



Nitrogen fixation, crop production and bacterial communities of common bean cultivars: a 77-year breeding perspective

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Received: 11 August 2025 / Accepted: 24 December 2025 / Published online: 21 February 2026

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Abstract

Background and aims Common bean (*Phaseolus vulgaris* L.) is an important food legume which contributes to sustainable agriculture by fixing atmospheric nitrogen (N). However, modern breeding programs have mostly focused on improving crop yield and agronomic traits, ignoring the crop's capacity for effective symbiotic nitrogen fixation and interactions with microbial communities. We sought to investigate how genotype, soil N availability, and environmental conditions, affect plant growth, N fixation, and the composition of root-associated bacterial communities of 16 bean cultivars released over 77 years of breeding history.

Methods Crop growth parameters of 14 pintos, 1 pink, and a non-nodulating navy bean R99 were evaluated in field trials conducted over two growing seasons under differing N soil fertility. The soil and root

microbiomes associated with these cultivars were analyzed using 16S rRNA amplicon sequencing.

Results The results revealed significant year-to-year differences in crop yield and SNF. While nodulation rates were consistent, N fixation efficiency declined under high soil N conditions. Cultivar-specific differences in microbiome composition were observed under N-limited conditions, with several taxa strongly associated with individual genotypes. Notably, modern cultivars showed reduced SNF, which was also more prominent under low N availability, suggesting potential trade-offs associated with breeding for high-input systems. The line R99 exhibited a distinct microbial profile and reduced *Rhizobium* abundance, indicating a complex genotype–microbiome interaction.

Conclusion These findings highlight the importance of both genotype and soil environment on bean performance and microbiome structure and underscore the need for breeding strategies aimed at improving N-use efficiency in bean production.

Responsible Editor: Ulrike Mathesius.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11104-025-08257-x>.

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Keywords Common bean · Microbiome · 16S rRNA amplicon sequencing · Symbiotic nitrogen fixation

Introduction

Common bean (*Phaseolus vulgaris* L.) is an important food legume, valued for its high protein content, dietary fiber, and micronutrients such as iron and

folate (Subar et al. 1989; Thompson and Brick 2016; Gouvêa et al. 2024). It serves as a dietary staple for millions, particularly in Latin America, Africa, and parts of Asia (Uebersax et al. 2023), while also playing a key role in sustainable agriculture through its symbiotic relationship with nitrogen-fixing rhizobia (Lisciani et al. 2024). This symbiotic nitrogen fixation (SNF) process reduces the need for synthetic nitrogen (N) fertilizers, making common bean an attractive crop in both low-input and environmentally conscious production systems (Uebersax et al. 2023).

In the United States (U.S.), dry beans are an important pulse crop mainly produced in North Dakota, Michigan, Nebraska, Minnesota, Idaho, and Washington (Grafel 2025). The U.S. is one of the top global producers and exporters of dry beans, with pinto, black, navy, great northern, and kidney beans being the most widely grown market classes (Hesami and Yoosefzadeh-Najafabadi 2025). In recent decades, breeding programs in the U.S., particularly in the northern Great Plains and Midwest, have focused on improving yield potential, disease resistance, plant architecture, and maturity timing to suit regional growing conditions (Assefa et al. 2019). However, these efforts have often taken place under conventional high-input systems, often relying on synthetic fertilizers, including N. As a result, the symbiotic efficiency of common bean remains relatively low (Isoi & Yoshida 1991; Martínez-Romero 2003). However, significant variation in SNF among bean cultivars has been reported (Sanyal et al. 2018; Wilker et al. 2019). Additionally, environmental factors, such as soil N availability, can inhibit nodulation and N fixation, while genetic differences among cultivars further influence the plant's ability to establish and maintain effective symbiosis. More specifically, high soil N levels may suppress rhizobial colonization and nodule function, resulting in a trade-off between maximizing yield and enhancing SNF (Reinprecht et al. 2020).

In parallel, a growing body of research has emphasized the role of the plant microbiome, particularly root-associated bacterial communities, in regulating plant health, nutrient uptake, and stress tolerance. These microbial communities are shaped by both abiotic and biotic factors, including soil nutrient content, plant genotype, and agricultural management practices (Araujo et al. 2025). However, despite the agronomic and ecological importance of common bean in the U.S., relatively little is known about how breeding history and variable soil fertility

conditions influence the composition and function of root-associated microbial communities, and how these changes may affect N fixation and plant performance.

In this study, we investigated a panel of common bean cultivars representing 77 years of U.S. breeding history (from 1945 to 2022) to explore how plant genetics and soil fertility, particularly N availability, interact to influence plant yield, SNF, and microbiome structure. Two field trials were conducted over consecutive years at the same research site in Washington State under differing soil fertility conditions. Our specific objectives were to: (i) evaluate the effects of cultivar and N availability on yield, nodulation, and % N derived from the atmosphere (%NDFA); (ii) determine how root-associated bacterial communities vary across cultivars and environmental conditions; and (iii) identify microbial taxa linked to cultivar-specific performance, with potential applications for microbiome-informed breeding. This study brings new insights into how modern breeding and soil management shape the symbiotic and microbial dynamics of common bean. These findings have direct implications for improving nitrogen-use efficiency and sustainability in U.S. dry bean production systems.

Material and methods

Site description

For this study, a select panel of 16 dry beans (described below) was planted at the WSU IAREC 'Roza' research farm in Prosser, WA (46°29' N and -119°74' W) for two years in 2023 and 2024 in a Warden (coarse-silty, mixed, superactive, mesic Xeric Haplocambids) soil type A. The experiments were conducted in small plots (6.1 m×2.24 m) planted in a randomized complete block design with four replications. Row width was 0.56 m. Seeding rate was 285,000 seeds ha⁻¹. Previous crop was Teff grass (*Eragrostis tef*). The trials were planted in adjacent fields in separate years in the dry bean 'purgatory plot' characterized by compacted soil, no supplemental fertilizer, a water deficit of at least 30%, and fluctuating populations of *Fusarium solani* pv. *phaseoli* and *Pythium ultimum* causing Fusarium and Pythium root rot diseases, respectively. This purgatory plot in existence since 2020 is used to select breeding lines with multiple stress resistance/tolerance. Outlook 1.0 L/ha and Sonalan (4.1 L/ha) tank mix was preplant incorporated for weed control. None

of the trials received any fertilizers or rhizobium inoculants. Mechanical and hand cultivation was used to further control weeds during the growing season. For these trials overhead irrigation applications were supplied by handlines throughout the growing season to maintain adequate soil moisture for normal plant growth. Weather data for the location are presented in Table S1.

