Microbiological Research 283 (2024) 127706

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

The interplay between the inoculation of plant growth-promoting rhizobacteria and the rhizosphere microbiome and their impact on plant phenotype

Izadora de Cássia Mesquita da Cunha [a,b], Ana Vitória Reina da Silva [a], Eduardo Henrique Marcandalli Boleta [a], Thierry Alexandre Pellegrinetti [a], Luis Felipe Guandalin Zagatto [a,c], Solange dos Santos Silva Zagatto [a], Miriam Gonçalves de Chaves [a], Rodrigo Mendes [d], Camila Maistro Patreze [e], Siu Mui Tsai [a], Lucas William Mendes [a,*]

[a] Cell and Molecular Biology Laboratory, Center for Nuclear Energy in Agriculture CENA, University of Sao Paulo USP, Piracicaba, SP 13416-000, Brazil
[b] Luiz de Queiroz College of Agriculture ESALQ, University of São Paulo USP, Piracicaba, SP 13418-900, Brazil
[c] Department of Terrestrial Ecology, Netherlands Institute of Ecology NIOO-KNAW, Wageningen NL-6700 AB, the Netherlands
[d] Laboratory of Environmental Microbiology, Embrapa Environment, Jaguariuna 18020-000, Brazil
[e] Institute of Biosciences, Federal University of the State of Rio de Janeiro, Rio de Janeiro, RJ 22290-240, Brazil

ARTICLE INFO

Keywords:
Common bean
Microbial ecology
16S rRNA
ITS

ABSTRACT

Microbial inoculation stands as a pivotal strategy, fostering symbiotic relationships between beneficial microorganisms and plants, thereby enhancing nutrient uptake, bolstering resilience against environmental stressors, and ultimately promoting healthier and more productive plant growth. However, while the advantageous roles of inoculants are widely acknowledged, the precise and nuanced impacts of inoculation on the intricate interactions of the rhizosphere microbiome remain significantly underexplored. This study explores the impact of bacterial inoculation on soil properties, plant growth, and the rhizosphere microbiome. By employing various bacterial strains and a synthetic community (SynCom) as inoculants in common bean plants, the bacterial and fungal communities in the rhizosphere were assessed through 16 S rRNA and ITS gene sequencing. Concurrently, soil chemical parameters, plant traits, and gene expression were evaluated. The findings revealed that bacterial inoculation generally decreased pH and V%, while increasing H+Al and m% in the rhizosphere. It also decreased gene expression in plants related to detoxification, photosynthesis, and defense mechanisms, while enhancing bacterial diversity in the rhizosphere, potentially benefiting plant health. Specific bacterial strains showed varied impacts on rhizosphere microbiome assembly, predominantly affecting rhizospheric bacteria more than fungi, indirectly influencing soil conditions and plants. Notably, *Paenibacillus polymyxa* inoculation improved plant nitrogen (by 5.2%) and iron levels (by 28.1%), whereas *Bacillus cereus* boosted mycorrhization rates (by 70%). Additionally, inoculation led to increased complexity in network interactions within the rhizosphere (~15%), potentially impacting plant health. Overall, the findings highlight the significant impact of introducing bacteria to the rhizosphere, enhancing nutrient availability, microbial diversity, and fostering beneficial plant-microbe interactions.

1. Introduction

During their life cycle, plants establish relationships with microorganisms in their environment, which are essential for their survival in a highly variable and competitive ecosystem (Imam et al., 2016). These ecological interactions can be negative, neutral, or positive, including communities of microorganisms in the rhizosphere soil. Beneficial interactions involve microorganisms aiding plant establishment and defense, by solubilizing nutrients, mineralizing organic compounds, producing phytohormones, and inhibiting pathogens. Conversely,

* Corresponding author.
E-mail address: lwmendes@cena.usp.br (L.W. Mendes).

https://doi.org/10.1016/j.micres.2024.127706
Received 22 December 2023; Received in revised form 26 March 2024; Accepted 27 March 2024
Available online 29 March 2024

negative interactions involve phytopathogenic microorganisms causing damage and economic losses through various attack strategies and preferences for specific target tissues (Santoyo, 2016; Wang, 2022).

The beneficial interactions have attracted significant research interest as they can lead to improved fitness and agricultural productivity (Compant et al., 2019). Microbial interactions are most pronounced in the rhizosphere, which is the soil region strongly influenced by roots and characterized by high microbial activity (Philippot et al., 2013). This proximity and the release of root exudates foster a thriving microbial community, leading to enhanced nutrient acquisition (Mendes et al., 2014), improved tolerance to abiotic stresses (Yang et al., 2009), and heightened protection against pathogens (Chapelle et al., 2016; Mendes et al., 2018, 2023). The rhizospheric effect is influenced by factors such as plant species, genotypes, developmental stages, and soil's physical and chemical properties (Araujo, 2019; Moroenyane et al., 2021; Sousa et al., 2020). These factors result in variations in the composition of root exudates, which directly impact the behavior of the microbial community in the rhizosphere, selectively attracting beneficial microorganisms (Berg and Smalla, 2009; Canarini, 2019; Williams, 2022).

Considering population growth projections and the need to boost agricultural output, employing microorganisms as bioinoculants has shown significant effectiveness (Suman, 2022). These bioinoculants not only enhance productivity and sustainability but also reduce reliance on mineral fertilizers (Bomfim et al., 2021). By harnessing the beneficial interactions between microorganisms and plants, bioinoculants contribute to a more sustainable and ecologically friendly agricultural management approach. Among the bacterial strains used as inoculants, the genera *Bacillus* and *Paenibacillus* are prominent bacterial strains with significant biotechnological potential as Plant Growth-Promoting Rhizobacteria (PGPR). The genus *Paenibacillus* comprises species that promote plant growth through various mechanisms, including the production of indole-3-acetic acid (IAA) and other auxins, the solubilization of inaccessible phosphorus into more labile forms, and some species are capable of atmospheric nitrogen fixation (Singh, 2018). The genus *Bacillus* contributes to the maintenance of plant health, development, and growth by inducing resistance pathways and producing hormones such as auxins, gibberellins, and cytokinins (Tsotetsi et al., 2022). These bacteria can also stimulate symbiosis with other beneficial microorganisms, enhancing, for example, endo- and ectomycorrhizal infection (Medina, 2003). Furthermore, *Brevibacillus* has been recognized for its multifaceted roles as plant growth-promoting bacteria, exhibiting nitrogen-fixing abilities, and demonstrating efficacy in soil bioremediation by removing toxic heavy metals from soils, water, and the atmosphere (Nehra et al., 2016). Accordingly, PGPR can be effectively employed as inoculants, either independently or in combination with other bacterial or fungal strains, to enhance plant growth (Goswami et al., 2016). This application demonstrates the recognized and developing importance of PGPR in biotechnology, offering solutions to challenges in food production based on a long history of successful use.

