 2023).

### 5.2 Disease suppression and stress resistance

Rhizosphere microbiota contribute to legume health by forming a first line of defense against soil-borne pathogens. Beneficial bacteria and fungi protect roots via antibiosis, competition for nutrients and niches, parasitism, and induction of systemic resistance (Tahat et al., 2020; Hakim et al., 2021). In common bean, cultivars bred for resistance to *Fusarium oxysporum* harbor rhizospheres enriched in *Pseudomonadaceae*, *Bacillaceae*, and *Cytophagaceae*, along with genes for antifungal metabolites, indicating that host genetics can co-select disease-suppressive communities.

Microbiome-mediated resistance also extends to abiotic stresses such as drought, salinity, and heat. Reviews on harnessing plant-microbe interactions and rhizosphere engineering note that tailored microbial consortia and stress-resilient PGPR can improve water use efficiency, modulate stress hormones, and maintain growth under adverse conditions (Yusuf et al., 2025). Specific PGPR strains from legume rhizospheres, such as *Pseudomonas chlororaphis* IRHB3 in soybean, both recruit functional bacteria involved in nutrient cycling and activate jasmonate-mediated resistance, thereby simultaneously enhancing growth and suppressing root rot in the field (Kumar and Dubey, 2020; Wei et al., 2024).

### 5.3 Soil health and ecosystem sustainability

Soil health is tightly linked to the diversity and activity of rhizosphere microorganisms, which regulate nutrient recycling, aggregate stability, and greenhouse gas fluxes (Tahat et al., 2020; Xing et al., 2025). Beneficial rhizosphere microbes in legume systems improve soil structure and organic matter turnover, support balanced nutrient cycles, and increase resilience of soil functions to disturbance (Hakim et al., 2021; Sharma et al., 2025). Leguminous cover crops and legume-based intercropping have been shown to enhance rhizosphere microbial diversity, enrich taxa involved in nitrogen fixation and organic matter decomposition, and improve soil pH, organic carbon, and nutrient availability relative to monocultures (Jalloh et al., 2024; Pokharel et al., 2025).

Microbial-based strategies are increasingly recognized as central to sustainable agriculture, providing eco-friendly alternatives to intensive chemical inputs. Reviews on soil microbial resources and rhizosphere manipulation emphasize that bio-inoculants and management practices that favor native beneficial communities can simultaneously enhance crop productivity, soil fertility, and environmental quality (Mahmud et al., 2021; Sharma et al., 2025). Dissecting rhizosphere microbiomes into environment-dominated and plant genetic-dominated components further suggests complementary levers-agronomic management and breeding-for designing legume systems that maintain functionally robust microbiomes and deliver long-term ecosystem services (Xun et al., 2024; Xing et al., 2025).

## 6 Molecular and Analytical Approaches in Rhizosphere Microbial Research

### 6.1 High-throughput sequencing technologies

Metagenomics and marker-gene amplicon sequencing are the core high-throughput tools for rhizosphere studies, enabling cultivation-independent profiling of complex communities. Review work highlights shotgun metagenomics for unbiased recovery of genomes and functional genes, and 16S rRNA or ITS amplicon sequencing for efficient taxonomic surveys of bacteria and fungi in root-associated soils (Rajguru et al., 2024). In legume rhizospheres, such approaches reveal dominant phyla and shifts in community structure under contrasting fertilization regimes, for example in soybean grown with organic versus inorganic inputs (Babalola et al., 2025).

Recent methodological advances focus on scalability and sensitivity for plant-associated samples. A high-throughput 16S rRNA library-preparation protocol using magnetic beads for DNA extraction directly from roots and exonuclease purification before the second PCR step improves handling and detection of minor bacterial taxa, yet produces community profiles comparable to commercial kits in roots and soils (Kumaishi et al., 2022). Standardized field-to-sequencing protocols have also been developed for collecting soil, rhizosphere, and root endosphere fractions and running validated 16S pipelines, facilitating cross-study comparisons across plant species and habitats.

