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RHIZOSPHERIC BACTERIAL COMMUNITIES AND SEEDLING GROWTH OF *Fraxinus uhdei* Wenz. Lingelsh.

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ABSTRACT

The application of plant growth-promoting rhizobacteria (PGPR) induces shifts in rhizospheric bacterial community composition. This study evaluated the effects of four *Azospirillum brasilense* strains in the rhizosphere of ash tree (*Fraxinus uhdei* Wenz. Lingelsh.) seedlings: two diazotrophic (nitrogen (N_2) fixing) and two phosphate solubilizing strains. The analysis encompassed bacterial community structure, potential metabolic functions, and seedling growth and nutrition responses. DNA was extracted from rhizospheric soil, and the V3–V4 hypervariable region of the 16S rRNA gene was amplified and sequenced. The rhizobiome of *A. brasilense* inoculated plants was predominantly composed of seven phyla, with Proteobacteria being the most abundant (54 %). Alpha diversity analysis assessed by the Chao 1 richness and Shannon Weaver indices indicated that inoculation did not significantly alter species richness but resulted in a reduction of diversity. Inoculation with nitrogen-fixing (NF) strains led to a sevenfold increase in the relative abundance of the genus *Azospirillum* compared to uninoculated plants (Psi). Beta diversity analysis based on Bray-Curtis dissimilarity revealed a significant separation between the bacterial communities of inoculated and control plants. Prediction of metabolic potential via the Kyoto Encyclopedia of Genes and Genomes (KEGG) indicated that inoculation significantly altered ($p \leq 0.05$) the relative abundance of 11 % of the predicted potential functional pathways. Analysis of variance (ANOVA) and the post-hoc Tukey test ($p \leq 0.05$) demonstrated that co-inoculation with both strains yielded the most significant growth enhancements, with increases of 11.4 to 36.9 % across measured variables. FN strains increased macronutrient content by 15 to 24 %. These findings demonstrate that *A. brasilense* inoculation influences the structure and

predicted functions of the rhizobacterial community and acts as an effective PGPR by enhancing the growth and nutrition of *F. uhdei* seedlings.

Keywords: *Azospirillum brasilense*, rhizosphere, 16S rRNA.

INTRODUCTION

With the advancement of omics sciences in the last decade, the perception of plants as a single individual has changed. They are no longer considered isolated organisms but rather holobionts, that is, larger entities made up of a host (plant) and its associated microorganisms (Cook *et al*., 2024). These phyllospheric, endophytic, and rhizospheric microorganisms affect plant development and growth. In the rhizosphere, which is the area surrounding the roots, bacteria have gained special interest due to their role in nutrient supply and the production of phytohormones and antimicrobial substances. For this reason, they are called plant growth-promoting rhizobacteria (PGPR) (Dutta *et al*., 2022).

In tree species, the composition of rhizosphere bacterial communities has been described during nursery plant growth. The core microbiome is composed of nitrogen-fixing (N_2) and plant growth-promoting taxa (García-Lemos *et al*., 2020). Bacterial groups involved in plant-microorganism interactions, which participate in plant metabolism and nutrition, have also been identified (Liu *et al*., 2022).

The diversity and composition of bacterial communities can be altered by agricultural practices such as the use of biofertilizers. In *Ulmus chenmoui* Cheng, the introduction of phosphate-dissolving bacteria changes the composition of the bacterial community. This benefits populations of Proteobacteria and Bacteroidetes, which are part of the carbon and nitrogen cycles (Song *et al*., 2021). These findings highlight the importance of evaluating the effects of PGPR biofertilizers on the diversity and composition of the bacterial microbiome, given that they can influence the formation of communities that favor plant growth (Gu *et al*., 2020).

The ash tree (*Fraxinus uhdei* Wenz. Lingelsh.) is commonly found in urban areas of Mexico. Its establishment is widely favored in parks and avenues, as it can survive in poorly developed and contaminated soils (Pérez-Baltazar *et al*., 2020). However, *F. uhdei* plants intended for urban tree planting could enhance their growth and nutrient availability with the use of PGPR-based biofertilizers.

The objective of this study was to determine whether inoculation with *Azospirillum brasilense*, a PGPR, using two strains capable of fixing nitrogen (N_2) and two strains capable of solubilizing phosphates, impacts the diversity, composition, and potential metabolic functions of the bacterial community in the rhizosphere of *F. uhdei* seedlings. The effect on their growth and nutrition was also evaluated. The null hypothesis states that inoculation with *A. brasilense*, either with one or both strain types, causes no changes in the structure of the rhizospheric bacterial community and does not favor plant growth and nutrition.