Dry bean panel

The select panel of 16 dry bean cultivars was comprised of 14 pintos, 1 pink, and 1 non-nodulating navy bean ‘R99’ (Park & Buttery 2006), encompassing 3 indeterminate growth habit types: upright, semi-upright and prostrate, and the determinate bush growth habit of R99 (Table S2). The pinto bean cultivars span 77 years of breeding from early 1945 to 2022 and include the ‘Common Pinto’ bean landrace that predates modern plant breeding activities. ‘UI-111’ released in 1945 represents a three-way cross between North American pinto, red, and great northern bean landraces. UI-111 represents the reference genome for race Durango of the Middle American gene pool (JGI 2025). ‘Roza’ pink and ‘GH 196’ pinto selected from a purgatory plot and released in the mid-1970s and –1980s, respectively, incorporate the tropical black bean PI 203958 introgression from race Mesoamerican used to improve root vigor and tolerance to *Fusarium* root rot in pinto beans and other race Durango market classes (great northern, pink, red). ‘Buster’ pinto released in 1998 was included because it performed poorly in the ‘purgatory plot’ in previous trials (Trapp et al. 2015). The pinto cultivars released in the twenty-first century possess upright architecture introgressed from tropical black beans using recurrent selection (Soltani et al. 2016). Recent upright pinto bean cultivars have improved yield potential and increased seed size compared to the first generation of upright pintos which had smaller seed size and lower yields. The ‘USDA Cody’, ‘USDA Diamondback’ (Miklas et al. 2024), and ‘USDA Rattler’ (Miklas et al. 2023) pinto bean cultivars were selected for tolerance to abiotic stresses in ‘purgatory plots’. The new ‘slow darkening’ pinto bean market class is represented by USDA Diamondback and ‘Wildcat’ (Urrea et al. 2022). Some of the cultivars were used to generate inbred populations to study genetics of tolerance i) to abiotic stresses (Buster/Roza) (Trapp et al. 2015); ii) white mold disease (*Sclerotinia sclerotiorum*) (USDA Cody/

USPT-WM-12) (Miklas et al. 2006; Soler-Garzon et al. 2025) and; iii) drought (DrWood/Stampede and USDA Rattler/Stampede, research pending). If these parental lines exhibit significant differences for SNF related traits in this study, they could be used to study the genetic inheritance of those differences.

Field data collection and analysis

Soil chemical analysis: Soil samples were collected a few weeks prior to planting using a 30 cm length probe with 3 cm diameter. The samples across the field were bulked and air dried before they were sent to Northwest Agricultural Consultants (Kennewick, WA) for analysis of macro- and micro-nutrients, and general soil characteristics such as pH, organic matter, and soluble salts (Table S3).

Plant parameters: Emergence was scored at the V2 growth stage on a scale from 1 = complete to 9 = no plants emerged. Lodging was scored at physiological maturity (R7 to R8) on a scale from 1 = no lodging to 9 = completely lodged. Seed yield (kg ha^{-1}) was obtained from the center two harvested rows and seed weight was based on $\text{g } 100 \text{ seeds}^{-1}$. Flowering date was the number of days after planting when 50% of the plants within a plot had at least one open flower. Harvest maturity was the number of days after planting when 80% of pods were dry.

The ^{15}N natural abundance method was used to assess biological N_2 fixation (Peoples et al. 2015). Fifty seeds from each plot were dried in an oven at 40°C for 24 h, then sent to the Stable Isotopes Lab at Washington State University (Pullman, WA) for sample preparation and analysis. Ground seed samples were converted to di-nitrogen gas (N_2) using a ECS 4010 Costech Analytical combustion system and analyzed with a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen) (Brenna et al. 1997; Qi et al. 2003). $\delta^{15}\text{N}$ ratios were normalized using the ATM-N International scale using two-point normalization after corrections for time and size (Coplen 2002). %NDRF was calculated using the equation below, where a β value of -2.45 was based on previous β values reported in the literature (Polania et al. 2016) for genotypes of Middle American origin similar to cultivars tested in this study and was used across cultivars and replications. Separate $\delta^{15}\text{N}$ values for each replication of the non-fixing reference plant (R-99) were used.

$$\% \text{NDFA} = \frac{\delta^{15}\text{N non-fixing reference plant} - \delta^{15}\text{N of N}_2 \text{ fixing legume}}{\delta^{15}\text{N non-fixing reference plant} - \beta} \times 100$$

β = $\delta^{15}\text{N}$ of N – fixing plant grown in no nitrogen conditions

Glutamic acid was used in a multi-point correction to estimate N% in the seed.

Nodulation Scoring

Nodulation rate was assessed approximately six weeks after planting, at the R1 growth stage. Six plants (three per subplot) including roots were extracted from each plot using a shovel, placed in sterile bags, and transported to the laboratory on ice. The root sections of each plant were rinsed with tap water and then evaluated for nodulation based on appearance of healthy looking pink nodules. Nodulation was scored on a scale from 0 to 6 based on a combination of nodule density and size, where:

- 0 = no nodules
- 1 = very few small nodules
- 2 = few small nodules
- 3 = moderate number of small to medium nodules
- 4 = many medium-sized nodules
- 5 = many large nodules
- 6 = abundant large nodules

The plot-level nodulation score was calculated as the average of five plants per plot.

Sample collection for amplicon sequencing

From the five plants above with attached roots, one plant per plot in the 2023 trial and two plants per plot in the 2024 trial were washed and sonicated three times in 10% glycerol as previously described (Yurgel and Mcgee 2025). Nodules and roots were then separated, washed in 10% glycerol, placed into cryogenic vials, and stored at -80°C prior to DNA isolation. Frozen roots were ground into a fine powder in liquid nitrogen, and 0.25 g of root tissue was used for DNA extraction. Nodules were immersed in 70% ethanol for 30 s, then transferred to 2.5% sodium hypochlorite for 5 min, followed by five rinses in 10% glycerol. The nodules were then crushed using disposable

Pellet Pestles (Fisherbrand), and 0.100 g of the tissue was used for DNA extraction. Ten soil samples were randomly collected from the top 15 cm of soil in each trial. The soil samples were collected from between the rows of planted beans in time of plant collection for microbiome analysis. Soil samples were placed on ice and transported to the laboratory, then sieved through a 2 mm mesh and stored at -80°C . For each sample, 0.25 g (wet weight) of soil was used for DNA extraction. DNA was extracted using the Omega Bio-Tek DNA extraction kit (Omega Bio-Tek, Inc., Norcross, Georgia), following the manufacturer's protocol. DNA quality and concentration were measured using a BioTek Synergy H1 Hybrid Multi-Mode Reader and the Gen5 software (version 3.08).

PCR amplification and Illumina sequencing

A total of 10 μL (containing at least 50 ng) of DNA per sample was sent to the Dalhousie University Centre for Comparative Genomics and Evolutionary Bioinformatics – Integrated Microbiome Resource (CGEB-IMR) for V6–V8 16S rRNA gene library preparation and sequencing. The V6–V8 region of the 16S rRNA gene was amplified using the primers: forward 5'-ACGCGHNRAACCTTACC-3' and reverse 5'-ACGGGCRGTGWGTRCAA-3'. Samples were multiplexed using a dual-indexing approach and sequenced on an Illumina MiSeq i100 platform with paired-end 300 $\times$ 300 bp reads. Illumina sequencing and multiplexing were performed as previously described (Comeau et al. 2017). The dataset generated for this study is available in the NCBI Sequence Read Archive (SRA) under the accession numbers PRJNA1292277, PRJNA1292281 and PRJNA1346669.

Amplicon sequence processing

The raw reads were processed as described previously (Yurgel and Mcgee 2025). Paired-end reads were first stitched together using Paired-End reAd mergeR (PEAR) (Zhang et al. 2014). Primer sequences were trimmed using Cutadapt v1.11 (Martin 2011), and the

reads were further processed using QIIME 2 version 2022.11 (Bolyen et al. 2019). Low-quality sequences were filtered using QIIME 2's q-score-joined function. Amplicon sequence variants (ASVs) were identified using the QIIME 2 Deblur plugin, which assigns ASVs at single-nucleotide resolution. Taxonomic classification of ASVs was performed using a Naïve Bayes RDP classifier trained on the SILVA 16S rRNA database (Quast et al. 2013). To account for internal sequencing errors associated with the Illumina MiSeq platform, ASVs with a mean relative abundance of less than 0.01% across the dataset were removed. Plant-derived ASVs, such as those from chloroplasts or mitochondria, as well as unannotated ASVs, were also excluded from further analysis.