While the advantageous impacts of PGPR as bioinoculants are well-documented, the nuanced effects these inoculants have on the rhizosphere microbiome warrant further exploration. Given the critical role of the plant microbiome in supporting plant health and growth, introducing inoculants represents a pivotal area of study for understanding and potentially optimizing microbial community interaction within the rhizosphere. This alteration can lead to increased plant growth by enhancing the complexity of microbial interactions in the rhizosphere. This study hypothesizes that the application of different bacterial strains as inoculants induces distinct changes in the composition of the rhizosphere microbiome, resulting in positive, neutral, or negative impacts on the community, ultimately influencing plant health and growth. The observed effects can be either direct, influenced by the inoculated organism itself, or indirect, through modulation of the microbiome. A comprehensive assessment of the rhizosphere microbiome of common bean was conducted using the bacterial 16 S rRNA gene and the fungal ITS to evaluate the impact of plant growth-promoting rhizobacteria (PGPR) on plant growth and the interactions of the microbiome.

2. Materials and methods

2.1. Rhizobacteria strains and inoculum preparation

Ten bacterial strains (Table S1) belonging to the Cellular and Molecular Biology Laboratory (CENA/USP) were used in this study. The strains were isolated from the rhizosphere of common bean cultivars with different levels of resistance to *Fusarium oxysporum*, grown in Amazonian dark earth soils (Mendes et al., 2019), and taxonomically classified as belonging to the genera *Bacillus, Paenibacillus*, *Fictibacillus*, and *Brevibacillus* (Pellegrinetti et al., 2023, 2024). Previous tests showed that four of these isolates (*Brevibacillus agri* CENA-BCM005, *Bacillus cereus* CENA-BCM007, *Paenibacillus polymyxa* CENA-BCM009, and *Paenibacillus polymyxa* CENA-BCM010) have growth-promoting potential, and were selected for evaluation in greenhouse experiments. Before preparing the inoculation treatment with the strains, compatibility among the candidate strains to compose the bacterial consortium was confirmed using a synergy assay following the streak plate dilution method. The isolates were considered compatible when no growth inhibition was observed when cultivated on the same plate (Figure S1). The compatible strains were: CENA-BCM001, CENA-BCM004, CENA-BCM006, CENA-BCM007, and CENA-BCM008. For the start of *in vivo* analyses, the chosen isolates were revived on KING agar culture medium, and subsequently transferred to 50 mL of KING liquid culture medium, based on the time stipulated by the bacterial growth curve (at the concentration of 10^8 CFU/mL, verified by $OD_{600} = 1.4$ for *Bacillus* and *Brevibacillus,* and $OD_{600} = 1.5$ for *Paenibacillus*; Figure S2). The cultures were incubated at 28 °C with constant agitation at 130 rpm. Bacterial cells were harvested by centrifugation for 10 minutes and then resuspended in 50 mL of sterile Phosphate-Buffered Saline solution (PBS). The draft genome and raw reads of the strains are submitted to the NCBI Sequence Read Archive under the identification BioProject PRJNA988090.

2.2. Bioassay and experimental design

Soil samples were collected from the 0–20 cm layer in an agricultural experimental area at the "Luiz de Queiroz" College of Agriculture (ESALQ/USP, Piracicaba, Brazil), located in Sertãozinho Farm (22°43'01.6" S and 47°36'49.0" W). The soil in the area is classified as a medium-textured Red-Yellow Latosol. The physical and chemical soil attributes were determined based on 500 g of soil sent for analysis, and the results are described in Table S2. The sampled soil was sieved through a 4 mm mesh, and pH was corrected with lime reaching 70% base saturation. To adjust the soil fertility, basal fertilization was carried out following the recommendations for common bean cultivation in the state of São Paulo and pot cultivation (Novais et al., 1991; Raij et al., 1997), considering the soil's chemical attributes. The soil was fertilized with 10 g per pot using a 04–14–08 NPK formulation as a source of macronutrients.

To analyze the influence of bacterial strain inoculation on the growth of common bean plants, nutrient availability, and rhizosphere microbial communities, the treatments consisted of plants without bacterial inoculation (Control); inoculation with *Brevibacillus agri* CENA-BCM005 (T1); *Bacillus cereus* CENA-BCM007 (T2); *Paenibacillus polymyxa* CENA-BCM009 (T3); *Paenibacillus polymyxa* CENA-BCM010 (T4); and the Synthetic Community (SynCom, composed of *P. vini* CENA-BCM001, *Fictibacillus* sp. CENA-BCM004, *P. polymyxa* CENA-BCM006, *B. cereus* CENA-BCM007, and *P. polymyxa* CENA-BCM008)(Table S1). Common bean seeds (*Phaseolus vulgaris* L.) of the cultivar IAC Milênio (Carbonell et al., 2014) were used in the experiment. Mesocosms were set up in polyethylene pots (30 cm height x 20 cm diameter) and approximately 7 kg of soil was used to fill the pots. Briefly, the seeds were carefully disinfested in a 10% NaClO solution and 70% alcohol for 2 minutes,

followed by rinsing with distilled water. Seed inoculation was performed for 2 minutes under manual agitation, using the inoculum solution (10^8 CFU mL^{-1}) at a dose of 1 mL per seed, while seeds in the Control treatment received only PBS Buffer solution without inoculum. Three seeds were sown per pot, and thinning was performed 20 days after planting, leaving only one plant per pot. The pots were arranged in a completely randomized design, with 4 replicates of each treatment, including four isolates, the SynCom, a control treatment with plants, and a control treatment without plants (bulk soil). Fifteen days after sowing, 1 mL of the inoculants was re-inoculated at the base of the plant stems to reinforce the inoculation. The Control pots received the same amount of PBS Buffer solution. Weeds were manually removed, and the soil moisture in the pots was maintained at 80% of field capacity, ceasing one day before dismantling to facilitate soil collection.

2.3. Plant growth parameters and rhizosphere sampling

The plants were collected at the R1 stage (early flower) at 35 days. Initially, measurements of stem diameter, plant height, and leaf area were taken. Subsequently, the third trifoliate leaf of each plant was collected for gene expression analysis and stored in an ultrafreezer at −80°C. The plants were carefully removed from the pots and the roots were shaken to remove the loose soil and the firmly attached soil, considered to be rhizosphere soil, was collected with a sterile spatula. Soil samples collected from the pots without plants were considered bulk soil. The bulk soil and rhizosphere samples were stored at −80ºC until further processing. For soil chemical analysis, approximately 100 g of soil was collected from the pots and stored in sealed bags. For soil enzyme analysis (arylsulfatase, β-glucosidase, and acid phosphatase), approximately 30 g of soil was collected and kept refrigerated until the analyses were performed. The delicate roots were separated for mycorrhizal colonization analysis and stored in an FAA solution (Acetic Acid: Alcohol: Formaldehyde, in a ratio of 5%:50%:10% + 35% water). At the end of the experiment, root length, above-ground dry mass, and root dry mass were evaluated.

2.4. Soil enzymatic activity and mycorrhizal colonization

The activity of β-glucosidase was determined following the method described by Tabatabai (1994). Briefly, 1.0 g of soil was treated with p-nitrophenyl β-glucopyranoside and incubated for one hour at 37 °C. Subsequently, the activity of β-glucosidase was measured by spectrophotometry at 410 nm. The activity of acid phosphatase was determined according to Tabatabai and Bremner (1969). In summary, 1.0 g of soil was incubated with disodium phosphate p-nitrophenyl as the substrate and incubated for one hour at 37 °C. Afterward, the activity was quantified by spectrophotometry at 420 nm. The activity of arylsulfatase was determined according to Spencer (1958). For this, 1.0 g of soil was treated with a potassium p-nitrophenyl sulfate solution and incubated at 37 °C for one hour.