MATERIALS AND METHODS

Site location and experimental conditions

The trial was established from January 23rd to August 15th, 2024, in Texcoco, State of Mexico (19° 29′ 23.74″ N, 98° 53′ 4.09″ W, at an altitude of 2250 m), under greenhouse conditions, covered with 50 % shade netting. There was no artificial control of temperature and humidity. The average, minimum, and maximum temperature values were 17.2, 3.8, and 30.1 °C, respectively, while relative humidity was 47.2, 12.2, and 81.4 %. These records were obtained with an RC-4HC datalogger thermo-hygrometer (Elitech, Mexico) with a storage capacity of 16 000 readings, programmed to take measurements every hour.

The substrate used for sowing, production, and inoculation of *F. uhdei* plants consisted of a mixture of Sunshine® Mix N3 peat, perlite, and vermiculite in a 60:30:10 (v/v) ratio. The substrate was sterilized three times with water vapor for 5 h. Multicote® 18-6-12 (N-P-K) controlled-release fertilizer was used as a nutrient source. According to the manufacturer, nutrients are released within 8–9 months at a constant temperature of 21°C.

Biological material

The *A. brasilense* inoculant with N_2-fixing strains was prepared in a sterile, semi-solid nitrogen-salt malate (Nfb) medium supplemented with malic acid, mineral salts, vitamins, and a nitrogen source. Incubation was carried out at 32 °C, with shaking at 220 rpm for 48 h. The bacterial concentration achieved was 5×10^8 colony-forming units per milliliter (CFU L^{-1}). In addition to their N_2-fixing capacity, these strains produce indoleacetic acid (IAA). Molecular identification and biochemical characterization were performed according to Carcaño-Montiel *et al*. (2006).

The *A. brasilense* inoculant with phosphate-solubilizing and IAA-producing strains was prepared in nutrient broth or trypticase soy broth. Incubation was carried out at 32 °C, shaking at 180 rpm for 24 h, until a population density of 8 × 108 CFU mL^{-1} was reached. Information on the molecular and biochemical characterization of these strains is not available due to their confidentiality (registration number 1160672, Mexican Institute of Industrial Property). Both inoculum sources belong to and were provided by the Soil Microbiology Laboratory of the Center for Research in Microbiological Sciences at the Meritorious Autonomous University of Puebla, Mexico.

The *F. uhdei* seeds were collected from adult trees located in the "El Ranchito" experimental field at the Autonomous University of Chapingo, in the municipality of Texcoco, State of Mexico, Mexico. They were disinfested with 30 % H_2O_2 for 20 min. They were subsequently rinsed three times with sterile distilled water. Sowing was carried out in 380 cm^3 polypropylene forest tubes, ensuring the growth of one seedling per tube by placing two seeds in each. At 26 d after sowing, the germination rate was 76 %. Irrigation was provided every third day with potable water. Inoculation was carried out at planting, adding 10 mL of inoculum to each tube. A second inoculation was carried out 90 d after planting, adding 15 mL of inoculum per plant.

Sample collection for bacterial communities

Samples were obtained from six-month-old plants grown with a fertilizer dose of 4 g L^{-1} of substrate. Under aseptic conditions for instruments and consumables, soil firmly adhered to fine roots was obtained, covering the surface, center, and bottom of the root ball. In 2 mL Eppendorf ONiLAB® conical-base tubes, 250 mg of soil were placed for each plant, adding 400 µL of DNA/RNA Shield protective reagent (Zymo Research, USA). The samples were kept at -20 °C until further laboratory analysis.

DNA extraction and sequencing of the 16S rRNA gene

Substrate sterilization with water vapor or other methods, such as cobalt-60 soil irradiation, does not prevent the rapid reconstruction of microbial communities from diverse environmental sources (Ferrarezi *et al*., 2023). Therefore, rhizosphere soil samples were sent to BGI Innomics Inc. (Tai Po, Hong Kong) for DNA extraction and amplicon sequencing. DNA was extracted using the QIAGEN DNeasy PowerSoil Pro Kit (Hilden, Germany) isolation kit, following the manufacturer's extraction protocol. DNA concentration and purity were assessed by fluorescence in a microplate reader. Amplicons were obtained from the V3–V4 variable region of the 16S rRNA gene by polymerase chain reaction (PCR), using the primer pair 341F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT), and sequenced using the DNBSEQ platform (Hu *et al*., 2024).

Sequencing data processing

Sequencing data were analyzed using USEARCH v7.0.1090 (Farooq *et al*., 2024) and filtered to obtain high-quality, clean readings. Readings with low-quality base pairs (20–25 base pairs less than the average) were truncated, and those that after truncation retained 75 % or less of their original length were eliminated. In addition, readings contaminated with adapter sequences (allowing up to three bases of discrepancy), ambiguous readings (N), and low-complexity readings (10 consecutive repeated bases) were discarded.

Chimera sequences were subsequently removed using the UCHIME algorithm. Genetic barcodes, used to align readings and ensure accurate assignment to the corresponding sample (zero-base mismatch), were also removed. The sequences were grouped into operational taxonomic units (OTUs) with a similarity level of 97 %.

