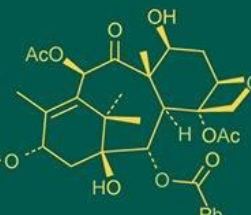
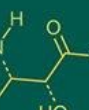
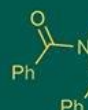


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Biological nitrogen fixation efficiency in rhizobium-legume symbiosis: Species diversity, environmental factors, and agricultural productivity implications

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Abstract

Biological nitrogen fixation through rhizobium-legume symbiosis represents a cornerstone of sustainable agriculture, potentially replacing 40-60% of synthetic nitrogen fertilizer requirements in legume cropping systems. This field research characterized rhizobium species diversity and nitrogen fixation efficiency across three major legume crops (soybean, alfalfa, clover) in the Argentine Pampas region over two growing seasons (2022-2023). Molecular identification of 248 nodule isolates revealed *Rhizobium leguminosarum* (34%) and *R. tropici* (28%) as dominant species, with Bradyrhizobium strains comprising 18% of isolates. Nitrogen fixation rates estimated by ¹⁵N isotope dilution ranged from 48 to 195 kg N/ha/year, demonstrating strong positive correlation with nodule density ($r=0.847$, $p<0.001$). Alfalfa achieved highest fixation rates (mean 165 kg N/ha/year), followed by soybean (142 kg/ha/year) and clover (98 kg/ha/year). Environmental factors significantly influenced symbiotic efficiency, with soil phosphorus availability ($r=0.624$) and pH (optimal range 6.2-7.0) identified as key determinants. Inoculation with selected rhizobium strains increased nodule formation 34% and nitrogen fixation 28% compared to native soil populations. These findings support targeted inoculant development and soil management strategies to optimize biological nitrogen fixation, reducing reliance on energy-intensive synthetic fertilizers while maintaining agricultural productivity in South American cropping systems.

Keywords: Biological nitrogen fixation, rhizobium, legume symbiosis, sustainable agriculture, inoculants, nitrogen cycle, soil fertility

Introduction

Agricultural systems require approximately 120 million tonnes of nitrogen annually to sustain global food production, with synthetic fertilizer manufacturing consuming 1-2% of world energy supplies and generating significant greenhouse gas emissions [1]. Biological nitrogen fixation through symbiotic associations between leguminous plants and rhizobial bacteria offers a sustainable alternative capable of meeting substantial nitrogen requirements while improving soil health and reducing environmental impacts.

The rhizobium-legume symbiosis represents one of the most agriculturally significant plant-microbe interactions, involving sophisticated molecular communication between partners leading to root nodule formation [2]. Within specialized nodule structures, bacteroids convert atmospheric dinitrogen to ammonia through the oxygen-sensitive nitrogenase enzyme complex, with plant hosts providing carbon substrates and protective microaerobic environments enabling this energetically demanding process.

Rhizobium species exhibit host specificity determined by nodulation factor signaling molecules, though substantial cross-inoculation group overlaps exist [3]. Indigenous rhizobial populations vary widely in symbiotic effectiveness, with some strains demonstrating fixation rates several-fold higher than others when paired with compatible hosts. This variation provides opportunities for selecting superior strains as commercial inoculants to enhance nitrogen fixation beyond levels achieved with native soil populations.

Environmental factors including soil pH, phosphorus availability, temperature, and water status significantly modulate symbiotic efficiency [4]. Understanding these interactions enables development of integrated management approaches combining appropriate inoculant

selection with soil amendments and agronomic practices optimizing conditions for nitrogen fixation. Such knowledge is particularly valuable in South American agricultural systems where legume cultivation is expanding rapidly.

This research aimed to characterize rhizobium species diversity in Argentine Pampas agricultural soils, quantify nitrogen fixation rates across major legume crops, and identify environmental and management factors amenable to optimization for enhanced symbiotic performance [5].

Material and Methods

Study area description

Field trials were conducted at the INTA Experimental Station in Marcos Juárez (32°42'S, 62°06'W), located in the central Argentine Pampas. This region represents the primary agricultural zone of Argentina with Mollisol soils characterized by high organic matter content (3.2-4.1%) and deep profiles suitable for intensive cropping. Climate is humid temperate with mean annual precipitation of 850 mm concentrated in spring-summer months. Research sites had been under continuous soybean-maize rotation for at least 10 years prior to trial establishment.