Data analysis and statistics

Root and Soil Microbiomes: After deblurring and filtering, 6,464,512 16S rRNA reads were retained for analysis (median frequency=25,821). One sample with a sequencing depth below 9,848 reads was excluded. **Nodule Microbiome:** After deblurring and filtering, 4,056,274 16S rRNA reads were retained for analysis (median frequency=34,500). All nodule samples were retained for downstream analysis. For alpha-diversity estimation, the datasets were rarefied to a depth of 9,848 and 21,364 reads for root and nodule samples, respectively. Shannon diversity and beta(β)-diversity metrics were calculated using the core-metrics-phylogenetic plugin in QIIME2. Differences in microbial alpha(α)-diversity among groups were assessed using the Kruskal–Wallis pairwise test ($P < 0.05$) with Benjamini–Hochberg false discovery rate (FDR) correction for multiple testing. Bray–Curtis dissimilarity matrices were used to evaluate β -diversity differences among groups using permutational multivariate analysis of variance (PERMANOVA, also known as Adonis) with 999 permutations in QIIME 2. Non-metric multidimensional scaling (NMDS) plots and hierarchical cluster analysis based on Bray–Curtis distances were built using the *vegan* package in R. NMDS plots were visualized using the *ggplot2* package in R (Wickham 2016). Differential relative abundances were identified using the Analysis of Compositions of Microbiome (ANCOM) method implemented in the ANCOM R package with 1% FDR threshold (Mandal et al. 2015). All relative abundances of bacterial taxonomic groups are reported

as percentages of total 16S rRNA reads. Associations of ASVs and each plant cultivar was determined using correlation-based indicator species analysis (multiplatt function) with 9,999 permutations in the *indicspecies* package (De Cáceres and Legendre 2009). The ASVs with an indicator value (IndVal) ≥ 0.5 and a $P \leq 0.05$ were considered potential indicator taxa.

Results

Site weather conditions and chemical and biological properties

The experiments were conducted in 2023 and 2024 at the 'Roza' research farm in Prosser, Washington. Note that total rainfall precipitation was negligible during the growing season both years, 19.38 mm vs 8.58 mm., respectively (Table S1). Therefore, ~228 mm of supplemental precipitation from overhead irrigations of 25 mm applied at 10 d intervals were required to maintain adequate soil moisture conditions each season. Average temperature was also similar between years. The two sites differed significantly in soil chemical composition. The 2023 site had higher levels of inorganic nitrogen ($\text{NO}_3^- + \text{NH}_4^+$), sulfur potassium, magnesium, and copper, but lower organic matter (OM) compared to the 2024 site (Table 1; Fig. 1). Both sites had similar neutral pH: 7.03 and 7.25 in 2023 and 2024, respectively (Table S3). Based on optimum dry bean nutrient requirements of nitrogen (N) at 112 kg/ha, phosphorus (P) at 7 ppm (bicarbonate method), and potassium (K) at 120 ppm, the 2024 trial was very limited in N, and slightly limiting in P and K fertility compared to the 2023 trial, which was only slightly limited for N fertility.

Soil microbiome structure and diversity differed significantly between the two years. Approximately 19% of variation in the bacterial community was explained by the year of the experiment (Table 2). Shannon diversity was significantly higher in soils from the 2023 field trials compared to those from 2024, with mean values of 9.83 and 9.56, respectively (Fig. 2A). The most relatively abundant taxa in both trials included *Gemmatimonadaceae*, *Actinobacteriota* MB-A2-108, *Chloroflexi* KD4-96, and *Micrococcaceae*, although together they accounted for only a small proportion of total soil 16S rRNA reads, ranging between 4.7 and 3.6%

Table 1 Soil chemical properties. For each variable, data followed by different letters are significantly different between years according to a t-test ($P < 0.05$)

Parameters	2023	2024
$\text{NO}_3^- + \text{NH}_4$, kg/ha	79.3 ^A	32.2 ^B
Organic matter, %	0.86 ^B	1.14 ^A
Sulfur, ppm	4.74 ^A	3.5 ^B
Potassium, ppm	127.5 ^A	90.75 ^B
Magnesium, meq/100 g	2.97 ^A	2.24 ^B
Copper, ppm	4.65 ^A	1.3 ^B

(Table S4). Minor differences in bacterial composition were observed between years, with only two relatively low abundant genera differentially represented. Actinobacteria *Saccharothrix* and *Pseudarthrobacter* were significantly overrepresented in 2023 soils compared to 2024 (Table S5).

Crop growth parameters

In 2023, the average crop yield, hundred-seed dry weight (100 SDWT), and percentage of nitrogen (N) in seed (% N) were significantly higher, while

percentage of N derived from atmosphere (% NDFA) was significantly lower, compared to 2024 (Fig. 1; Table S6). Specifically, the average crop yield for all cultivars evaluated in the trials was 4,080 kg/ha in 2023 versus 2,447 kg/ha in 2024; 100 SDWT was 45 g in 2023 versus 42 g in 2024; % N in seed was 3.8% in 2023 versus 3.5% in 2024; and % NDFA, not including R99, was 14% in 2023 versus 31% in 2024. The average nodulation score did not differ significantly between years and remained relatively low, with values of 2.6 and 2.4 (not including R99) for 2023 and 2024, respectively (Fig. 1C), indicating a small number of nodules per plant. When examining individual cultivars, % NDFA was generally lower in 2023, while crop yield, % N in seed, and 100 SDWT were generally higher compared to the 2024 trials (Fig. 3B, C; Table S6). Specifically, in 2023% NDFA, not including R99, ranged from 6.3–11.7% (R99, USPT-WM-12, Wildcat, ND-Falcon, USDA Cody, USDA Diamondback, Buster, and USDA Rattler) to 14.1–27.1% (UI-111, LaPaz, Stampede, UI-114, Common Pinto, GH-196, DrWood, and Roza), whereas in 2024, it ranged from 20.4–26.9% (GH-196, USDA Diamondback, USDA Cody, ND-Falcon, Wildcat, Buster, and USDA Rattler) to 31.5–40.3%