For the analysis of mycorrhizal colonization, the roots were treated with a 10% KOH solution for 30 minutes at 90 °C and then washed and incubated in a 0.3 M HCl solution for 30 minutes. Subsequently, a 0.5% dilution of Parker Quink Washable Blue ink (in a ratio of 1:10) was added and the roots were incubated for 8 minutes at 90 °C. The dye was removed, and the roots were transferred to slides containing 50% glycerol. The roots were arranged linearly on microscope slides, with ten root fragments per slide. Each fragment was divided into ten parts to facilitate the visualization of the presence or absence of mycorrhizal fungi. The roots were observed and photographed using a Zeiss AXIO Zoom V16 stereoscopic microscope.

2.5. Foliar nutritional analysis

For the evaluation of foliar macro and micronutrients, common bean leaves were air-dried and ground in a Wiley mill with a 1 mm sieve (20 mesh). The samples were then sent to the Laboratory of Plant Nutrition at the Faculty of Engineering of Ilha Solteira, São Paulo State University "Júlio de Mesquita Filho" (UNESP), for nutritional analysis. The contents of P, K, Ca, S, Mg, B, Cu, Fe, Mn, and Zn were determined using ICP-MS. The total N content was determined through sulfuric digestion and Kjeldahl distillation (Malavolta et al., 1997).

2.6. Foliar RNA extraction and gene expression analysis

Frozen common bean leaves were macerated in liquid nitrogen and stored in an ultra freezer at −80°C until further processing. Total RNA from the plant material was extracted using the SV Total RNA Isolation System kit (Promega, Madison WI, USA), following the manufacturer's instructions. The quality and purity of the isolated total RNA were analyzed using a spectrophotometer (NanoDrop 2000c) and its integrity was verified by 1.5% agarose gel electrophoresis (Figure S3). Subsequently, 1 μg of total RNA was used for cDNA synthesis. Reverse transcription was performed using the SuperScript III First-Strand Synthesis SuperMix kit (Invitrogen®, Waltham MA, USA), with Oligo(dT)20 as the primer, following the manufacturer's protocol.

To evaluate relative gene expression, the product of the first-strand cDNA reaction was diluted 1:45 in nuclease-free water. Gene expression was assessed in common bean leaves subjected to six treatments (Control, T1, T2, T3, T4, and SynCom). Primers were selected based on gene expression studies in common beans, with actin (ACT) as the reference gene, and the target genes were *chlorophyll a/b-binding proteins* (CAB), *2,4-D inducible glutathione S-transferase* (GST), and *WRKY transcription factors* (WRKY) (Table S3). Primer efficiencies were estimated for each experimental set using the LinRegPCR program (Untergasser et al., 2021), and the obtained values were used in subsequent analyses. Real-time quantitative PCR (qPCR) analyses were conducted using a StepOnePlusTM Real-Time PCR System (Applied Biosystems, Waltham MA, USA). The reaction mix was prepared as follows: 2.8 μl of diluted cDNA (1:50), 5 μl of SYBR Green Master Mix PowerUp, 0.5 μl (5 pmol) of each primer, 0.2 μl of BSA, and 1 μl of nuclease-free water in a final volume of 10 μl. The amplification conditions consisted of an initial denaturation at 95°C for 10 min, followed by 40 cycles of 15 s denaturation at 95°C, 30 s annealing at 60°C, and 30 s extension at 72°C (with data collection). The melting curve analysis involved denaturation at 95°C for 15 s, and annealing at 60°C for 1 min, with data collection during annealing and extension for every 0.7°C increase (from 60°C to 95°C). On each plate, in addition to the reference gene (*ACT*), the target genes, and negative controls were included. The results of the analyses were recorded at each amplification cycle using StepOneTM Software version 2.2.2. The amplification curves of each gene+sample were analyzed, ensuring that the technical replicates had Ct differences smaller than 0.5. Additionally, the melting curves for each gene+sample were examined to observe the specificity of the reactions (Figure S4).

2.7. Soil DNA extraction, sequencing, and data processing

Total DNA extraction from bulk soil and rhizosphere samples was carried out using the DNeasy PowerSoil® Kit (MoBio Laboratories, Carlsbad, CA, USA), according to the manufacturer's protocol. Measurements of DNA quality and quantity were performed by 1% sodium boric acid agarose gel electrophoresis and NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, EUA). For taxonomic profiling, a total of 56 DNA samples were sequenced, which includes 7 treatments (bulk soil, control, and 5 inoculated) x 4 replicates per treatment x 2 genes (16 S and ITS). The sequencing targeted the bacterial V3-V4 region of the 16 S rRNA gene with the primers 341 F (5-CCTAYGGGRBGCASCAG-3) e 806 R (5-GGACTACNNGGGTATCTAAT-3) (Yu et al., 2005), and the fungal ITS region with the primers ITS5–1737 F (5-GGAAGTAAAAGTTCGTAACAAGG-3) e ITS2–2043R (5-GCTGCGTTCTTCATCGATGC-3) (Bellemain et al., 2010) on an Illumina Miseq Sequencing System (Illumina, San Diego CA, USA)

according to the company's protocol (Novogene Corporation Inc, Sacramento CA, USA).

The sequences obtained from 16 S rRNA and ITS were preprocessed using the QIIME2 v2021.11 software (Bolyen et al., 2019). The files containing the forward and reverse sequences were merged using the PEAR software (Zhang et al., 2014). Then the sequences were demultiplexed and quality control was carried out using DADA2 (Callahan et al., 2016), using the consensus method to remove any remaining chimeric and low-quality sequences (< q20). The 16 S rRNA sequencing approach generated approximately 4547,000 reads with <10% of chimera. After trimming, 3320,643 high-quality sequences remained with an average of 118,600 sequences per sample. Afterward, samples were rarefied to 99,430 sequences, following the number of the lowest sample, and singletons and doubletons were removed. For the ITS, the sequencing generated approximately 3397,000 reads with 2928,016 high-quality sequences after trimming, with an average of 104,572 sequences per sample. Afterward, samples were rarefied to 50,680 sequences, following the number of the lowest sample, and singletons and doubletons were removed. Taxonomic classification of the 16 S rRNA region was performed using QIIME2, using the qiime-feature-classifier tool trained with the SILVA database version 132 (97%) (Quast et al., 2012). For the ITS region, classification was performed using the UNITE 9.0 database (Nilsson et al., 2018). The sequences are submitted to the NCBI Sequence Read Archive under the identification BioProject PRJNA1054175.

2.8. Data analysis

For statistical analysis of gene expression, the raw amplification data were exported in RDML format and analyzed using the LinRegPCR program (Untergasser et al., 2021), which corrected the baselines for each gene+sample. The program generated linear regression data to obtain Ct values for each sample (the number of cycles required for the fluorescent signal to reach the detection threshold) and calculate Efficiency (E) values for each gene. Gene expression data and statistical analyses were performed using the REST (Relative Expression Software Tool) program (Pfaffl et al., 2002), where p≤ 0.05* and p≤0.01** indicated treatments that significantly differed from the Control treatment.