The alpha diversity analysis of the bacterial communities was performed using the Mothur software v.1.31.2 (Schloss, 2020), which was used to obtain rarefaction curves and estimate the Chao 1 richness and Shannon Weaver alpha diversity indices. For the beta diversity analysis, QIIME v1.8.0 (Ibarra-Sánchez and Romero-Salas, 2024) was used. The similarity and dissimilarity of the bacterial communities was visualized using the principal coordinates analysis (PCoA) with the Bray-Curtis dissimilarity test. To evaluate differences between groups, the non-parametric statistical test of similarity analysis (ANOSIM) was performed.

Potential metabolic functions were predicted from the Kyoto Encyclopedia of Genes and Genomes (KEGG) using PICRUSt2 v2.3.0-b (Douglas *et al*., 2019). Amplicon

sequence variants (ASVs) were obtained using the DADA2 algorithm. ASVs were inserted into a reference phylogenetic tree obtained from prokaryotic genomes available in the Integrated Microbial Genomes system (https://img.jgi.doe.gov/) to determine their most likely phylogenetic position. Subsequently, hidden state prediction (HSP) was performed using the Castor algorithm based on the evolutionary relationship of the ASVs to microorganisms with known genome-wide sequences. The predicted functional genes, identified from KO identifiers (KEGG Orthology), were grouped according to the functional hierarchy provided by the KEGG database. Finally, potential metabolic functions at level three (specific metabolic pathways) were obtained, and their relative abundance in each sample was determined.

Morphological variables

The growth of *F. uhdei* seedlings was evaluated based on their total height, stem diameter at the root collar, leaf area, root and above-ground biomass, and total dry biomass. Total height was measured with a measuring tape, starting at the root collar and ending at the apex of each plant. Stem diameter was measured with a KEATRONIC digital caliper. Leaf area was obtained with a leaf area integrator (LI-300, LI-COR; Lincoln, NE, USA). Biomass was determined by drying the above-ground and root components for 3 d at 70 °C in a forced-air oven (Riossa, HCF-125D, Mexico). The material was weighed on an analytical balance (OHAUS model E01140, NJ, USA). Leaf area and biomass were measured from four randomly selected plants per treatment. Total height and root collar diameter were recorded for all experimental units.

Nutritional analysis

At the end of the trial (206 d), four plants per treatment were randomly selected for nutrient analysis. Atomic absorption spectrometry was used to determine the P, K, Ca, and Mg contents based on the methods described by Alcántar-González and Sandoval-Villa (1999). Nitrogen content was obtained by the micro-Kjeldahl method (Bremner, 1965).

Experimental design and statistical analysis

The assay for analysis of bacterial communities was established under a completely randomized experimental design. The established treatments were: 1) inoculation with N_2-fixing *A. brasilense*, 2) simultaneous inoculation with N_2-fixing *A. brasilense* and phosphate-solubilizing *A. brasilense*, and 3) control without inoculation. For each treatment, four *F. uhdei* plants (biological replicates) were randomly selected from a total of 20 plants per treatment.

The plant growth and nutrition trial was conducted using a completely randomized, factorial design, with two doses of controlled-release fertilizer and four inocula (2 × 4). Fertilization levels were established with the application of 2 and 4 g L^{-1} of substrate. The inoculum sources were: N_2-fixing *A. brasilense* (FN), phosphate-solubilizing *A. brasilense* (SF), the simultaneous combination of both (*A. brasilense* FN+SF), and a

non-inoculated control (Psi). The combination of factors and levels resulted in eight treatments. Each treatment was replicated 20 times, considering one plant as the experimental unit for a total of 160 plants.

Bacterial community results were analyzed using the Kruskal-Wallis test. Analysis of variance (ANOVA) was used for growth and nutrient analysis after verifying normality assumptions (Shapiro-Wilk test). When treatment effects were present, means were compared using Tukey's honest significant difference test with a significance level of $\alpha = 0.05$. Statistical analysis was performed using the R statistical package (R Core Team, 2024).

RESULTS AND DISCUSSION

Composition of bacterial communities

The bacterial community composition of the three inoculation groups generated 2969 bacterial OTUs (1601 ± 18.8 per sample). The OTUs were assigned to a single kingdom, 33 phyla, 62 classes, 110 orders, 203 families, 418 genera, and 443 species. Seven bacterial phyla accounted for 86.4 % of the relative abundance; of these, *Candidatus* Saccharimonadota was significantly affected in abundance ($p \leq 0.05$) by the FN+SF strain combination (Figure 1A). This phylum is distributed in diverse environments; in the rhizosphere of *Larix decidua* Mill, it constitutes up to 10 % of the total relative abundance. However, knowledge of its functions is still limited (Praeg and Illmer, 2020).