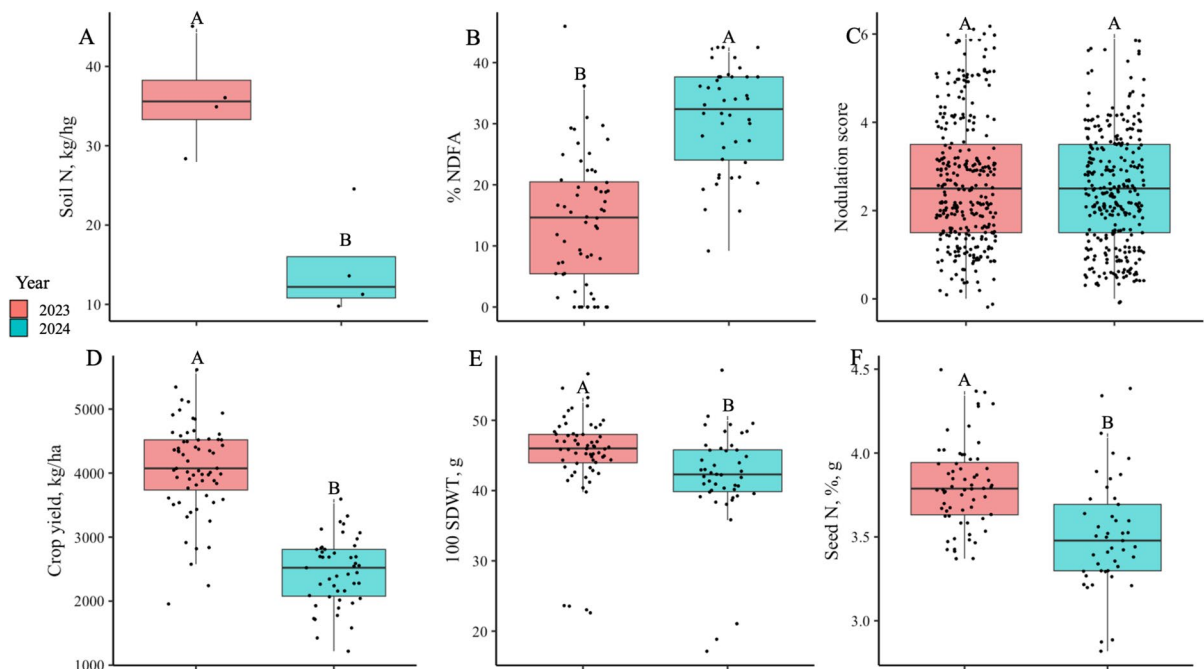


Fig. 1 Bean (*Phaseolus vulgaris*) plant agronomic data across trials. For each variable, data followed by different letters are significantly different according to a t-test ($P < 0.05$). **A** Soil N,

kg/ha; B % NDFA; C Nodulation score; **D** Crop yield, kg/ha; **E** 100 SDWT; **F** Seed N, g

Table 2 Variation in sample groupings as explained by weighted Bray–Curtis dissimilarity distances

Factors	R ²
Soil	
Year	0.185***
Root and soil, 2023 and 2024 combined	
Tissue	0.132***
Year	0.088***
Tissue:Year	0.016***
Root 2023 and 2024	
Cultivar	0.089*
Year	0.102***
Root 2023 and 2024, no R99	
Cultivar	0.077
Growth habit	0.012
Year	0.123***
Root 2023	
Cultivar	0.235**
Root 2023, no R99	
Cultivar	0.296**
Growth habit	0.065**
Root 2024	
Cultivar	0.143
Root 2024, no R99	
Cultivar	0.116
Growth habit	0.021

Adonis tests were used to assess whether beta-diversity is related to sample groupings, 999 permutations, R², * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$

(*Stampede*, *LaPaz*, *DrWood*, *USPT-WM-12*, *UI-114*, *UI-111*, *Roza*, and *Common Pinto*). *Common Pinto* landrace was among the best N fixers in both years, and *USPT-WM-12* showed the greatest increase in % NDFA between 2023 and 2024 trials (> 30%). Additionally, *USPT-WM-12* accumulated the most % seed N in both years, indicating an efficiency to partition and remobilize both soil and symbiotically fixed N.

A significant positive correlation was detected between nodulation score, crop yield, 100 SDWT, and days to maturation between 2023 and 2024 trials. Specifically, 100 SDWT exhibited a very strong correlation of 0.88***, while nodulation score, crop yield and maturity showed moderate correlation between years, 0.63**, 0.51*, and 0.62**, respectively (Table 3). However, no significant correlations were found for % NDFA or % N in seed between 2023 and 2024 trials.

We detected an effect of cultivar release date on several plant parameters, including % NDFA, nodulation score, and crop yield (Table 3; Fig. 3D, F and G). However, the strength and direction of these effects varied depending on the year of the trial and the specific parameter. For instance, % NDFA was strongly and negatively correlated with release date in both 2023 and 2024 trials, with correlation coefficients of $R = -0.61^*$ and -0.67^{**} , respectively (Fig. 3F). In contrast, significant correlations between release date and other traits were only observed in 2023: a strong negative correlation with nodulation score ($R = -0.72^{**}$) and a moderate positive correlation with crop yield ($R = 0.63^*$) (Fig. 3D and G). We also observed year-specific differences for nodule formation among cultivars. *Buster*, *Common Pinto*, *Roza*, and *Stampede* exhibited significantly higher nodulation scores in 2023 than in 2024, whereas *USPT-WM-12* had a significantly lower nodulation score in 2023 (Fig. 3A). Additionally, a moderately positive correlation between % NDFA and nodulation score ($R = 0.58^*$) was detected in 2023 trial, but not in 2024 (Fig. 3E; Table 3).

Root microbiome

Bacterial alpha- and beta-diversity differ significantly between the soil and root microbiomes. Shannon diversity was notably higher in soil (9.7) than in root (5.0) (Fig. 2A), and more than 13% of the variation in bacterial community structure was explained by tissue type (root vs. soil) (Table 2; Fig. 2B). In total, 200 bacterial taxa were differentially represented between the soil and root microbial communities (Table S7). The root microbiome was dominated by *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* group (hereafter referred to as *Rhizobium*), which accounted for over 48% of total root 16S rRNA reads attributed to this taxon. In contrast, less than 1% of total soil 16S rRNA reads were assigned to *Rhizobium* (Fig. 2D). Other prominent taxa overrepresented in the roots compared to soils included *Niastella*, *Chitinophagaceae* family, *Ohtaekwangia* and *Bradyrhizobium* (Table S7; Fig. 2C).

Variation of root microbiome across the trials

The year of the trial was a significant factor influencing bacterial community structure and diversity in the root microbiome. Specifically, it accounted for