The data from plant biometric analyses, soil and plant chemistry, and enzyme assays were subjected to the Shapiro-Wilk test for normality and Levene's test for homoscedasticity. All statistical analyses were performed using the R software. The variation of these data compared to the Control and their respective significances were obtained using the ExpDes.pt package, which performs ANOVA with post-hoc tests using the Tukey test. The community structures of bacteria and fungi were evaluated using redundancy analysis (RDA) with Bray-Curtis distance for microbiological variables and Euclidean distance for environmental variables. The environmental variables with the greatest influence on community structure were selected using the Vegan package with the envfit function. The RDA ordination plot was generated using the microeco and ggplot2 packages for R. To test the significant difference in functional profiles among different treatments, the PERMANOVA test (Anderson, 2001) was used. Richness and alpha diversity (Shannon index) analyses were calculated using the microeco package and tested for differences among treatments using ANOVA. To explore the relationships between the relative abundance of microbial groups at the phylum level and plant properties, Spearman's rank correlation coefficients were calculated using the 'multtest' package in R, with corrections applied using the Benjamini-Hochberg FDR method.

Differential abundance between the inoculated treatments and the Control was obtained using the two-sided Welch's t-test with the STAMP software (Parks et al., 2014). Network analysis was performed using the abundance tables of bacteria and fungi (asv_table) in the microeco package, using the trans_network function with parameters: cor_method = "sparcc", sparcc_method = "SpiecEasi", filter_thres = 0.0005. The network was generated using the cal_network function with parameters: COR_p_thres = 0.001 and COR_cut = 0.8. Finally, the networks were generated and saved in "gexf" format to be plotted in the Gephi software (Bastian and Jacomy, 2009). In Gephi, the Fruchterman Reingold layout was selected, node size represents the number of interactions of each ASV (average_degree), and the color of each node represents the domains "Bacteria" and "Fungi". The color of each edge is represented in blue for positive correlations and red for negative correlations. Network statistics were generated by the Gephi software.

3. Results

3.1. Effect of the rhizosphere and microbial inoculation on soil chemical properties

Comparing bulk soil with the rhizosphere, it was observed that the values of sulfur (S), pH, and base saturation (V%) were higher in the bulk soil. On the other hand, the rhizosphere soil exhibited higher levels of aluminum saturation percentage (m%) and potential acidity (H+Al). Additionally, organic matter content varied among the treatments (Table S2). Analyzing the impact of inoculation on the chemical parameters of rhizosphere soil, it was observed that pH and V% tended to decrease in the inoculated treatments. Specifically, the treatment with SynCom exhibited lower pH, while plants inoculated with *B. cereus* CENA-BCM007 (T2) showed lower V% compared to the control ($P < 0.05$). Conversely, inoculation tended to increase H+Al and m%, with the inoculation of *P. polymyxa* CENA-BCM010 (T4) resulting in higher H+Al compared to the control ($P < 0.05$). The analysis of soil enzymatic activity showed significant differences only for the acid phosphatase in the interaction between the inoculated and non-inoculated treatments, while no significant differences were observed for the β-glucosidase and arylsulfatase enzymes (Table S4).

3.2. Effect of microbial inoculation on plant parameters

The growth parameters of the evaluated plants did not show a significant difference between the control and the inoculated treatments ($P > 0.05$, Table S5). Significant differences were observed in the leaf chemistry analysis for Nitrogen (N), Magnesium (Mg), Copper (Cu), and Iron (Fe) among treatments ($P < 0.05$) (Table S6). Inoculation with *P. polymyxa* CENA-BCM010 (T4) exhibited the highest concentrations of N (54.0 g Kg^{-1}), while inoculation with *P. polymyxa* CENA-BCM009 (T3) showed the lowest concentrations (49.5 g Kg^{-1}, $P < 0.05$). Similarly, for Mg, the treatment with *P. polymyxa* CENA-BCM010 (T4) had the highest foliar content (6.8 g Kg^{-1}, $P < 0.05$). In terms of Cu leaf content, *P. polymyxa* CENA-BCM009 (T3) and the SynCom had the highest values (43.8 and 45.3 mg Kg^{-1}, respectively, while for Fe, the treatments with *P. polymyxa* (T3 and T4) showed higher values (191.2 and 189.8 mg Kg^{-1}, respectively). Overall, it is evident that the inoculation with the *Paenibacillus polymyxa* yielded the best results for foliar nutrition (Table S6).

The relative expression of the CAB, GST, and WRKY genes in plant leaves following inoculation with different isolates was then evaluated (Figure S5). The findings indicated consistent down-regulation of the CAB and GST genes across all treatments. Moreover, repression of the WRKY gene was noted in treatments with B. agri CENA-BCM005 (T1), P. polymyxa CENA-BCM010 (T4), and SynCom. Notably, a significant up-regulation of the WRKY gene was observed in treatments with B. cereus CENA-BCM007 (T2) and *P. polymyxa* CENA-BCM009 (T3), which did not differ from the Control.

An assessment of the symbiosis between plant roots and mycorrhizal fungi, essential for plant nutrition and development, was also conducted. The presence of mycorrhizae on the roots was confirmed through microscopic observation of stained mycorrhizal structures (hyphae) in all plants (Figure S6). The inoculation distinctly affected the mycorrhization rate among the treatments, with the highest mycorrhization rate

observed in plants inoculated with B. cereus CENA-BCM007 (T2) (Figure S6C).

3.3. Effects of rhizobacteria inoculation on the prokaryotic rhizosphere microbiome assembly

After performing quality trimming, approximately 3.33 million 16 S rRNA sequences were obtained, and a total of 14,933 prokaryotic Amplicon Sequence Variant (ASVs) were identified at a 97% sequence similarity threshold. Taxonomic classification at the phylum level showed that samples were dominated by Actinobacteria (40.6% of all sequences), followed by Proteobacteria (28.1%), Firmicutes (17%), Acidobacteria (4.7%), Chloroflexi (2.8%), Gemmatimonadetes (1.9%), and Bacteroidetes (1.7%) (Fig. 1A). The phylum Actinobacteria was more abundant in the rhizosphere after the inoculation of *B. agri* CENA-BCM005 (T1), *P. polymyxa* CENA-BCM010 (T4), and SynCom, while Proteobacteria was more abundant in the rhizosphere of the Control treatment ($P < 0.05$). Upon examining diversity measurements, it was found that prokaryotic richness did not exhibit significant differences among the treatments. However, diversity increased in the rhizosphere after the inoculation (Fig. 1B).

The RDA analysis was conducted to investigate the structure of prokaryotic communities and their correlation with soil characteristics and plant parameters, as shown in Fig. 1C. The results demonstrated that the microbial inoculation with a single strain acted as a driver for the rhizosphere microbial community structure. The first two axes of the RDA explained 68% of the data variation, and significant differences were observed between the inoculated and non-inoculated groups. Additionally, differences were found between Bulk soil and rhizosphere samples. The structure of the rhizosphere bacterial community in the Control strongly correlated with soil pH and Zinc. Conversely, the bacterial community in the inoculated treatments showed correlations with plant height, root length, and foliar content of magnesium, boron, and phosphorus. To conduct a more detailed analysis, the Bulk soil samples were excluded from the analysis, and each inoculated treatment was compared to the Control using Principal Coordinates Analysis (PCoA) with Bray-Curtis distance (Fig. 1D). The results revealed a significant effect of inoculation on the community structure in the rhizosphere across all treatments ($P < 0.05$). However, the treatment with *B. cereus* CENA-BCM007 (T2) exhibited a less pronounced difference in community structure compared to the Control ($P = 0.084$).