The bacterial community was dominated by the phyla Proteobacteria (54.7 ± 0.7 %) and Bacteroidota (14 ± 2 %). The genera exhibiting the highest relative abundance collectively accounted for 18.8 % of the total community (Figure 1B). These findings contrast with prior studies on forest soils. For instance, Proteobacteria was reported at a lower relative abundance (35.3 to 37 %) in the root system soil of various tree genera, including *Fraxinus*, *Pinus*, *Larix*, *Quercus*, *Fagus*, and *Carpinus* (Staszel-Szlachta *et al*., 2024). A community composition more similar to the one observed here was reported in the study of *Alnus nepalensis* D. Don, where Proteobacteria (37.2 %) and Bacteroidota (12 %) were also the dominant phyla (Sen *et al*., 2022). These phyla were associated with nitrogen-fixing bacterial taxa, which were enriched in the rhizospheric soil. Approximately 50 % of the bacterial community in the rhizosphere possessed N_2 fixation mechanisms (Sen *et al*., 2022).

The increased presence of NF taxa in the rhizosphere of *F. uhdei* could be enhanced by inoculation with NF bacterium *A. brasilense*, which showed a significant effect ($p \leq 0.05$) by increasing the abundance of the genus *Azospirillum* by 7- and 4-fold compared to Psi and FN+SF, respectively (Figure 1B). The genus *Ohtaekwangia,* belonging to Bacteroidota, decreased 1- and 2-fold in FN and FN+SF. This genus has been identified in the rhizosphere of agricultural crops such as olive (*Olea europaea* L.) and is considered a biomarker of the optimal state of soil amendments (Palla *et al*., 2022). The influence

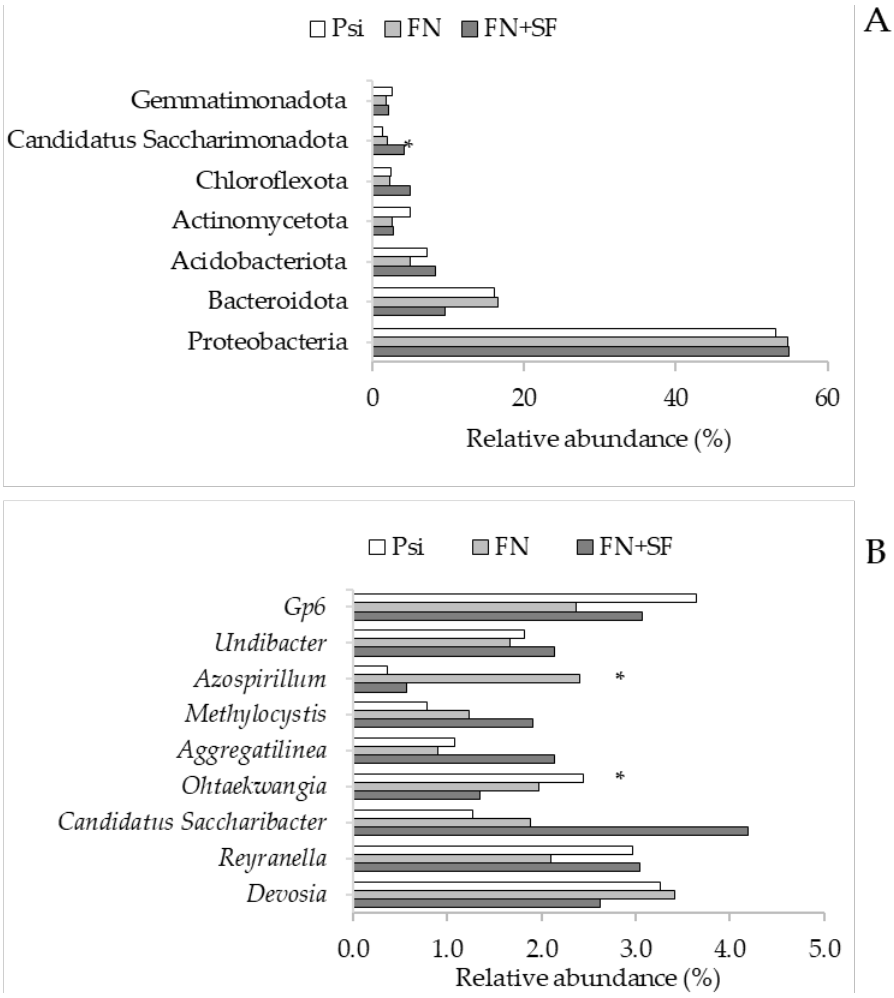

Figure 1. Composition of bacterial communities associated with the rhizosphere of *Fraxinus uhdei* Wenz. Lingelsh. A: Bacterial phyla with the highest relative abundance (%); B: Bacterial genera with the highest relative abundance (%). Psi: Uninoculated plants; FN: plants inoculated with N_2-fixing *Azospirillum brasilense*; FN+SF: plants inoculated with N_2-fixing *A. brasilense* and phosphate-solubilizing *A. brasilense*.

of inoculation treatments on a single phylum and bacterial genus indicates that the rhizospheric microbiome of *F. uhdei* shows minimal changes due to the use of PGPR.