### 6.2 Bioinformatics and data analysis

Downstream of sequencing, dedicated bioinformatics pipelines convert read data into diversity metrics, taxonomic profiles, and functional inferences. A practical guide summarizes recommended workflows for amplicon and metagenomic analyses, detailing quality control, denoising or clustering, taxonomic assignment, diversity estimation, and advanced methods such as network analysis and machine learning to extract ecological meaning from microbiome datasets (Liu et al., 2020). Habitat-specific optimization is increasingly emphasized: evaluation of 35,889 microbe species and >150,000 microbiomes produced Qscore, a framework to select optimal 16S regions and strategies for different ecosystems, achieving profiling precision close to shotgun metagenomes (Zhang et al., 2023).

Pipeline choice can strongly bias apparent community structure and diversity. Comparative assessments of 16S amplicon workflows using mock communities and environmental datasets show large differences among tools such as Mothur, QIIME1, QIIME2, MEGAN, DADA2, and others; in one study, QIIME2 markedly reduced false positives and improved taxonomic and diversity estimates relative to alternatives (Straub et al., 2020). Another comparison of OTU- and ASV-based pipelines found that ASV methods such as DADA2 and USEARCH-UNOISE3 improved resolution and specificity, while some OTU workflows inflated richness and spurious taxa, underscoring the need for careful pipeline selection in rhizosphere research (Prodan et al., 2020).

### 6.3 Experimental models and cultivation techniques

Sequencing-based surveys are increasingly complemented by experimental models that allow mechanistic tests of plant-microbe interactions. Synthetic microbial communities (SynComs) constructed from cultured rhizosphere isolates have been systematically reviewed as tools to bridge complexity and control; SynComs ranging from a few to ~190 strains, typically dominated by Proteobacteria, Actinobacteria, and Firmicutes, are deployed on diverse plant hosts and growth systems to dissect functions such as colonization, competition, and plant growth promotion (Marín et al., 2021). A 16-member synthetic soil community derived from a single rhizosphere was further optimized for reproducibility, tunable starting composition, long-term cryopreservation, and use in standardized fabricated ecosystem devices (EcoFABs), enabling controlled plant-microbe experiments across laboratories (Coker et al., 2022).

Cultivation remains crucial for isolating functional strains and validating metagenomic predictions, but many rhizosphere microbes are recalcitrant to standard media. Improved culture-dependent strategies-such as modifying gelling agents and autoclaving steps-enhanced recovery of wheat rhizosphere bacteria from <1% to up to ~2.5% of metagenomic OTUs and yielded isolates with multiple plant growth-promoting traits (Youseif et al., 2021). Microcosm and multitrophic systems, supported by detailed manuals on soil sterilization, isolation, inoculation, and microcosm design, further allow controlled investigation of bacteria, fungi, protists, and nematodes together, better reflecting the complexity of rhizosphere food webs.

## 7 Case Study: Rhizosphere Microbial Diversity in Soybean Cropping Systems

### 7.1 Overview of soybean rhizosphere microbiota

Soybean rhizospheres host complex, multi-kingdom microbial communities whose composition and function vary strongly across soils and regions. A metagenomic survey across 13 major soybean-producing regions in China identified over 43,000 microbial species (bacteria, archaea, fungi and viruses), with clear site-specific clustering and 556 hub taxa correlated with yield and involved in C, N, P and S cycling (Ren et al., 2025). Comparative work further shows that rhizosphere communities differ markedly from bulk soil, with enrichment of genera such as *Rhizobium*, *Novosphingobium*, *Phenylobacterium*, *Streptomyces* and *Nocardioideis* and convergence in functional pathways linked to xenobiotic degradation, plant-microbe interactions and nutrient transport.

Soil background and plant genetics jointly modulate soybean rhizosphere assembly. Across contrasting soils and genotypes, soil type has the dominant effect, but soybean genotype subtly “tunes” recruitment and microbe-microbe networks, with wild *Glycine soja* maintaining higher rhizosphere diversity than domesticated lines (Figure 2). Other studies show that rhizocompartments (bulk soil, rhizosphere, roots, nodules) host distinct bacterial assemblages, and that rhizosphere networks include strong correlations between rhizobia and non-rhizobial taxa, which can in turn influence nodulation patterns and symbiotic efficiency (Han et al., 2020).