To investigate changes in community composition, a differential taxonomic abundance analysis was conducted at the ASV level, revealing specific differences in bacterial abundances between the Bulk soil and treatments with and without bacterial inoculation in the rhizosphere (Fig. 2A). For instance, Cluster II comprises 44 ASVs significantly more abundant in the Bulk soil ($P < 0.05$), while the other clusters exhibit ASVs enriched in the rhizosphere. For example, Cluster IV shows 14 ASVs enriched in the SynCom treatment, and clusters I, II, and V display ASVs enriched in treatments with a single bacterial strain inoculation. This analysis primarily indicates a rhizospheric effect that modulates microbial relationships by altering the community's

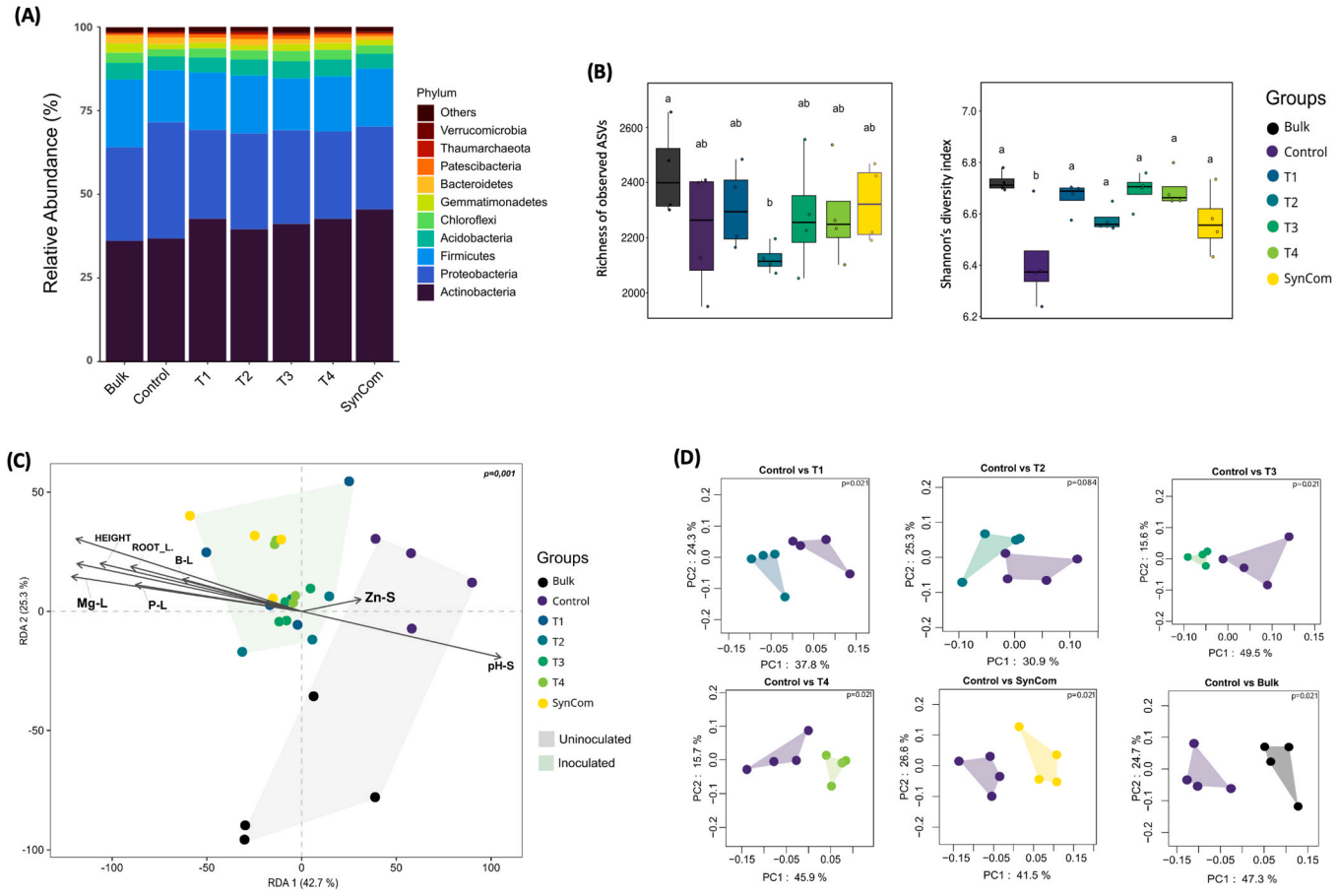

Fig. 1. Prokaryotic community composition, structure, and diversity in bulk soil and rhizosphere of common bean inoculated with different bacterial strains. The taxonomic profiling was based on the 16 S rRNA gene affiliated to the Silva database at 97% of similarity. **(A)** Barplot showing the composition at the phylum level. **(B)** Richness and diversity measurements based on ASV level. Different lower-case letters indicate significant differences based on Tukey's HSD test ($P < 0.05$). **(C)** Redundancy analysis (RDA) of the prokaryotic community and correlation with plant and soil parameters. **(D)** Principal coordinate analysis (PCoA) for pairwise comparison between control and treatments with inoculation using different bacterial strains. The color groups within the plots indicate a grouping based on PERMANOVA ($P < 0.05$). Root_L.: root length; Mg-L: leaf magnesium; P-L: leaf phosphorus; B-L: leaf borum; Zn-S: soil zinc; pH-S: soil acidity.