Richness and diversity of the bacterial rhizosphere

Rarefaction curves based on the coverage index were stable from 20 000 to 60 000 sequences (close to 1.0), suggesting sufficient sampling depth to capture most of the bacterial diversity (Figure 2A). The overlap of the rarefaction curves indicates that the rhizospheric bacterial microbiome of *F. uhdei* does not show volatility upon PGPR inoculation. The Chao 1 estimator based on rare species counts showed that bacterial

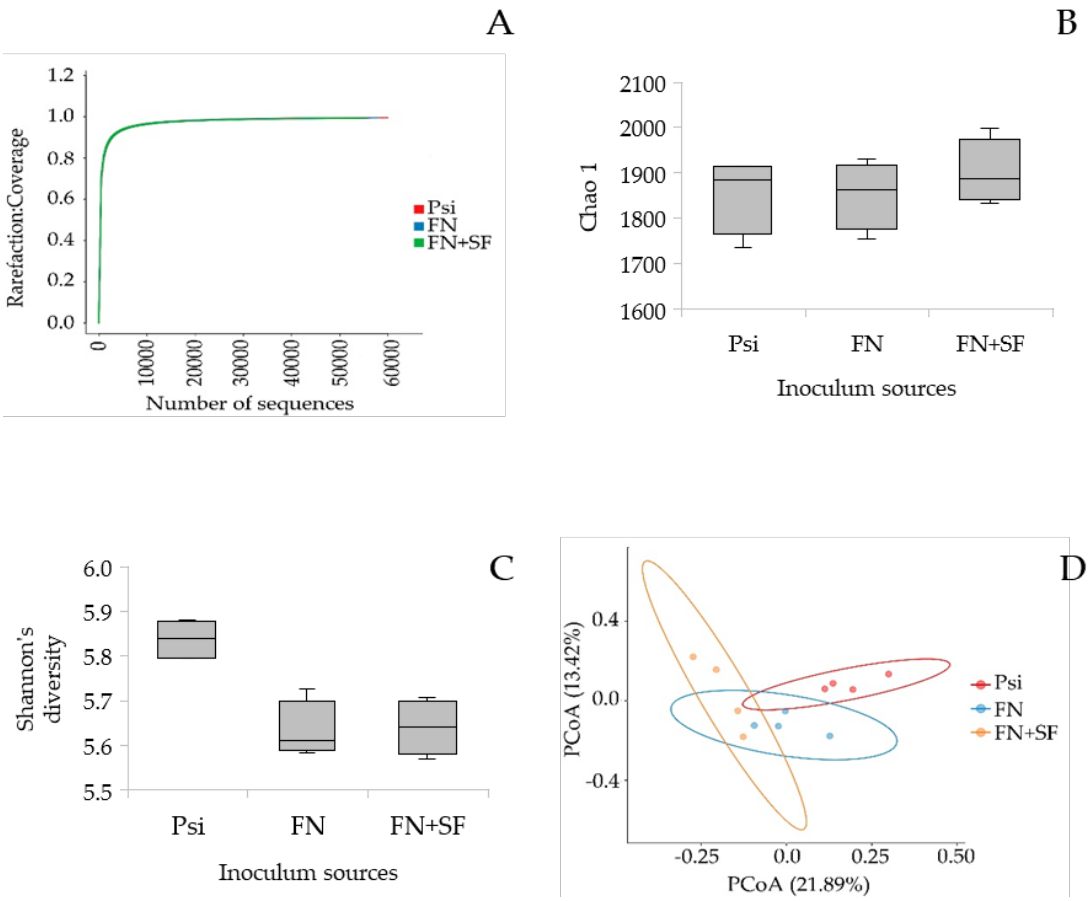

Figure 2. Diversity of bacterial communities associated with the rhizosphere of *Fraxinus uhdei* Wenz. Lingelsh. A: Rarefaction curves based on the observed diversity proportion; B: Chao 1 richness index; C: alpha diversity index; D: principal coordinate analysis (PCoA); circles enclosed by ellipses represent samples (biological replicates) from each treatment. Psi: Uninoculated plants; FN: plants inoculated with N_2-fixing *Azospirillum brasilense*; FN+SF: plants inoculated with N_2-fixing *A. brasilense* and phosphate-solubilizing *A. brasilense.*

community richness ranged from 1736 to 1998 taxa and was not influenced ($p < 0.05$) by inoculation with *A. brasilense* strains (Figure 2B). Alpha diversity was affected by inoculation treatments ($p \leq 0.05$), with greater diversity observed in the Psi rhizosphere compared to the FN and FN+SF treatments (Figure 2C), which showed the same levels of diversity (Shannon index).