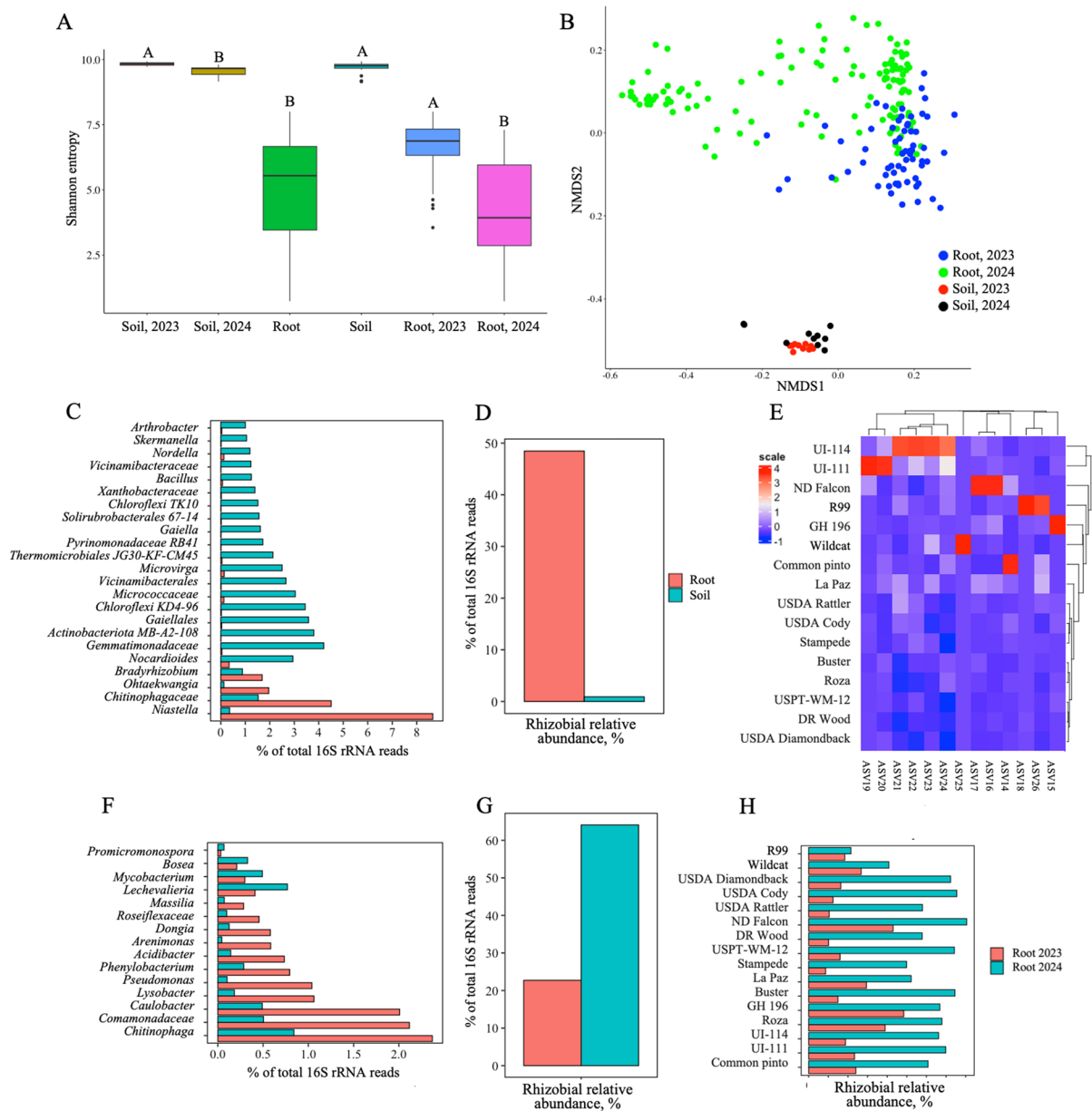


Fig. 2 Bacterial population in bean microbiome. **A** Estimated Shannon diversity of bacterial community. For each variable, data followed by different letters are significantly different within each group (soil or root) according to Kruskal–Wallis pairwise test ($P < 0.05$). **B** Bacterial beta-diversity in bean microbiome. Nonmetric multidimensional scaling (NMDS, based on Bray–Curtis dissimilarity distances) of bacterial communities at amplicon sequence variants (ASVs) level. **C**, **D** Relative abundances of genera differentially represented between soil and root tissue based on ANCOM test with 1% FDR. **C** all genera excluding *Rhizobium*; **D** *Rhizobium*.

E Bacterial ASVs which were detected as unique indicators in root-associated microbiome in each cultivar. Indicator species analysis, 2024 trial only. $0.5 < \text{IndVal} < 0.6$. **F**, **G** Relative abundances of root-associated genera differentially represented between trials based on ANCOM test with 1% FDR. **F** – all genera excluding *Rhizobium*; **G** – *Rhizobium*. **H** Rhizobial distribution in the root microbiome across cultivars and trials. **C**, **D**, **F**, **G**, **H** Corrected P -values were calculated based on Benjamini–Hochberg false discovery rate (FDR) multiple test correction

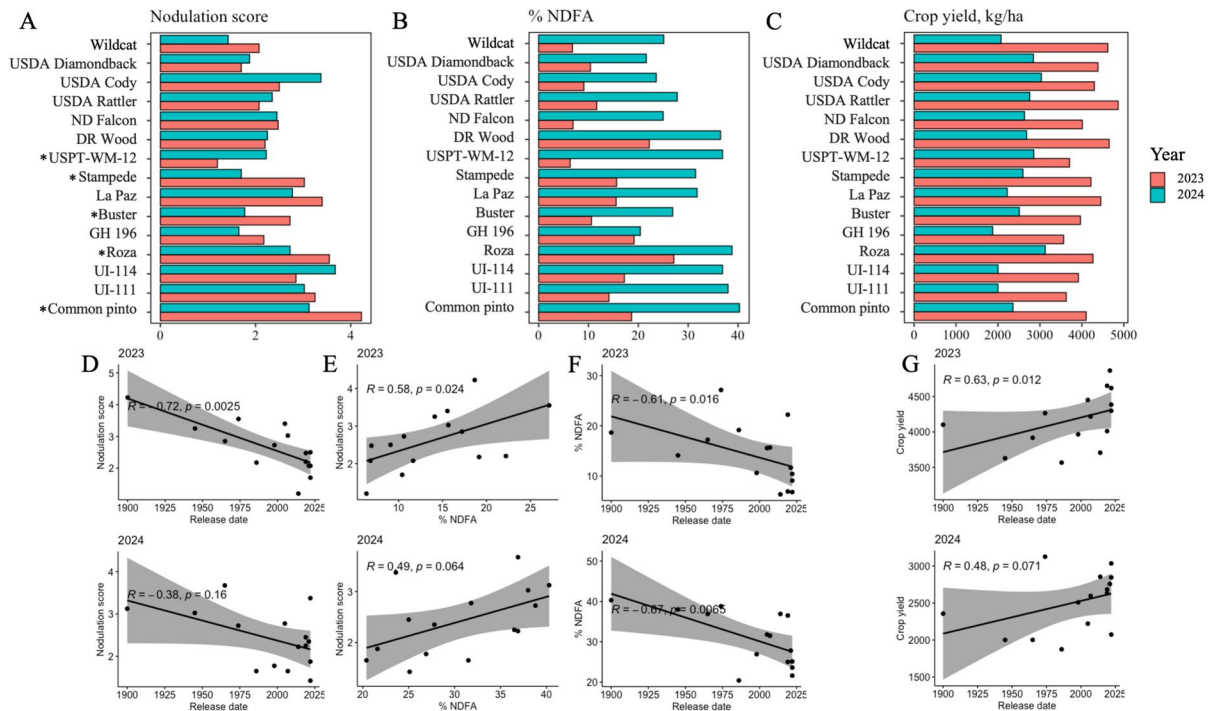


Fig. 3 Crop parameters across cultivars. **A–C** variation of crop parameters, **A** nodulation score; **B** % Nitrogen derived from atmosphere (NDFA); **C** crop yield. * – Cultivars with nodulation score differed across the trial according to a t-test ($P < 0.05$). **D–G** pairwise Spearman correlation between:

D nodulation score and cultivar release date; **E** nodulation score and % NDFA; **F** % NDFA and cultivar release date; and **G** crop yield and cultivar release date. Upper panel – 2023 trial; lower panel – 2024 trial

Table 3 Correlation between crop growth parameters

Parameters	2023 vs. 2024	2023	2024
Nodulation score	0.63**		
Yield	0.51*		
100 SDWT	0.88***		
Maturity	0.62**		
% NDFA	0.41		
% Seed N	0.42		
Release date vs. % NDFA		-0.61*	-0.67**
Release date vs. nodulation score		-0.72**	-0.38
Release date vs. yield		0.63*	0.48
Release date vs. % Seed N		-0.06	0.11
Release date vs. 100 SDWT		0.11	0.42
Release date vs. root rhizobial (%)		-0.33	0.03
% NDFA vs. Nodulation score		0.58**	0.49
% NDFA vs. Yield		0.16	0.18
Nodulation score vs. yield		0.05	0.11
Nodulation score vs. root rhizobial abundance		0.21	0.25

Pairwise Spearman correlation, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$

10% of the variation in root-associated bacterial communities (Table 2; Fig. 2B). The root microbiome collected in 2023 exhibited a higher Shannon diversity index (6.6), compared to that from 2024 (4.3) (Fig. 2A). In total 81 root-associated bacterial genera were differentially represented between the two years. Notably, the genus *Rhizobium* was overrepresented in 2024 roots, comprising 64% of total 16S rRNA reads, compared to 23% in 2023 (Table S8; Fig. 2G). In contrast, several non-rhizobial taxa were overrepresented in 2023 roots compared to 2024, including *Chitinophaga*, Comamonadaceae, *Caulobacter*, *Lysobacter*, *Pseudomonas*, *Phenylobacterium*, and *Acidibacter* (Fig. 2F). Bacterial taxa enriched in 2024 roots relative to 2023 roots included *Lechevalieria*, *Mycobacterium*, *Bosea*, and *Promicromonospora*.

