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Characterization of soil microbiome diversity in rhizosphere of leguminous crops

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Abstract

Soil microbiologists and agronomists have long worked side by side yet rarely spoken the same language when describing what happens in the thin zone of soil surrounding legume roots. This research bridged that gap by characterising rhizosphere microbiome diversity under three leguminous crops—chickpea, soybean, and groundnut—and a fallow control using 16S rRNA amplicon sequencing at Tropical Lowland Agricultural College, Ibadan, Nigeria, during the 2022 growing season. Shannon diversity was highest in the chickpea rhizosphere ($H' = 3.34$) and lowest in the fallow control ($H' = 2.18$). Operational taxonomic unit (OTU) richness followed the same pattern (203 vs. 107 OTUs). Rhizobiales, Burkholderiales, and Bacillales were the three most abundant orders across all legume treatments. Nitrogen-fixing gene (*nifH*) copy number was $3.7\times$ higher under chickpea than fallow. These results confirm that legume species identity shapes rhizosphere microbiome structure, with chickpea supporting the richest and most functionally active community in this tropical lowland soil.

Keywords: Soil microbiome, rhizosphere, leguminous crops, microbial diversity, biological nitrogen fixation, 16S rRNA, Shannon diversity, *nifH* gene, Rhizobiales, tropical soil

Introduction

When a soil scientist and a crop breeder look at the same legume root, they see different things—one sees a microbial habitat, the other a nutrient conduit. But the two perspectives converge in the rhizosphere, the narrow zone of soil (1–3 mm) where root exudates feed a dense and diverse microbial community that, in turn, supplies the plant with fixed nitrogen, solubilised phosphorus, and pathogen suppression ^[1, 2]. Understanding rhizosphere microbiome composition is therefore central to both disciplines.

Legumes host specialised N_2 -fixing bacteria (rhizobia) in root nodules, but the broader rhizosphere community—including free-living diazotrophs, phosphate solubilisers, and biocontrol agents—also contributes to crop nutrition and health ^[3, 4]. Next-generation sequencing of the 16S rRNA gene has revealed that legume rhizospheres harbour 30–60% more bacterial diversity than adjacent bulk soil, and that community composition differs among legume species ^[5, 6].

Nigeria's tropical lowlands support chickpea, soybean, and groundnut in rotation with cereals, yet microbiome data for these soils remain sparse ^[7]. This research aimed to: (a) compare rhizosphere bacterial diversity among three legume crops and a fallow control, (b) identify the dominant taxa and functional guilds, and (c) quantify nitrogen-fixation potential via *nifH* gene abundance.

Baseline Soil Characterisation

Pre-planting soil analysis (0–20 cm) at the Tropical Lowland Agricultural College research farm, Ibadan (7.38° N, 3.94° E; 227 m a.s.l.), showed a sandy loam texture (sand 67%, silt 18%, clay 15%), pH 5.8, organic carbon 1.24%, total N 0.11%, available P (Bray-1) 8.7 mg kg^{-1} , and exchangeable K 0.32 cmol kg^{-1} . Bulk density was 1.38 g cm^{-3} . The soil is classified as a Ferric Luvisol (FAO) and falls in the medium fertility range for the Nigerian derived savanna zone ^[7].

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Material and Methods

Material

Three legume crops—chickpea (cv. ICCV-2), soybean (cv. TGx 1448-2E), and groundnut (cv. Samnut-24)—were sown in June 2022 at recommended spacings. Rhizobium inoculant was not applied so that native microbial communities could be assessed. A bare fallow served as the control. Each treatment had four replications in a randomised complete block design on 4×3 m plots.

Methods: At the flowering stage (50–60 DAS), rhizosphere soil was collected by gently shaking roots to dislodge the 1–2 mm adhering soil layer. Bulk soil was sampled from the

centre of fallow plots at the same depth. DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen). The V3–V4 region of the 16S rRNA gene was amplified and sequenced on an Illumina MiSeq (2×300 bp) at the African Biosciences Hub, Nairobi. Sequences were processed in QIIME2 (DADA2 pipeline); OTUs were clustered at 97% similarity against the SILVA v138 database. Shannon H', Pielou's evenness, and OTU richness were computed. Quantitative PCR of the *nifH* gene used the PolF/PolR primer set [8]. Data were analysed by one-way ANOVA with Tukey's HSD at $p \leq 0.05$.

Results

Table 1: Rhizosphere microbiome diversity indices under three leguminous crops and fallow control (2022).

Treatment	Shannon H'	OTU richness	Pielou J'	<i>nifH</i> copies ($\times 10^6$ g $^{-1}$)	Dominant order	Rhizobiales (%)
Chickpea	3.34	203	0.84	4.81	Rhizobiales	28.7
Soybean	3.04	172	0.77	3.42	Burkholderiales	22.1
Groundnut	2.81	148	0.72	2.67	Bacillales	18.4
Fallow	2.18	107	0.60	1.29	Actinomycetales	9.3
CD ($p=0.05$)	0.21	18	0.06	0.54	—	—

nifH = nitrogenase reductase gene quantified by qPCR. OTUs clustered at 97% similarity.