Metagenomic analyses following inoculation with *A. brasilense* strains are limited and mostly focus on maize and rice crops. Reported results are contrasting, with effects ranging from null or minimal to clearly positive on bacterial community structure (Nievas *et al.*, 2023). Inoculation with PGPR is usually associated with a decrease in alpha diversity. For example, in the rhizosphere of maize inoculated with commercial *A. brasilense* strains, a reduction in alpha diversity has been observed, even

accompanied by modifications in the native microbiome. However, it has also been reported that greater diversity does not necessarily translate into a more favorable bacterial community for plant-PGPR interaction (Ferrarezi *et al*., 2023).

Beta diversity was also influenced by inoculation treatments (ANOSIM R = 0.59, $p \leq 0.05$). The principal coordinates analysis (PCoA) explained 35.3 % of the variation and showed a separation of bacterial communities between inoculated and uninoculated plants (Figure 2D). However, the overlap of the uninoculated plant ellipse with the inoculation treatment ellipses (95 % confidence interval) shows that changes in bacterial diversity are smaller.

The evidence available from sequencing methods indicates that the inoculation of plants with *Azospirillum* does not significantly alter microbial diversity but does favor microbial functional groups, such as diazotrophs (Renoud *et al*., 2022). This was observed with significant increases in the abundance of the genus *Azospirillum* (Figure 1B), such that *A. brasilense* strains exhibit the ability to interact with some bacterial groups in the *F. uhdei* rhizosphere.

Potential metabolic functions of bacterial communities

Prediction of potential metabolic functions at level three, based on species-specific KEGG pathways, showed that inoculation with *A. brasilense* had a significant effect ($p \leq 0.05$) on the relative abundance of 11 % of these functions (Figure 3). In particular, the

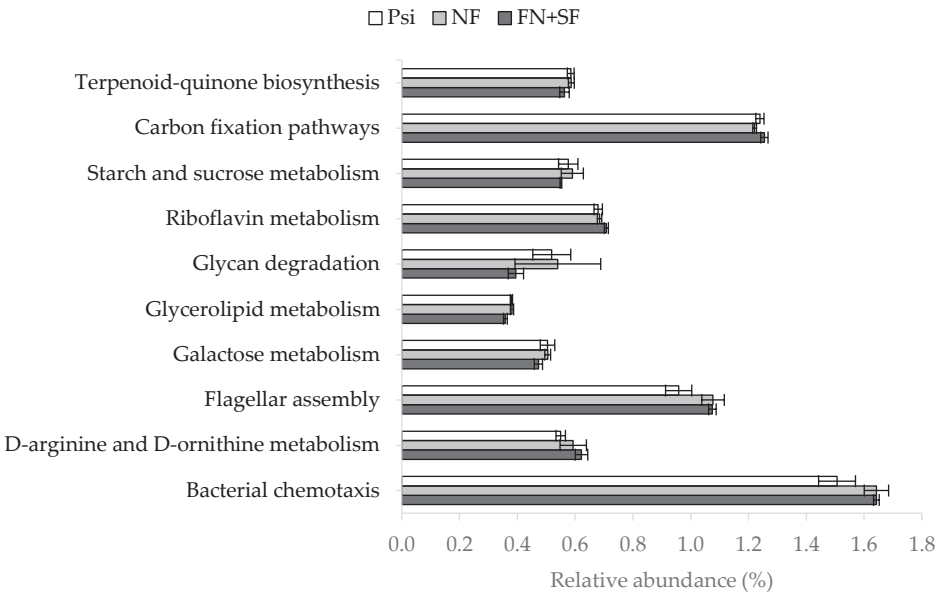

Figure 3. Relative abundance of metabolic functions influenced by inoculation treatments. Psi: Uninoculated plants; FN: plants inoculated with N_2-fixing *Azospirillum brasilense*; FN+SF: plants inoculated with N_2-fixing *A. brasilense* and phosphate-solubilizing *A. brasilense.*

chemotaxis function increased by 9 % with the FN treatment, while flagellum assembly increased by 12.4 % in FN+SF, both compared to Psi. The motility and chemotaxis of *A. brasilense* have been analyzed in real time during root colonization of various crops, showing that the accumulation of this PGPR in the rhizosphere responds to chemical gradients generated by root exudates that act as attractive or repellent signals (O'Neal *et al*., 2020). These results suggest that the strains used present a competitive chemotaxis that favors their colonization in the rhizosphere of *F. uhdei*.

The FN+SF treatment improved the abundance of functions related to disease resistance mechanisms and environmental adaptation. Riboflavin, D-arginine, and D-ornithine metabolism increased by 4.1 and 13.1 %, respectively. The remaining functions presented similar relative abundances between treatments. The metabolic functions of PGPR, particularly those focused on the production of secondary metabolites, are a strategy designed to occupy a niche close to the plant root so that they can access the supply of root exudates, establishing the PGPR-plant interaction (Naureen *et al*., 2022). In maize cultivation, the use of PGPR bacterium *Azospirillum* has favored the production of indoleacetic acid (IAA) in the rhizosphere, which has been associated with a higher abundance of certain metabolic functions (Coniglio *et al*., 2024). The *A. brasilense* FN strains used in this study are IAA producers (Carcaño-Montiel *et al*., 2006), which may be one of the factors that enhance the abundance of metabolic functions.