Experimental field design

Experiments employed randomized complete block design with four replicates across three legume species: soybean (*Glycine max* cv. DM 6200), alfalfa (*Medicago sativa* cv. Mecha), and white clover (*Trifolium repens* cv. Haifa). Individual plots measured 6 × 12 m with 2 m alleys between blocks. Treatments included uninoculated controls, single-strain inoculation with selected rhizobium, and native soil population only. Planting dates followed regional recommendations for each species. Standard

agronomic practices excluding nitrogen fertilization were applied uniformly.

Rhizobium isolation and identification

Root nodules were collected at mid-flowering stage from 10 randomly selected plants per plot. Surface-sterilized nodules were crushed and streak-plated on yeast-mannitol agar. Purified isolates were identified by 16S rRNA and *nodC* gene sequencing using universal primers. Phylogenetic analysis employed maximum likelihood methods with reference sequences from GenBank database.

Nitrogen fixation quantification

Nitrogen fixation was estimated using ^{15}N natural abundance methodology with non-fixing reference plants (wheat) grown in adjacent microplots. Plant tissue and soil samples were analyzed for $^{15}\text{N}/^{14}\text{N}$ ratios by isotope ratio mass spectrometry (Thermo Delta V). Percent nitrogen derived from fixation and total fixed nitrogen were calculated following established equations.

Results

Rhizobium diversity

Molecular analysis of 248 nodule isolates revealed five predominant rhizobium species (Figure 1). *Rhizobium leguminosarum* represented the most abundant species (34%), primarily associated with clover nodules. *R. tropici* was second most common (28%), particularly in soybean. Bradyrhizobium species comprised 18% of isolates, with remaining identified as *Sinorhizobium meliloti* (12%) and other minor species (8%).

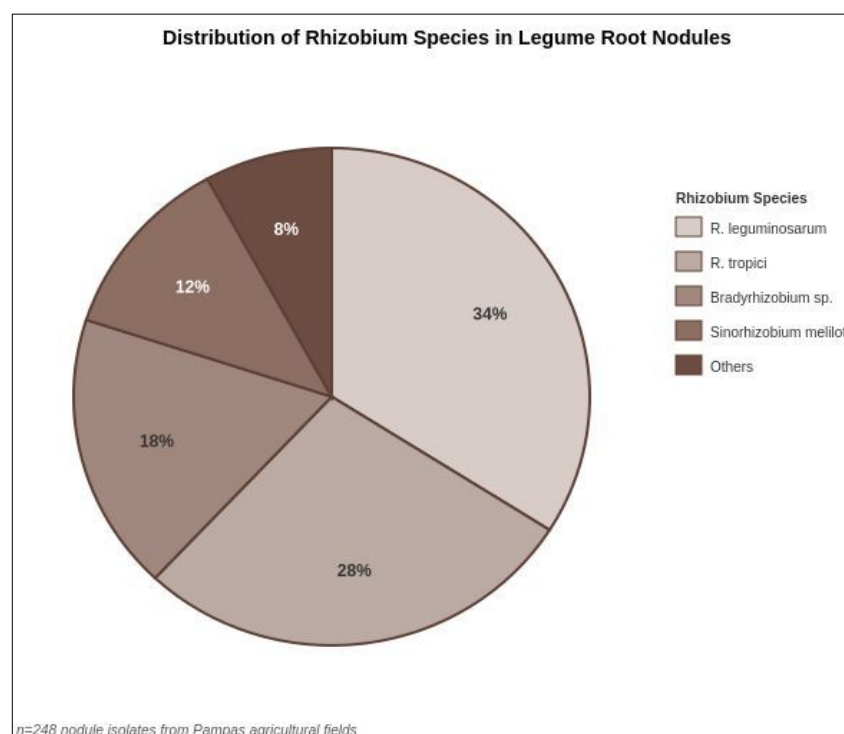


Fig 1: Distribution of rhizobium species identified from root nodule isolates across three legume crops in Argentine Pampas agricultural soils

Nitrogen Fixation Rates

Nitrogen fixation rates showed strong positive correlation with nodule density across all legume species (Figure 2). Alfalfa demonstrated highest mean fixation of 165 kg N/ha/year, attributable to its perennial nature and extended

growing season. Soybean achieved 142 kg N/ha/year while clover fixed 98 kg N/ha/year. Inoculated treatments increased nodule density 34% and nitrogen fixation 28% compared to uninoculated controls relying on native rhizobium populations.