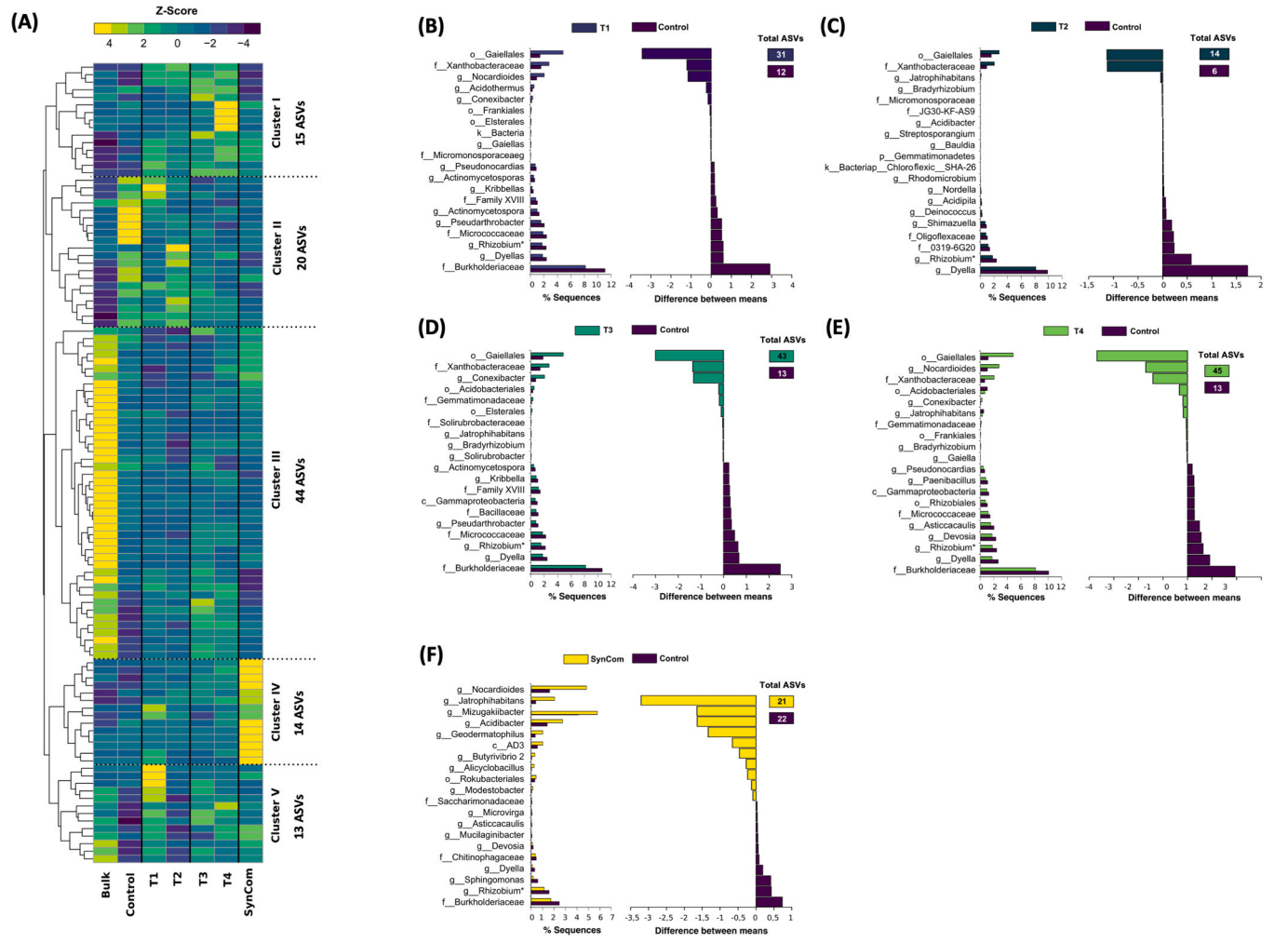

Fig. 2. Prokaryotic community composition in bulk soil and rhizosphere of common bean inoculated with different bacterial strains. The taxonomic profiling was based on the 16 S rRNA gene affiliated to the Silva database at 97% of similarity. **(A)** Heatmap showing the differential abundance of ASVs among all treatments. **(B)** Pairwise comparison between control and each treatment with inoculation. In all graphs, only significant different ASVs are shown, based on Welch's t-test with Benjamini-Hochberg correction ($P < 0.05$).

composition through changes in the abundance of specific microbial groups. Furthermore, inoculation with different bacterial species also resulted in differential ASV abundances across various clusters. For a more comprehensive analysis, a pairwise comparison was conducted between the Control and the treatments involving inoculation (Fig. 2B-F). Inoculating with a single microbial species resulted in an increased abundance of the order Gaiellales and the family Xanthobacteraceae. Across all inoculated treatments, there was a decrease in the abundance of the genera *Rhizobium* and *Dyella* compared to the Control. Notably, the groups *Bradyrhizobium*, Acidobacteriales, and Gemmatimonadaceae were consistently present in rhizospheric bacterial communities inoculated with *P. polymyxa* (Fig. 2D-E). When comparing the different inoculation treatments, distinct profiles emerged between applying a single strain and a bacterial consortium. The treatment with the SynCom exhibited an abundance of various organisms, with the genera *Nocardioides*, *Jatrophihabitans*, *Mizugakiibacter*, and *Acidibacter* being the most prevalent (Fig. 2F).

3.4. Effects of rhizobacteria inoculation on the fungal rhizosphere microbiome assembly

After quality trimming, approximately 2.9 million ITS sequences were obtained, with a total of 2070 fungal ASVs identified at a 97% sequence similarity threshold. Taxonomic classification at the phylum level showed that samples were dominated by Ascomycota (69% of the total sequences), followed by Basidiomycota (21.4%), and Chytridiomycota (1%), with a proportion of unidentified fungal ASVs (8.4%) (Fig. 3A). The phylum Ascomycota exhibited higher abundance in the Control, and inoculation with *B. cereus* CENA-BCM007 (T2), and *P. polymyxa* CENA-BCM009 (T3). Conversely, the phylum Basidiomycota was more abundant in the rhizosphere under treatments with *B. agri* CENA-BCM005 (T1), *P. polymyxa* CENA-BCM010 (T4), and SynCom. Additionally, the phylum Chytridiomycota was more abundant in the rhizosphere inoculated with *B. agri* CENA-BCM005 (T1) ($P < 0.05$). Examining diversity measurements, both richness and diversity varied between the treatments, with the highest values observed in the rhizosphere inoculated with *P. polymyxa* CENA-BCM010 (T4) (Fig. 3B).

The RDA analysis revealed that the inoculation had a lesser effect on the community structure compared to the prokaryotic community (Fig. 3C). The first two axes of the RDA explained 76.6% of the data variation and the comparison between Bulk soil and the rhizosphere of the Control treatment showed minimal differences in the community structure. Soil pH exerted a significant influence on the fungal structure in both the rhizosphere samples, regardless of the inoculation. In a pairwise comparison between the individual inoculated treatments and the Control, significant differences in the fungal community structure were observed only for the treatments involving *Paenibacillus polymyxa* (Fig. 3D).