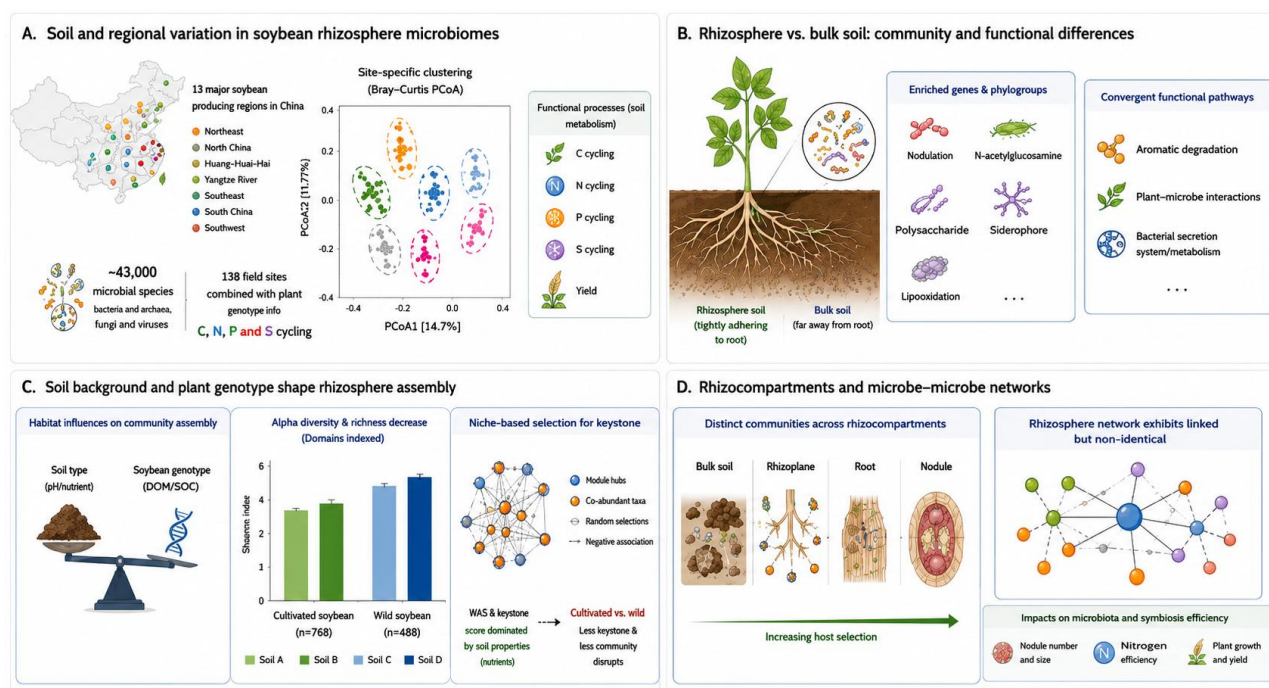


Figure 2 Multi-scale characterization of soybean rhizosphere microbiomes across contrasting soils, regions, and rhizocompartments

### 7.2 Effects of cropping patterns on microbial diversity

Cropping patterns substantially reshape soybean rhizosphere diversity, composition, and functional potential. In maize-soybean relay strip intercropping, soybean rhizosphere bacterial diversity increased compared with monoculture, with higher richness of *Pseudomonas*, *Bacillus* and other antagonists; several intercropping-derived strains suppressed *Fusarium* root rot and one *Pseudomonas chlororaphis* strain (IRHB3) promoted root growth and seedling survival under pathogen pressure (Chang et al., 2022). In coastal saline soils, soybean-corn intercropping altered soil C, N, P and salinity and significantly changed bacterial and fungal communities; intercropping increased Chao1 richness, shifted dominant phyla (Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi; Ascomycota, Mortierellomycota, Basidiomycota) and enriched taxa linked to nutrient cycling and bioremediation (Nyimbo et al., 2025).

At finer scales, belt/strip planting and intercropping layouts under field conditions also modulate multi-kingdom communities and link to yield traits. Metagenomic analysis of soybean-maize strip systems showed that bacteria

and viruses dominate inter-root communities and are more sensitive to planting mode than fungi or archaea, with shifts in *Pseudomonas*, rhizobia and other genera across modes that favored either soybean or maize yields (Wang et al., 2024). In maize-soybean systems compared over three years, conversion from monocropping to intercropping increased microbiome network modularity and functional diversity and enriched genes for nitrate assimilation, nitrification and dissimilatory nitrate reduction, changes that were closely related to higher yields in intercropped soybean (Shu et al., 2024).