Variation of root microbiome across cultivars

When non-nodulating R99 was included in the analysis, cultivar identity was a significant, though minor, factor affecting bacterial root microbiome structure, explaining approximately 9% of the community variation (Table 2). However, when R99 was excluded from the analysis, the effect of cultivar on root microbiome composition was no longer significant. Similarly, growth habit (upright vs. semi-upright vs. prostrate) had no effect on the community structure (Table 2). Furthermore, no significant differences in the relative abundances of bacterial families or in Shannon diversity were detected across cultivars or growth habit when microbiome data from 2023 and 2024 were combined, regardless of whether R99 was included in the analysis.

In the 2023 trial, cultivar had a significant effect on bacterial root microbiome structure ($R^2=0.235$, $P<0.01$), whereas no significant effect of cultivar was detected in 2024 trial (Table 2). Similarly, growth habit was a significant factor, shaping root microbiome in the 2023 ($R^2=0.065$, $P<0.01$) but not in the 2024 trial ($R^2=0.021$, $P>0.05$). However, our data revealed a differential distribution of the genus *Rhizobium* among bean cultivars in 2024, but not in 2023 (Fig. 2H). Specifically, in 2024, *Rhizobium* was apparently underrepresented in non-nodulation cultivar R99 compared to all other cultivars. When R99 was excluded from the analysis, no differentially represented taxa were detected

across cultivars, suggesting that the depletion of *Rhizobium* in R99 roots was the primary driver of the observed variation in *Rhizobium* abundance. On the other hand, in the 2023 trial, no bacterial genera were found to be differentially represented across cultivars, regardless of whether R99 was included or excluded from the analysis.

Two relatively low abundant ASVs, ASV18 and ASV26, annotated as Chitinophagaceae and *Lechevalieria*, respectively, were differentially represented across cultivars in the 2024 trial. These ASVs were predominantly found in non-nodulating cultivar R99, suggesting a preferential association with this host (Table S9). However, the strength of these associations was modest, with IndVal values of 0.559 ($P=0.001$) for ASV18 and 0.425 ($P=0.003$) for ASV26. Overall, the correlation-based indicator species analysis revealed low persistence of bacterial indicator ASVs across specific cultivars. Only four ASVs had strong associations ($\text{IndVal}>0.6$): ASV16 (*Stenotrophomonas*) and ASV17 (*Microbacterium*) were associated with ND Falcon and ASV19 (*Sphingobacterium*) and ASV20 (*Dyella*) were indicative for UI 111. Additionally, seven ASVs showed significant but moderate association with specific cultivars ($0.5<\text{IndVal}<0.6$). This included ASV14 (*Chitinophaga*) associated with Common Pinto; ASV15 (*Taibaiella*) associated with GH 196; ASV21 (*Dyadobacter fermentans*), ASV22 (*Microbacterium*), ASV23 (*Shinella*), and ASV24 (*Comamonadaceae*) associated with UI 114; and ASV25 (*Sphingobium*) associated with Wildcat (Fig. 2E; Table S9).

Rhizobial population in bean root microbiome

A total of 61 *Rhizobium*-affiliated ASVs were identified across soil and root microbiomes (Fig. 4A and B; Table S10). All 32 *Rhizobium* ASVs detected in soil were also present in the root microbiome. In total, soils and roots shared the majority of rhizobial ASVs – 62% of soil ASVs and 72% of root ASVs. However, roots collected in 2024 contained a substantial proportion (26%) of unique *Rhizobium* ASVs, while only one unique ASV was identified in the 2023 root microbiome. Two *Rhizobium* ASVs (ASV2 and ASV11) were differentially represented in soil between the two years (Fig. 4C; Table S10). Specifically, ASV2 was highly abundant in 2023

soils (comprising 40% of rhizobial 16S rRNA reads) but nearly absent in 2024 (0.2%), while ASV11 was detected only in 2023 soils. Overall, six rhizobial ASVs (ASVs 2, 3, 4, 6, 7, and 9) exhibited significantly different relative abundances between soil and root microbiomes (Table S10). Several of these ASVs were among the most relatively abundant in both microbiomes (Fig. 4D, collectively representing 64% and 63% of rhizobial 16S rRNA reads in roots and soils, respectively). Interestingly, there was a strong apparent plant-mediated selection against ASV6, whose relative abundance was 11 times lower in the root microbiome compared to the soil, suggesting a potential exclusion or suppression mechanism.

Ten rhizobial ASVs were differentially represented in the roots between the two years and accounted for the majority of rhizobial 16S rRNA reads in root, 72% in 2023 and 2024 roots, respectively (Table S10). Among these, ASV1 was noticeably more abundant in 2024 roots, representing 35% of rhizobial reads

compared to only 1.5% in 2023 – a 23-fold increase. In contrast, ASV3, ASV4 and ASV6 were significantly overrepresented in 2023 roots. These three ASVs collectively comprised 68% of rhizobial reads in 2023 roots, but only 30% in 2024 roots (Fig. 4E, Table S10).

Rhizobial population in bean nodule microbiome

Due to constraints on the number of plants that could be collected from each plot, implemented to preserve the integrity of plant yield, we were unable to obtain sufficient material to perform microbiome analysis on the nodules from the 2024 roots. Therefore, only the bacterial microbiomes of nodules from the 2023 plants were analyzed. In total, 103 unique ASVs were identified in the nodule tissue (Table S11). Alpha- and beta-diversity analyses did not reveal any significant effects of cultivar on bacterial diversity or community structure in the nodules. Additionally, no bacterial ASVs were differentially represented across cultivars. The