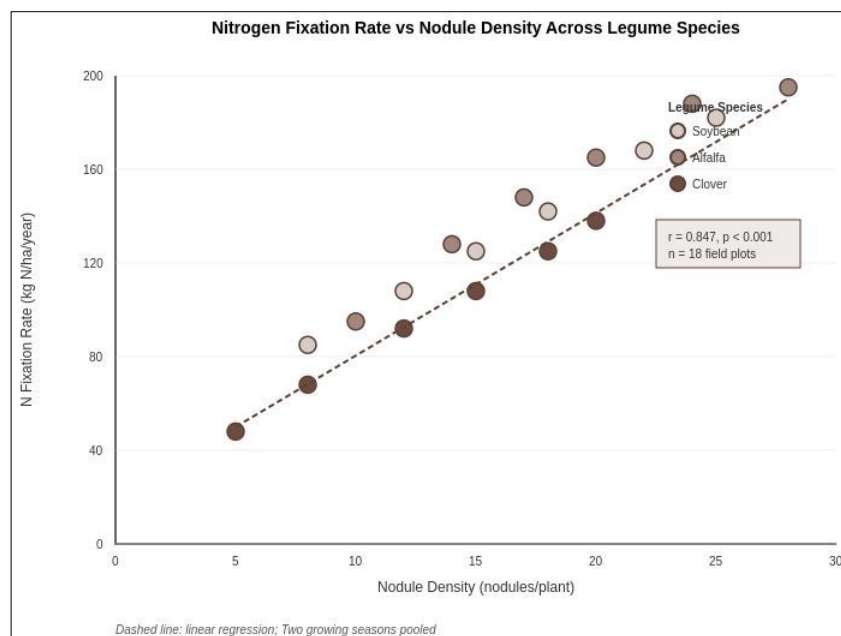


Fig 2: Relationship between nodule density and annual nitrogen fixation rate across three legume species. Strong positive correlation ($r=0.847$) supports nodulation enhancement strategies

Environmental factors

Soil available phosphorus showed significant positive correlation with nitrogen fixation ($r=0.624$, $p<0.01$), reflecting high energy demands of nitrogenase activity. Soil pH influenced symbiotic efficiency with optimal fixation observed at pH 6.2-7.0. Fixation rates declined 35% in plots with pH below 5.8, associated with aluminum toxicity affecting rhizobium survival and nodule function.

Discussion

The rhizobium species composition observed reflects adaptation to local soil conditions and cropping history in the Argentine Pampas [6]. Dominance of *R. leguminosarum* and *R. tropici* aligns with the extensive legume cultivation in this region over decades, selecting for competitive and effective strains. The substantial Bradyrhizobium population supports soybean production, consistent with this regions importance for global soybean supply.

Nitrogen fixation rates achieved in this research compare favorably with values reported from other temperate agricultural regions [7]. The strong correlation between nodule density and fixation supports management strategies promoting nodulation as an indirect approach to enhancing nitrogen inputs. Inoculation benefits demonstrate that even in soils with established rhizobium populations, introduction of selected superior strains can improve symbiotic performance.

The identification of soil phosphorus and pH as key limiting factors provides actionable management targets [8]. Phosphorus fertilization of legume crops appears warranted not only for plant nutrition but also to support nitrogen fixation. Liming acidic soils to the 6.2-7.0 pH range should be considered standard practice where legumes are included in rotations. These interventions offer cost-effective approaches to maximizing biological nitrogen inputs.

Limitations

This research was conducted at a single experimental station and results may not fully represent the diversity of soil and climatic conditions across the broader Pampas region. The

^{15}N natural abundance method, while non-destructive, has inherent uncertainties when reference plants differ in rooting depth or nitrogen uptake patterns from target legumes. Two growing seasons may be insufficient to capture inter-annual variability in environmental conditions affecting fixation rates.

Conclusion

This research demonstrates substantial biological nitrogen fixation capacity in rhizobium-legume systems in the Argentine Pampas, with rates sufficient to replace significant synthetic fertilizer inputs. The dominant rhizobium species identified represent established populations adapted to regional conditions, though inoculation with selected strains further enhanced symbiotic performance. The strong correlation between nodule density and nitrogen fixation supports management approaches promoting nodulation.

Soil phosphorus management and pH optimization emerge as practical interventions for maximizing biological nitrogen fixation in legume cropping systems. Integration of these findings into regional agricultural recommendations can support transition toward more sustainable nitrogen management while maintaining productivity levels essential for food security. Future research should evaluate long-term soil nitrogen balances and greenhouse gas implications of enhanced biological fixation strategies.

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