### 7.3 Implications for sustainable agriculture

Evidence from soybean systems indicates that managing rhizosphere microbiota offers powerful levers for sustainable intensification. Long-term comparisons of soybean continuous monocropping versus maize-soybean rotation show that both long-term continuous soybean (13 years) and rotation can elevate soil pH and available N, P, K, and increase network complexity, while enriching beneficial *Bradyrhizobium*, *Gemmatimonas* and *Mortierella* and reducing pathogenic *Fusarium* compared with short-term continuous soybean (Liu et al., 2020). Similarly, in wheat-soybean double-cropping, introducing wheat/soybean-wheat/maize rotations improved soybean yield and a soil fertility index and shifted rhizosphere fungi toward plant growth-promoting, nematophagous and biocontrol groups, while continuous wheat/soybean favored lignocellulose degraders and pathogens (Sun et al., 2022).

Conceptual and review work suggests that such cropping-based microbiome effects can be deliberately exploited. One framework proposes dividing rhizosphere microbiota into environment-dominated and plant genetic-dominated components, with agronomic practices (e.g., rotations, intercropping, reduced tillage) used to steer the former and breeding used to enhance the latter, thereby stabilizing beneficial consortia in crops like soybean (Xun et al., 2024). More broadly, rhizosphere microbiome engineering-using indigenous consortia and designed inoculants-has been highlighted as a route to reduce synthetic inputs, enhance yield and resilience, and align soybean production with long-term soil health and environmental sustainability goals (Mahmud et al., 2021).

## 8 Challenges, Future Perspectives, and Conclusions

Despite rapid methodological advances, major knowledge gaps limit the application of rhizosphere microbiomes in legume-based systems. A key challenge is the complex assembly mechanisms of rhizosphere communities, where environment-dominated and plant genetic-dominated components interact in ways that are still poorly quantified, especially under realistic field conditions. This complexity hampers prediction of microbiome responses to agronomic practices or legume genotypes, and constrains efforts to design stable microbial consortia for enhanced nitrogen fixation and stress resilience.

Translating microbiome insights into reliable bioinoculants also remains difficult. Many beneficial strains perform well in controlled experiments but fail under variable soils, climates, and management, in part because interactions with native microbiota and environmental heterogeneity are insufficiently understood. Challenges specific to nitrogen-fixing systems include the ecological competitiveness of inoculant strains, context-dependent performance of symbiotic and free-living diazotrophs, and the need to match microbial partners with host genetics and local microbiomes to achieve consistent field-level benefits.

Future rhizosphere research in legumes will likely focus on predictive and integrative frameworks that connect soil factors, plant genetics, and management to microbiome structure and function. Building data-driven, high-throughput models that quantify how soil properties and agronomic practices shape environment-dominated microbiome components is a priority for precise rhizosphere regulation in real cropping systems. At the same time, identifying genes and loci controlling plant genetic-dominated microbiome fractions will support breeding of “microbiome-assisted” legumes and potentially N-self-fertilizing crops.

There is also strong momentum toward microbiome engineering and synthetic communities tailored to legume growth stages and stresses. Multi-omics meta-analyses already reveal developmental stage-specific growth-promoting marker bacteria in legumes that could guide design of multi-species inoculants. Conceptual frameworks such as microbiome-mediated smart agriculture systems emphasize combining reduced tillage,

biofertilization, increasingly complex synthetic microbiomes, and even plant genome editing to recruit beneficial microbiota and improve resilience to drought and other stresses.

Research on rhizosphere microbial diversity in legume cropping systems has demonstrated that legumes assemble functionally specialized microbiomes with strong impacts on nitrogen fixation, nutrient cycling, and stress tolerance. Reviews of legume microbiomes highlight that rhizobia operate within broader rhizosphere and nodule communities, where non-rhizobial bacteria and other microbes contribute to nodule formation, legume fitness, and agroecosystem services, including reduced fertilizer needs and pollution. Harnessing these assemblages is therefore central to strategies aiming at sustainable intensification and climate-friendly nitrogen management.