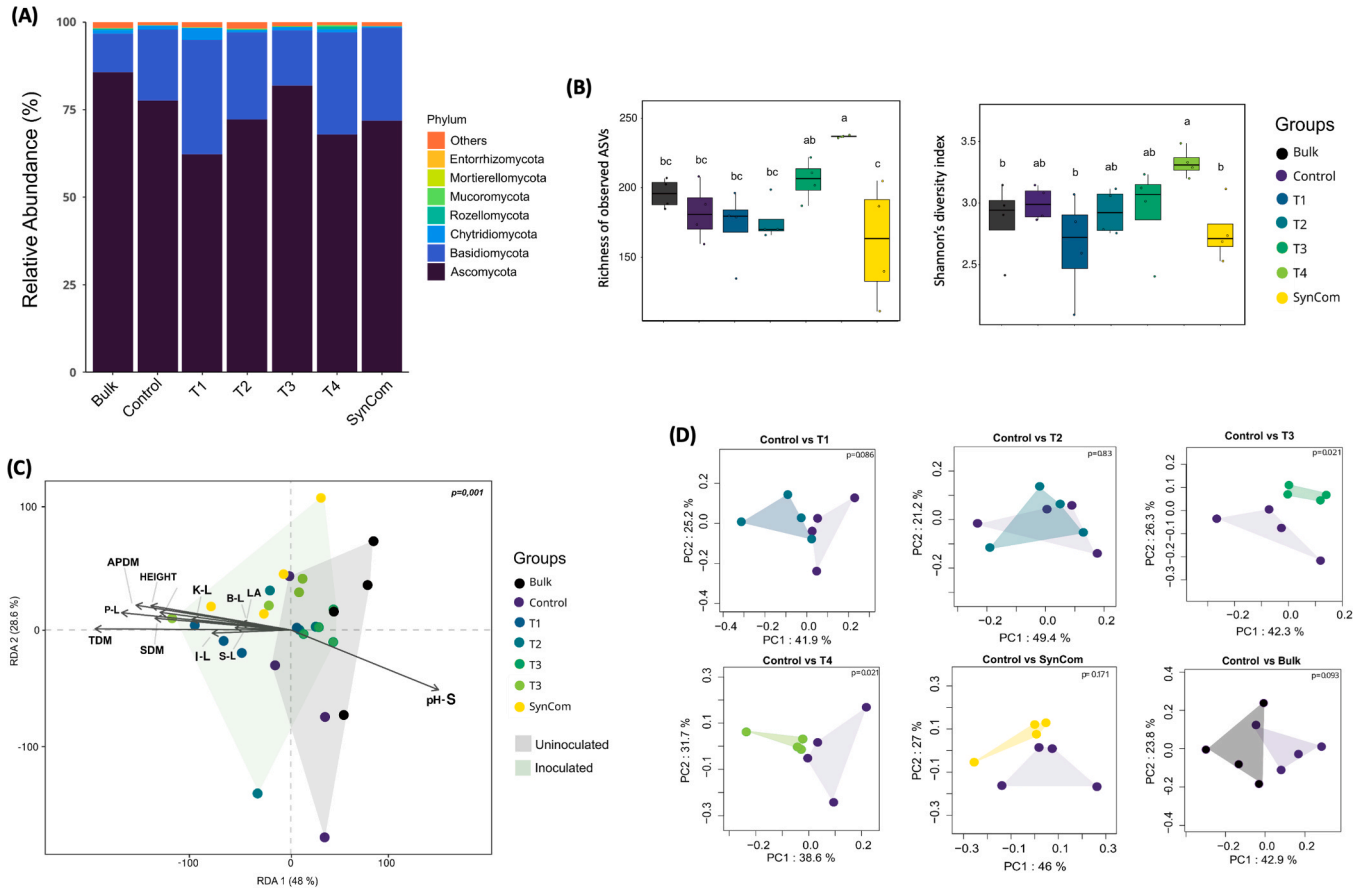

Fig. 3. Fungal community composition, structure, and diversity in bulk soil and rhizosphere of common bean inoculated with different bacterial strains. The taxonomic profiling was based on the ITS gene affiliated to the UNITE database at 97% of similarity. **(A)** Barplot showing the composition at the phylum level. **(B)** Richness and diversity measurements based on ASV level. Different lower-case letters indicate significant differences based on Tukey's HSD test ($P < 0.05$). **(C)** Redundancy analysis (RDA) of the fungal community and correlation with plant and soil parameters. **(D)** Principal coordinate analysis (PCoA) for pairwise comparison between control and treatments with inoculation using different bacterial strains. The color groups within the plots indicate a grouping based on PERMANOVA ($P < 0.05$). Root_L.: root leagth; Mg-L: leaf magnesium; P-L: leaf phosphorus; B-L: leaf borum; Zn-S: soil zinc; pH-S: soil acidity.

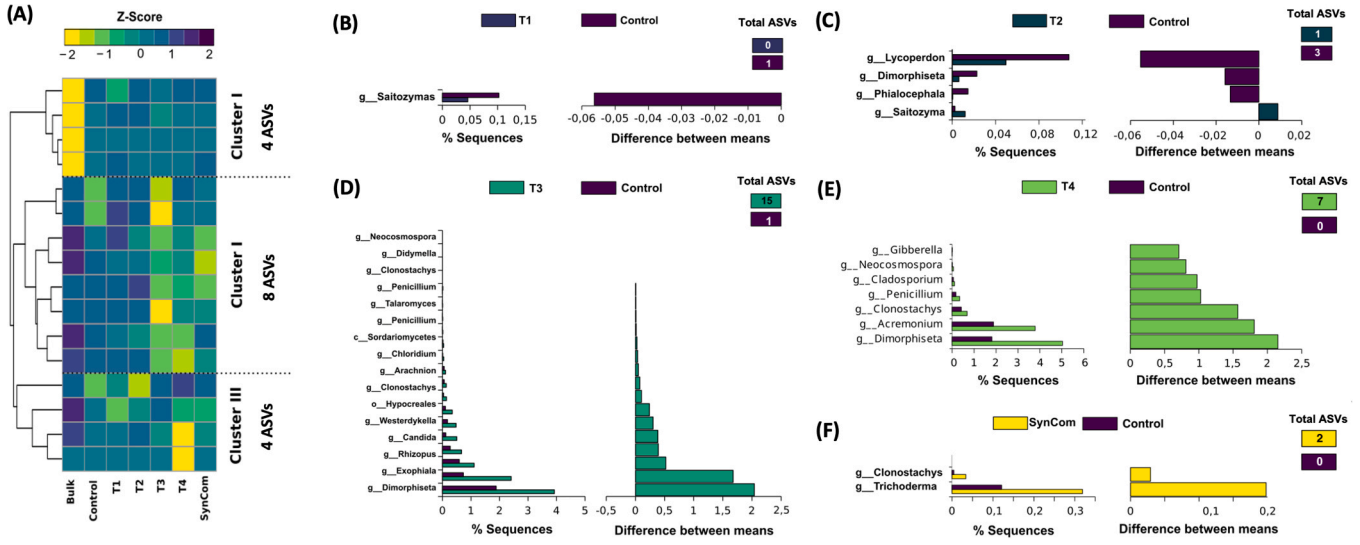

Fig. 4. Fungal community composition in bulk soil and rhizosphere of common bean inoculated with different bacterial strains. The taxonomic profiling was based on the ITS gene affiliated to the UNITE database at 97% of similarity. **(A)** Heatmap showing the differential abundance of ASVs among all treatments. **(B)** Pairwise comparison between control and each treatment with inoculation. In all graphs, only significant different ASVs are shown, based on Welch's t-test with Benjamini-Hochberg correction ($P < 0.05$).

In terms of the fungal community composition, the impact of bacterial strain inoculation on composition was less pronounced compared to the prokaryotic community, with fewer ASVs responding to the treatments (Fig. 4A). For instance, Cluster II exhibited an increase in some ASVs when *P. polymyxa* and SynCom were applied. Apart from the rhizospheric effect of the plant, inoculation with different bacterial species also influenced the abundance of specific ASVs in various clusters within the soil fungal community in the rhizosphere. The pairwise comparison between the Control and the rhizosphere inoculated with *B. agri* (T1) revealed a reduction in the abundance of the genus *Saitozymas* (Fig. 4B), while inoculation with *B. cereus* (T2) increased its abundance and reduced the abundance of the genera *Lycoperdon*, *Dimorphiseta*, and *Phialocephala* (Fig. 4C). The genera *Dimorphiseta* and *Penicillium* were commonly present in the fungal community of rhizospheres inoculated with *P. polymyxa* (Fig. 4D-E). It is worth noting that the strain *P. polymyxa* CENA-BCM009 reduced the genus *Neocosmospora*, while the strain CENA-BCM010 increased. When comparing *P. polymyxa* CENA-BCM009 and the Control, it can be observed that the strain increased the abundance of the genera *Exophiala*, *Rhizopus*, *Candida*, and *Westerdykella* (Fig. 4D). The treatment with P. polymyxa CENA-BCM010 increased the abundance of the genera *Acremonium*, *Clonostachys*, *Cladosporium*, and *Gibberella* (Fig. 4E). Finally, the treatment with SynCom increased the abundance of certain genera such as *Trichoderma* and *Clonostachys* (Fig. 4F).