Seedling growth

The growth of *F. uhdei* seedlings showed a significant interaction ($p \leq 0.05$) between inoculation and controlled-release fertilizer, but only in stem biomass. The FN × 4 g L^{-1} substrate interaction generated greater accumulation of shoot biomass (9.8 ± 1.3 g), while the Psi × 2 g L^{-1} interaction recorded less accumulation (6 ± 0.3 g). The fertilizer dose affected ($p \leq 0.05$) the total height; the application of 4 g L^{-1} substrate showed greater height compared to the use of 2 g L^{-1} substrate (34.1 ± 1 and 31.2 ± 0.8 cm, respectively).

Regarding the inoculation treatment, an effect ($p \leq 0.05$) was found on stem diameter at the root collar, leaf area, root dry biomass, and total dry biomass (Table 1). The inoculant combination (FN+SF) generated greater growth, with increases of 11.4 % in stem diameter and 33.8 % in leaf area, compared to Psi. It also increased root biomass by 36.9 % and total biomass by 24.7 %, compared to the FN and SF treatments, respectively (Table 1). These values are lower than those reported for *Cecropia pachystachya* Trécul, where inoculation with *A. brasilense* increased leaf area and total biomass by 70.4 % (Calzavara *et al*., 2021). The differences are attributed to the higher growth rate of *C. pachystachya*, which is a warm-zone species with a high photosynthetic rate.

In the olive cultivar 'Arbequina,' under a pH gradient in the substrate, inoculation with *A. brasilense* strains almost doubled the total height of the plants compared to the controls, although it had no effect on the stem diameter (Boeni *et al*., 2024). This result contrasts with what was observed in *F. uhdei* plants, where inoculation did not favor height growth but did increase the stem diameter. The strains used in *F. uhdei*

Table 1. Growth in stem diameter at the root collar, leaf area, and dry biomass in *Fraxinus uhdei* Wenz. Lingelsh. seedlings inoculated with *Azospirillum brasilense* strains.

Sources of inoculum	Total height (cm)	Diameter (mm)	Leaf area (cm^2)	Shoot biomass (g)	Root biomass (g)	Total biomass (g)
Psi	31.3 ns	7.0 b	479.43 b	6.2 ns	5.2 ab	11.4 ab
FN	35.4 ns	7.3 ab	629.33 ab	8.1 ns	4.6 b	12.7 ab
SF	33.0 ns	7.5 ab	491.73 ab	6.6 ns	4.7 b	11.3 b
FN+SF	31.0 ns	7.8 a	641.60 a	7.8 ns	6.3 a	14.1 a
SEM	0.65	0.09	24.93	0.33	0.23	0.38

Mean values per column with a different letter are statistically different ($p \leq 0.05$). Psi: Uninoculated plants; FN: plants inoculated with N_2-fixing *Azospirillum brasilense*; SF: plants inoculated with phosphate-solubilizing *A. brasilense*; FN+SF: plants inoculated with N_2-fixing *A. brasilense* and phosphate-solubilizing *A. brasilense*; SEM: standard error of the mean.

are producers of IAA, an auxin that in *F. uhdei* promotes root development whose absorption through the root system, followed by its translocation to the stem via xylem, stimulates cell elongation in the shoots (Kargapolova *et al*., 2020). Since the FN+SF strain combination significantly increased root biomass, it is likely that IAA accumulated in the root system and not in the shoot.

Seedling nutrition

The macronutrient content, with the exception of K, responded significantly ($p \leq 0.05$) to the interaction between inoculation and fertilizer dose. Compared to the Psi treatment, the FN × 4 g L^{-1} substrate interaction promoted greater nutrient accumulation in the whole plant, with increases of 24.4 % in N (Figure 4A), 44.7 % in P (Figure 4B), 29.6 % in Ca (Figure 4C), and 45.8 % in Mg (Figure 4D). Furthermore, the interaction revealed that when inocula were combined with the lowest fertilizer dose (treatment FN+SF × 2 g L^{-1}), the substrate tended to favor larger nutrient accumulation, showing significant differences ($p \leq 0.05$) in N, Ca, and Mg.

The results show a relationship with those found in *Araucaria angustifolia* (Bertol.) Kuntze, where inoculation with *A. brasilense* favored N and P contents (10.4 and 2.3 g kg^{-1}, respectively) compared to inoculation with *Bacillus subtilis* (8.1 and 1.7 g kg^{-1}, respectively) (Kondo *et al*., 2024). The benefits of PGPR in nutrient acquisition have been described in detail, showing their participation in the activation of ion transport systems within roots. In maize crops, *A. brasilense* strains excrete different amounts of NH_4^+ depending on the N source supplied (Pedrosa *et al*., 2019). The strains used in *F. uhdei* showed the ability to promote nutrient transfer, considering that the main source of mineral supply came from a controlled-release fertilizer. However, plants inoculated with FN had lower growth than those inoculated with FN+SF, which may be a factor in the higher nutrient content.