Moving forward, realizing the full potential of legume-associated rhizosphere microbiomes will require coordinated advances from microns to field scales. Sustainable agriculture perspectives stress that exploiting nitrogen-fixing rhizobacteria and other plant growth promoters depends on overcoming challenges in bioinoculant consistency, integrating omics-based discovery with agronomy, and fostering large-scale collaboration among researchers, industry, and farmers. By combining predictive microbiome management with breeding, intercropping, and reduced-chemical inputs, legume systems can become key platforms for microbiome-based solutions that support soil health, productivity, and ecosystem sustainability.

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### Conflict of Interest Disclosure

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

### References

- Abd-Alla M.H., Al-Amri S.M., and El-Enany A.W.E., 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>
- Alimi A.A., Adeleke R.A., and Moteetee A.N., 2021, Soil environmental factors shape the rhizosphere arbuscular mycorrhizal fungal communities in South African indigenous legumes (Fabaceae), *Biodiversitas*, 22(5): 2466-2476.  
<https://doi.org/10.13057/biodiv/d220503>
- Alimi A.A., Ezeokoli O.T., Adeleke R.A., and Moteetee A.N., 2025, Arbuscular mycorrhizal fungal communities and relationship with edaphic factors in the rhizospheric soil of Fabaceae in semi-arid South Africa, *Scientific African*, 27: e02997.  
<https://doi.org/10.1016/j.sciaf.2025.e02997>
- Amaya-Gómez C., Flórez-Martínez D., Cayuela M.L., and Tortosa G., 2025, Compost and vermicompost improve symbiotic nitrogen fixation, physiology and yield of the Rhizobium-legume symbiosis: a systematic review, *Applied Soil Ecology*, 210: 106051.  
<https://doi.org/10.1016/j.apsoil.2025.106051>
- Babalola O.O., Osuji I.J., and Akanmu A.O., 2025, Amplicon-based metagenomic survey of microbes associated with the organic and inorganic rhizosphere soil of *Glycine max* L., *BMC Genomic Data*, 26(1): 40.  
<https://doi.org/10.1186/s12863-025-01333-2>
- Braga L.P.P., Spor A., Kot W., Breuil M.C., Hansen L.H., Setubal J.C., and Philippot L., 2020, Impact of phages on soil bacterial communities and nitrogen availability under different assembly scenarios, *Microbiome*, 8(1): 52.  
<https://doi.org/10.1186/s40168-020-00822-z>
- Brown S.P., Grillo M.A., Podowski J.C., and Heath K.D., 2020, Soil origin and plant genotype structure distinct microbiome compartments in the model legume *Medicago truncatula*, *Microbiome*, 8(1): 139.  
<https://doi.org/10.1186/s40168-020-00915-9>
- Chang X., Wei D., Zeng Y., Zhao X., Hu Y., Wu X., Song C., Gong G., Chen H., Yang C., Zhang M., Liu T., Chen W., and Yang W., 2022, Maize-soybean relay strip intercropping reshapes the rhizosphere bacterial community and recruits beneficial bacteria to suppress *Fusarium* root rot of soybean, *Frontiers in Microbiology*, 13: 1009689.  
<https://doi.org/10.3389/fmicb.2022.1009689>
- Chen D., Wang X., Carrión V.J., Yin S., Yue Z., Liao Y., Dong Y., and Li X., 2022, Acidic amelioration of soil amendments improves soil health by impacting rhizosphere microbial assemblies, *Soil Biology and Biochemistry*, 167: 108599.  
<https://doi.org/10.1016/j.soilbio.2022.108599>