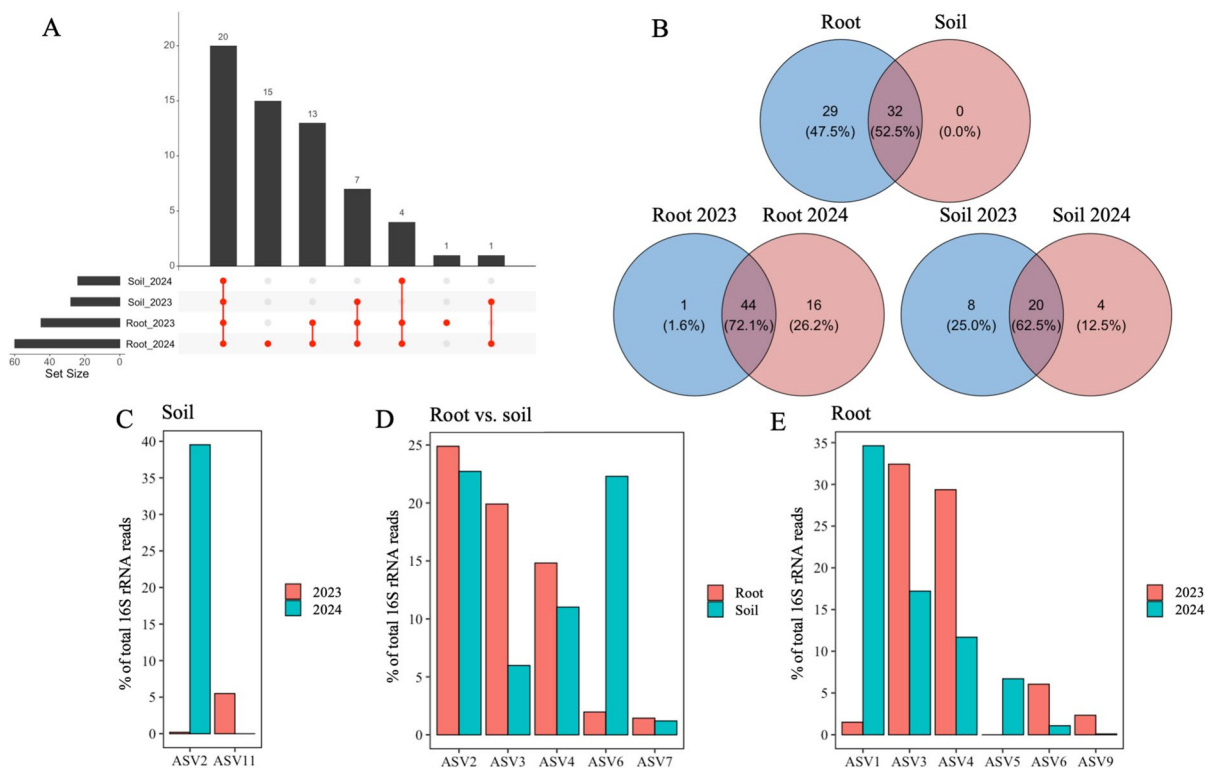


Fig. 4 Rhizobial population in bean root microbiome. **A**, **B** Distribution of rhizobial ASVs across niches. **C–E** Most relatively highly abundant rhizobia differentially represented

between the trials, based on ANCOM test with 1% false discovery rate. The values represent % of the reads in a group

nodule microbiome was dominated by rhizobia, which accounted for 99.7% of the total reads and were distributed across 23 ASVs. Thirteen of these ASVs were also found in bean roots. These shared ASVs were highly abundant in both tissues, representing 99.9% and 96.8% of rhizobial reads in the nodule and root microbiomes, respectively (Fig. 5A). Two of the three most abundant ASVs in the root microbiome (ASV2 and ASV3) were further enriched in the nodules, comprising 36% and 40% of the total nodule reads, respectively. ASV1, although not highly abundant in the 2023 roots (1.5% of root reads), was substantially enriched in the nodules, accounting for 21% of 2023 nodule reads. The relative abundance of ASV4 was reduced in the nodule microbiome, 2% compared to 29% of 16S rRNA reads in the nodule and root microbiomes, respectively.

Discussion

Bean growth parameters

Variations in soil fertility conditions likely contributed to the significant differences observed in bean crop yield and symbiotic N fixation across years. While both trials were conducted at the same site with similar soil type, supplemental irrigation, and average temperatures, the 2024 trial had much lower

soil fertility with significantly lower levels of N and K, and several micronutrients, such as S, Mg, and Cu. However, the most limiting nutrient was N. Previous evaluation of the responses of dry bean genotypes to high soil N availability indicated a positive effect on crop yield and a negative effect on N fixation (% NDFA) (Reinprecht et al. 2020). Similarly, we detected a significant decrease in overall bean symbiotic N fixation, measured by % NDFA, and an increase in crop yield in 2023 with higher N availability compared to the 2024 trial. These results are consistent with previous reports of the inhibitory effect of soil N on bean symbiotic performance (Tsai et al. 1993; Polania et al. 2016; Ma et al. 2025).

There were no significant differences in overall bean nodulation score between the trials, suggesting the decrease in N fixation may instead be attributed to reduced effectiveness of the nodules formed in 2023 under high soil N conditions. This is consistent with a previous report showing soil nitrogen significantly affects symbiotic nitrogen fixation but not nodule number (Tsai et al. 1993). Similarly, we detected a significant decrease in nodulation and an apparent increase in % NDFA in Common Pinto, Roza, Buster and Stampede in 2024, compared to 2023 trial, suggesting more efficient N fixation in the nodules formed for these cultivars under N limitation.

Several studies have investigated the influence of common bean genotype, environment, and genotype

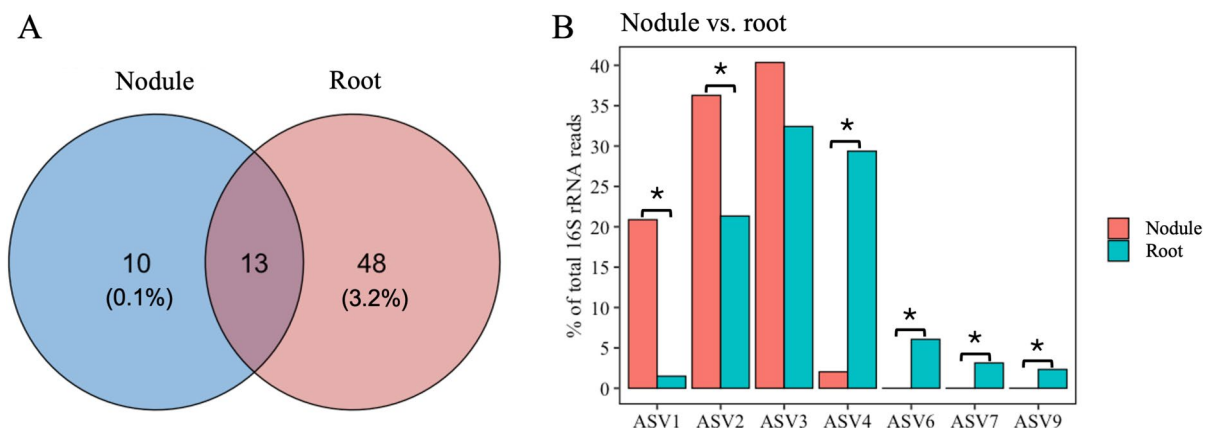


Fig. 5 Rhizobial population in bean nodules from 2023 trial. **A** Distribution of rhizobial ASVs between nodule and root tissue. The numbers in parentheses indicate percentage of rhizobial reads unique to nodule or root microbiome, respectively. **B** Representation of most relatively highly abundant rhizobia

in root in nodule microbiomes. The asterisks indicate ASVs differentially represented between root and nodule tissue, based on ANCOM test with 1% false discovery rate. The values represent % of the reads in each tissue

x environment interaction on plant growth parameters (Barili et al. 2015; Philipo et al. 2021). The correlation analysis of growth parameters across the trials herein indicated the cultivars were environmentally stable with respect to nodulation score, crop yield, and 100 SDWT. However, no significant correlation in N fixation among cultivars was detected between years, suggesting that genotype x environment interaction had a strong effect on cultivar ability to acquire N from the atmosphere. Soil N content was one of the major soil characteristics that differed between trials, suggesting soil N was the main factor affecting cultivar N fixation.

We took advantage of the panel of bean cultivars spanning 77 years of breeding history, from 1945 to 2022 to examine the effect of breeding progress on plant growth parameters. A significant negative correlation was detected between plant % NDFA and cultivar release date, which is consistent with previous reports on the poor symbiotic performance of modern bean cultivars (Hardarson et al. 1993). Similarly, a relatively strong negative correlation between nodulation score and cultivar release date was observed in the 2023 trial. However, no significant correlation between these parameters was found in 2024, which could potentially be explained by stronger inhibitory effect of higher soil N on nodulation in modern beans cultivars.