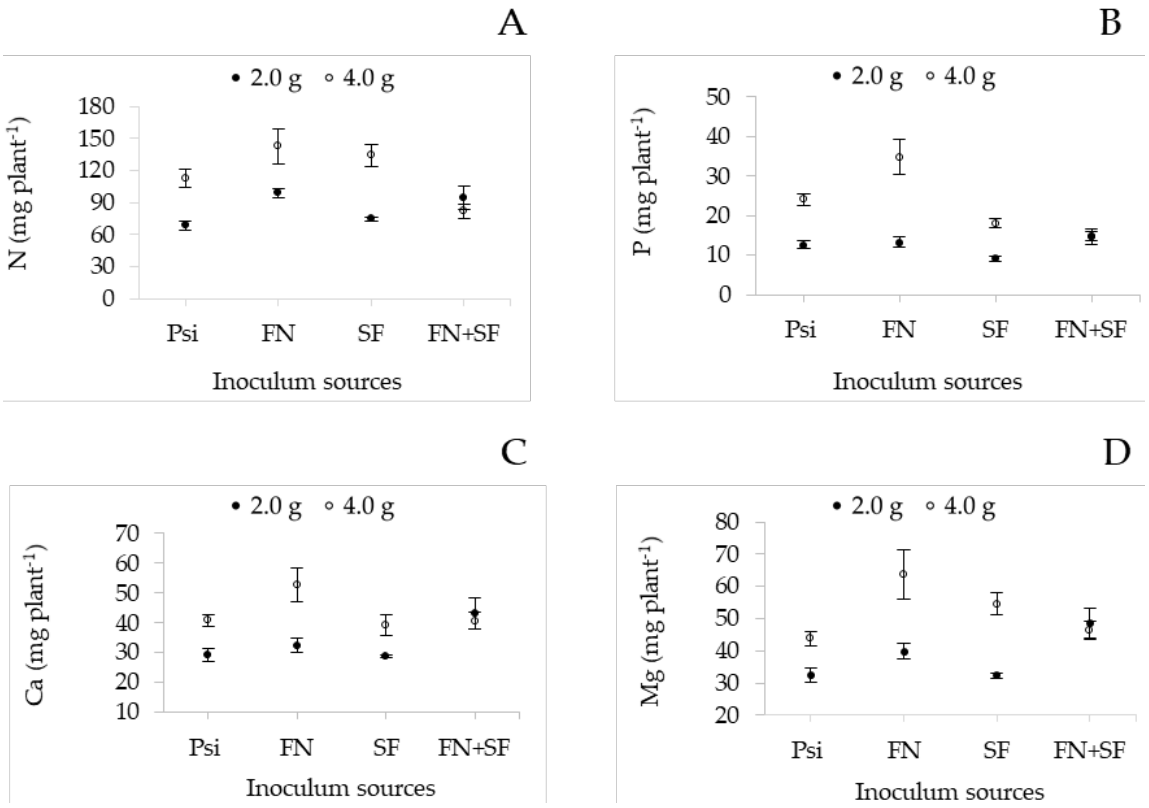

Figure 4. Macronutrient content in *Fraxinus uhdei* seedlings under the interaction of *Azospirillum brasilense* inocula × fertilizer dose (2 and 4 g L^{-1} of substrate). A: nitrogen content; B: phosphorus content; C: calcium content; D: magnesium content. Psi: Uninoculated plants; FN: plants inoculated with N_2-fixing *Azospirillum brasilense*; SF: plants inoculated with phosphate-solubilizing *A. brasilense*; FN+SF: plants inoculated with N_2-fixing *A. brasilense* and phosphate-solubilizing *A. brasilense*. The bars above the points indicate the standard error of the mean.

CONCLUSIONS

Inoculation with *Azospirillum brasilense* strains did not induce significant alteration in the overall composition of the rhizospheric bacterial community of *Fraxinus uhdei*. However, it influenced community diversity and the relative abundance of genes associated with potential metabolic functions. Seedlings inoculated with the combination of nitrogen-fixing and phosphate-solubilizing strains exhibited enhanced growth, whereas applications of the nitrogen-fixing strain alone resulted in higher macronutrient contents. These results indicated that the use of commercial *A. brasilense* strains as plant growth-promoting rhizobacteria may serve as an effective biotechnological strategy for the production of *F. uhdei* plants destined for urban environments. Furthermore, this approach is compatible with conventional

inputs, such as peat-based substrates and controlled-release fertilizers, fostering the establishment of bacterial communities implicated in nutrient cycling.

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