- Chen L., and Liu Y., 2024, The function of root exudates in the root colonization by beneficial soil rhizobacteria, *Biology*, 13(2): 95.  
<https://doi.org/10.3390/biology13020095>
- Chen Z., Wang W., Chen L., Zhang P., Liu Z., Yang X., Shao J., Ding Y., and Mi Y., 2024, Effects of pepper-maize intercropping on the physicochemical properties, microbial communities, and metabolites of rhizosphere and bulk soils, *Environmental Microbiome*, 19(1): 108.  
<https://doi.org/10.1186/s40793-024-00653-7>
- Chukwuneme C.F., and Babalola O.O., 2025, Microbial diversity and function in the rhizosphere microbiome: Driving forces and monitoring approaches, *Agrosystems, Geosciences and Environment*, 8(3): e70169.  
<https://doi.org/10.1002/agg2.70169>
- Coker J.A., Zhalnina K., Marotz C., Thiruppathy D., Tjuanta M., D'Elia G., Hailu R., Mahosky T., Rowan M., Northen T.R., and Zengler K., 2022, A reproducible and tunable synthetic soil microbial community provides new insights into microbial ecology, *mSystems*, 7(6): e00951-22.  
<https://doi.org/10.1128/msystems.00951-22>
- De Andrade L.A., Santos C.H.B., Frezarin E.T., Sales L.R., and Rigobelo E.C., 2023, Plant growth-promoting rhizobacteria for sustainable agricultural production, *Microorganisms*, 11(4): 1088.  
<https://doi.org/10.3390/microorganisms11041088>
- Enagbonma B.J., Modise D.M., and Babalola O.O., 2025, Effects of legume-cereal rotation on sorghum rhizosphere microbial community structure and nitrogen-cycling functions, *MicrobiologyOpen*, 14(5): e70085.  
<https://doi.org/10.1002/mbo3.70085>
- Fu X., Huang Y., Fu Q., Qiu Y., Zhao J., Li J., Wu X., Yang Y., Liu H., Yang X., and Chen H., 2023, Critical transition of soil microbial diversity and composition triggered by plant rhizosphere effects, *Frontiers in Plant Science*, 14: 1252821.  
<https://doi.org/10.3389/fpls.2023.1252821>
- Gong X., Feng Y., Dang K., Jiang Y., Qi H., and Feng B., 2023, Linkages of microbial community structure and root exudates: Evidence from microbial nitrogen limitation in soils of crop families, *Science of the Total Environment*, 881: 163536.  
<https://doi.org/10.1016/j.scitotenv.2023.163536>
- Guo T., Yao X., Wu K., Guo A., and Yao Y., 2024, Response of the rhizosphere soil microbial diversity to different nitrogen and phosphorus application rates in a hulless barley and pea mixed-cropping system, *Applied Soil Ecology*, 195: 105262.  
<https://doi.org/10.1016/j.apsoil.2023.105262>
- Hakim S., Naqqash T., Nawaz M.S., Laraib I., Siddique M.J., Zia R., Mirza M.S., and Imran A., 2021, Rhizosphere engineering with plant growth-promoting microorganisms for agriculture and ecological sustainability, *Frontiers in Sustainable Food Systems*, 5: 617157.  
<https://doi.org/10.3389/fsufs.2021.617157>
- Han Q., Ma Q., Chen Y., Tian B., Xu L., Bai Y., Chen W., and Li X., 2020, Variation in rhizosphere microbial communities and its association with the symbiotic efficiency of rhizobia in soybean, *The ISME Journal*, 14(8): 1915-1928.  
<https://doi.org/10.1038/s41396-020-0648-9>
- Jalloh A., Mutyambai D., Yusuf A.A., Subramanian S., and Khamis F.M., 2024, Maize edible-legumes intercropping systems for enhancing agrobiodiversity and belowground ecosystem services, *Scientific Reports*, 14(1): 14355.  
<https://doi.org/10.1038/s41598-024-64138-w>
- Kumaishi K., Usui E., Suzuki K., Kobori S., Sato T., Toda Y., Takanashi H., Shinozaki S., Noda M., Takakura A., Matsumoto K., Yamasaki Y., Tsujimoto H., Iwata H., and Ichihashi Y., 2022, High throughput method of 16S rRNA gene sequencing library preparation for plant root microbial community profiling, *Scientific Reports*, 12(1): 19289.  
<https://doi.org/10.1038/s41598-022-23943-x>
- Kumar A., and Dubey A., 2020, Rhizosphere microbiome: Engineering bacterial competitiveness for enhancing crop production, *Journal of Advanced Research*, 24: 337-352.  
<https://doi.org/10.1016/j.jare.2020.04.014>
- Kumar G., Kumar S., Bhardwaj R., Swapnil P., Meena M., Seth C., and Yadav A.N., 2024, Recent advancements in multifaceted roles of flavonoids in plant-rhizomicrobiome interactions, *Frontiers in Plant Science*, 14: 1297706.  
<https://doi.org/10.3389/fpls.2023.1297706>

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