Bean microbiome

While common beans are widely cultivated across the world, limited information is available regarding the microbial communities associated with this crop, particularly the root-associated microbiome (Baraza et al. 2020; Sendi et al. 2020; Luo et al. 2022; Medina-Paz et al. 2022; Mendes et al. 2023). Differences in soil community structure and diversity between the trials suggest that variation in edaphic factors may have influenced microbial composition. Previously we reported a stronger correlation between bacterial community composition and soil concentrations of magnesium, sulfur, and potassium, compared to other soil fertility parameters such as organic matter and carbon concentration (Yurgel et al. 2017). Therefore, the increased concentration of these elements in the 2023 soil, relative to 2024, could partially explain the observed differences in bacterial composition between trials.

Several bacterial taxa belonging to the Chitinophagaceae family, as well as Ohtaekwangia and

Bradyrhizobium, were enriched in the root microbiome compared to the surrounding soil. Many species within the Chitinophagaceae family and the Bradyrhizobium genus are capable of fixing atmospheric nitrogen, which can be utilized by the host plant and may also contribute to the suppression of pathogenic infections (Carrión2019; Takatsu et al. 2022). Additionally, some of these taxa possess plant growth-promoting properties that may play a role in enhancing plant fitness and productivity (Kim et al. 2024; Sharma et al. 2025). Therefore, the enrichment of these bacterial taxa in the root microbiome suggests a selective recruitment by the plant to improve nutrient acquisition and bolster defense mechanisms. Future research should focus on isolating and characterizing these microorganisms to better understand their functional roles and interactions with host plants.

In our study, the rhizobial population was strongly enriched in beans roots compared to surrounding soil, consistent with a previous study of winter pea-associated microbiome (Yurgel and Mcgee 2025). Similarly, nearly 95% of bean root microbiome was attributed to *Rhizobium* genus (Mendes et al. 2023). The proportion of *Rhizobium* in the root microbial community was significantly higher in 2024 compared to the 2023 trial. This might be explained by lower N availability in the 2024 soils, which could stimulate production of plant flavonoids and increase the expression of rhizobial *nod* genes (Dusha et al. 1989; Wang and Stacey 1990; Coronado et al. 1995; Liu and Murray 2016). The overrepresentation of *Rhizobium* in bean roots may account for the decrease in bacterial alpha-diversity and the relative abundances of several other bacterial genera in 2024 compared to the 2023 trial, since a high abundance of rhizobia may have reduced the detection or relative contribution of non-rhizobial taxa in the root microbiome in 2024.

Our analysis of the root-associated rhizobial community revealed several major ASVs that were differentially represented between the trials. Although rhizobial colonization of root does not guarantee successful nodulation or the formation of an effective N-fixing symbiosis, our previous study showed that the most abundant root-associated rhizobia were also the major ASVs found in winter pea nodules (Yurgel and Mcgee 2025). In agreement with this, two of the three major rhizobial ASVs in the 2023 roots (ASV2 and ASV3) were also dominant in the 2023 nodules.

ASV1 and ASV2 were significantly enriched in the nodules, indicating the potential of the rhizobia represented by these three ASVs to form symbiotic associations with beans. Interestingly, the relative abundances of ASV4, ASV6, ASV7, and ASV9 were significantly lower in the nodule microbiome compared to the roots, suggesting a low symbiotic potential for the rhizobia represented by these ASVs. Due to limitations in the number of plants collected in the 2024 trial, we did not evaluate the rhizobial population within the 2024 nodules. Therefore, further isolation and characterization of rhizobia from both 2023 and 2024 nodules are recommended to better understand strain diversity and identify candidates for improving symbiotic nitrogen fixation in beans.

Plant domestication and breeding alter plant phenotypic, genomic, and metabolic characteristics to meet the demand for efficient crop production and enhanced nutritional quality (Purugganan and Fuller 2009; Abbo et al. 2014; Smykal et al. 2018). For example, a key difference between old and new cultivars is their growth habit/architecture: indeterminate prostrate (TYPE III) vs indeterminate upright (TYPE II), respectively. In TYPE II plants, more root energy is used to support upright architecture, whereas in TYPE III plants, root energy can be expended to explore more soil, resulting in a greater overall root mass. These changes can significantly influence how plants select and shape their associated microbiomes (Pérez-Jaramillo et al. 2017, 2019; da Silva et al. 2023). Similarly, our data indicated that cultivar and growth habit had a significant effect on root-associated bacterial communities in beans. However, this effect was only observed in plants grown under low N availability, suggesting differences in host plant bacterial selection under N limitation. Additionally, ASV16 (*Stenotrophomonas*) and ASV17 (*Microbacterium*) were strongly associated with the ND Falcon cultivar with upright architecture. Conversely, ASV19 (*Sphingobacterium*) and ASV20 (*Dyella*) were strongly associated with UI 111 with prostrate growth habit. But these specific ASV associations nor others observed among the cultivars were specific to growth habit types.

The microbiome structure of non-nodulating cultivar R99 differed from that of other cultivars capable of establishment of symbiosis with rhizobia. For example, inclusions of R99 in a PERMANOVA analysis of combined 2023 and 2024 root microbiome

data revealed a minor but significant effect of cultivar on microbiome structure – an effect not observed when R99 was excluded from the analysis. We also detected a significant decrease in *Rhizobium* abundance in R99 roots compared to other cultivars, suggesting a complex effect of *nnd-2* mutation on the bean-*Rhizobium* interaction (Park and Buttery 1994).

In conclusion, this study demonstrated environmental factors significantly influenced plant growth, SNF, and root-associated microbiomes. The observed variation in yield and symbiotic nitrogen fixation between trials was largely attributed to differences in soil nutrient availability. While nodulation rates remained relatively consistent, N fixation efficiency (%Ndfa) varied significantly between trials, highlighting the inhibitory effect of high soil N on the symbiotic process. Our results also indicated the role of bean genotype in shaping microbial communities, particularly under N-limited conditions. Several cultivar-specific bacterial associations were identified, with distinct microbial signatures linked to the ND Falcon and non-nodulating R99 cultivars. The overrepresentation of *Rhizobium* in root microbiomes under low N availability suggest a host selection and potential competition within the rhizosphere that may suppress other bacterial taxa. Our data revealed a negative correlation between cultivar release year and SNF, suggesting that breeding efforts may have reduced symbiotic efficiency in modern cultivars. The identification of specific rhizobial ASVs associated with improved performance under N limitation points to opportunities for employing beneficial microbes in breeding and agronomic strategies. Together, these findings highlight the importance of integrating plant genotype, soil conditions, and microbiome interactions into sustainable crop improvement programs to enhancing N use efficiency and bean productivity.

Author contributions Conceptualization, S.N.Y. and P.N.M.; methodology, S.N.Y, P.N.M. and L.D.P.; formal analysis, S.N.Y.; writing—original draft preparation, S.N.Y.; writing—review and editing, S.N.Y, P.N.M. and L.D.P.. All authors have read and agreed to the published version of the manuscript.

Funding This research was funded by USDA ARS Projects 2090–21600-040-000D.

Data availability The datasets generated for this study can be found in the NCBI sequence read archive under the accession numbers PRJNA1292277 and PRJNA1292281.

Declarations

Conflict of interest The authors declare no conflicts of interest.

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