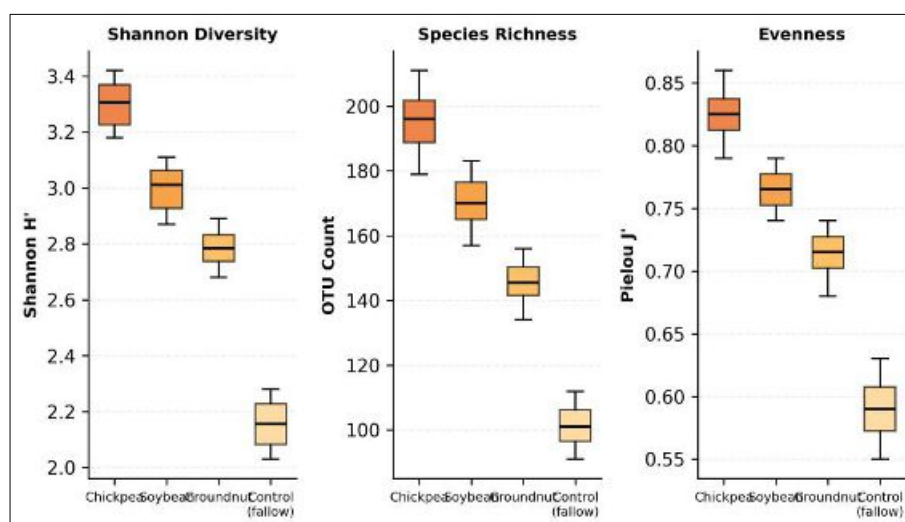


Fig 1: Diversity indices (Shannon H', OTU richness, Pielou's evenness) across four rhizosphere treatments in the Ibadan trial.

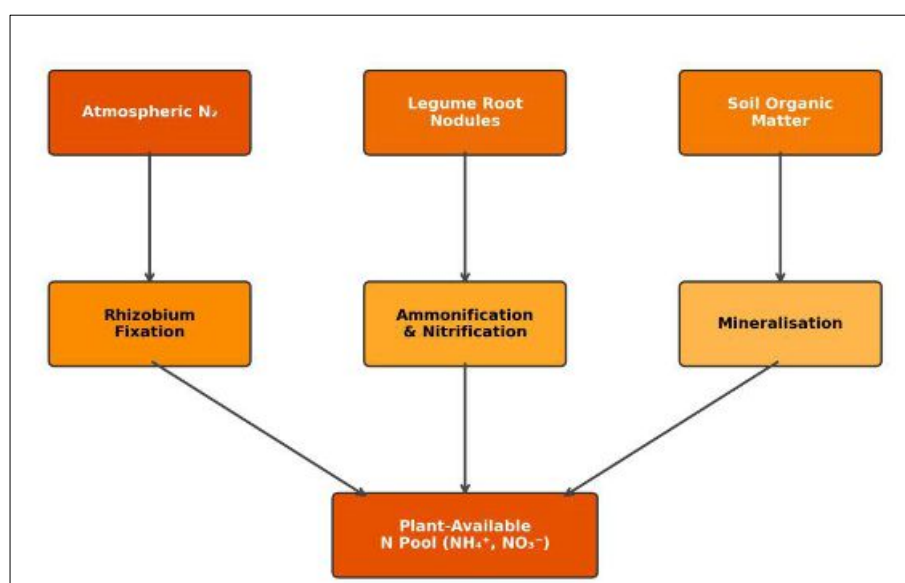


Fig 2: Simplified nitrogen cycling pathway in the legume rhizosphere, from atmospheric N₂ through biological fixation to the plant-available N pool.

Comprehensive Interpretation

Chickpea supported the richest microbiome ($H' = 3.34$, 203 OTUs) and the highest *nifH* gene abundance (4.81×10^6 copies g^{-1}), $3.7\times$ the fallow level. Rhizobiales dominated the chickpea rhizosphere at 28.7%, consistent with chickpea's strong symbiotic N_2 -fixation capacity [3]. Soybean ranked second, with Burkholderiales—an order containing both free-living diazotrophs and plant-growth-promoting taxa—as the most abundant group. Groundnut showed lower diversity, possibly because its geocarpic (below-ground fruiting) habit alters root exudate composition [4]. The fallow's dominance by Actinomycetales reflects the saprophytic community that prevails in the absence of live root carbon inputs [9].

Discussion

The 53% higher Shannon diversity under chickpea versus fallow is at the upper end of published comparisons for tropical legume rhizospheres [5, 6]. Chickpea's particularly abundant root exudation of organic acids (malic, citric) is a likely driver, as these compounds selectively recruit Rhizobiales and phosphate-solubilising Pseudomonadales [10, 11].

The practical takeaway is that including chickpea in cereal rotations can enrich the soil microbiome beyond the direct N_2 -fixation benefit. Farmers in the Nigerian derived savanna who rotate chickpea before maize may see improved P availability and disease suppression in the subsequent crop—benefits that are hard to capture in a single-season yield comparison [12, 13].

A limitation is the single-season, single-site scope and the use of 16S rRNA (which identifies bacteria only, not fungi or archaea). Shotgun metagenomic sequencing would provide a more complete picture of functional potential [14].

Conclusion

Chickpea rhizosphere harboured the highest bacterial diversity ($H' = 3.34$) and *nifH* gene abundance (4.81×10^6 copies g^{-1}) among three legume crops, confirming its value as a soil-health-building component in tropical rotations. Soybean and groundnut also supported significantly richer communities than fallow. Incorporating chickpea into cereal-based rotations is recommended for both nitrogen economy and microbiome enrichment in the Nigerian derived savanna.

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