3.5. Effects of rhizobacteria inoculation on the rhizosphere microbial interaction

The analysis of niche occupancy revealed that, in general, the rare and generalist species surpassed the specialists, as shown in Figure S7. Moreover, the inoculation of microbial species had a varying impact on the percentage of specialists in the rhizosphere, resulting in an increase ranging from 0.6% to 2%, depending on the inoculated species. Notably, the treatment inoculated with the SynCom exhibited the highest percentage of specialists.

Furthermore, to investigate the response of the rhizosphere microbiome to microbial inoculation, co-occurrence networks were constructed using SparCC correlations. These networks displayed distinct characteristics among the treatments, with a decrease in community complexity observed from the bulk soil to the rhizosphere (Fig. 5 and Table S7). Specifically, the bulk soil presented the highest complexity (nodes = 550, edges = 2539, average degree = 9.23). Interestingly, the

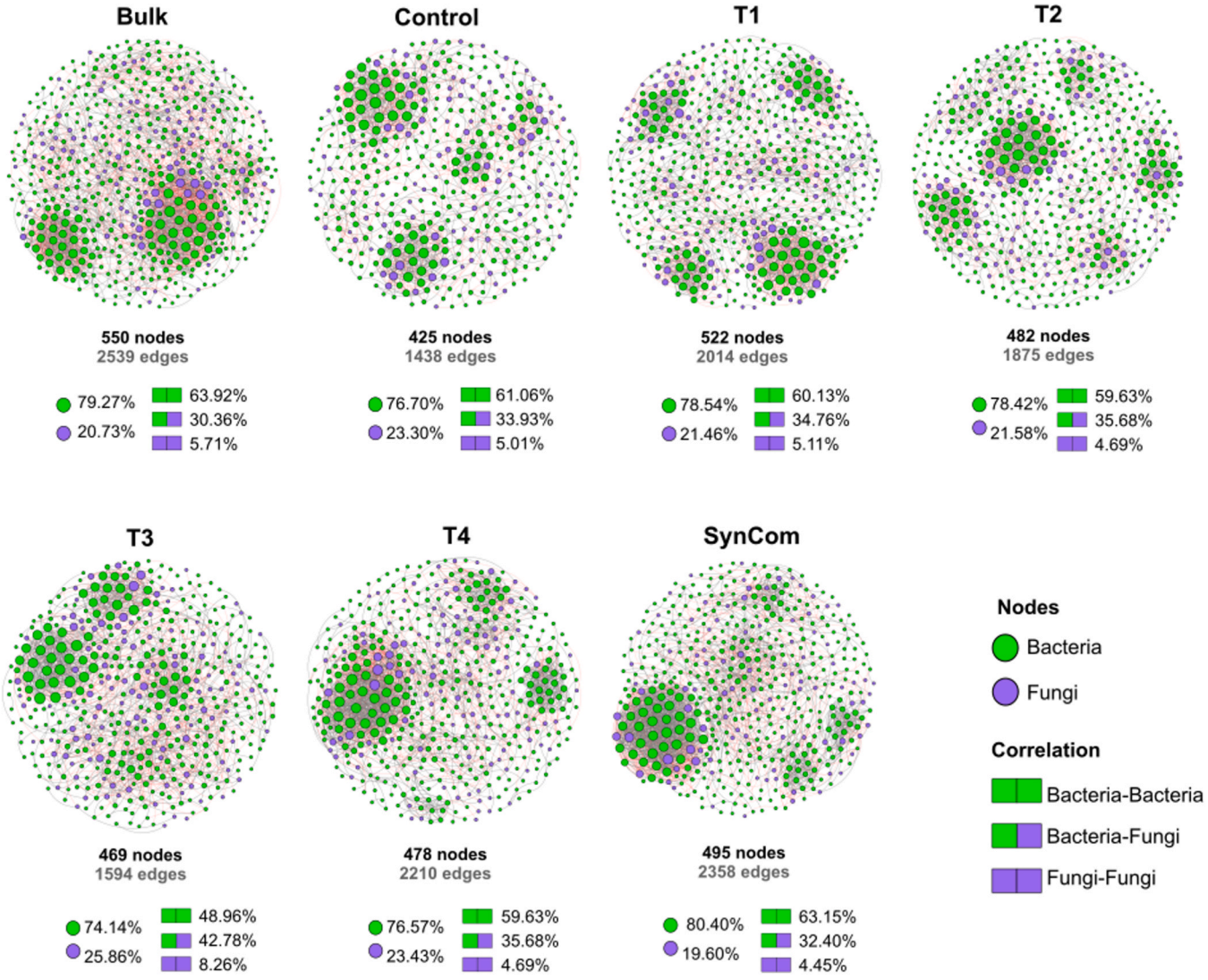

Fig. 5. Co-occurrence network analysis of the prokaryotic and fungal communities in bulk soil and rhizosphere of common bean inoculated with different bacterial strains based on the 16 S rRNA and ITS genes. A connection stands for SparCC correlation with magnitude >0.8 (positive correlation–black edges) or <− 0.8 (negative correlation–red edges) and statistically significant ($p \leq 0.01$). Each node represents taxa at ASV level, and the size of the node is proportional to the number of connections (that is, degree). The color of the nodes is based on the betweenness centrality, where darker colors indicate higher values.

inoculation led to increased complexity in the rhizosphere microbiome, with the SynCom treatment displaying the highest complexity (nodes = 495, edges = 2358, average degree = 9.52) compared to the Control (nodes = 425, edges = 1438, average degree = 6.76). Furthermore, the network analysis unveiled a notable observation regarding the proportion of interactions between fungi and bacteria. While the overall proportion of fungi and bacteria remained relatively stable, the interactions between these domains varied significantly among different inoculants. For instance, the inoculation of *P. polymyxa* and SynCom resulted in decreased proportions of bacteria-bacteria interactions (48.96%, 59.63%, and 53.15%, respectively). Conversely, fungal-fungal interactions increased in the treatment with *P. polymyxa* CENA-BCM009 but decreased in the *B. cereus*, *P. polymyxa* CENA-BCM010, and SynCom treatments (4.69%, 4.69%, and 4.45%, respectively). Finally, it was observed that the relationship between fungi and bacteria was substantially modified only in the *P. polymyxa* CENA-BCM009 treatment. These findings highlight that the inoculation of different isolated species not only influences the structure and complexity of relationships but also affects the types of interactions between distinct taxonomic domains.

3.6. Correlation between bacterial community and plant traits

To explore the correlations between specific bacterial groups, microbial diversity, and community complexity with plant nutrients and biometrics, all possible Spearman's rank correlations were computed (Fig. 6). The results revealed that the majority of bacterial and fungal phyla exhibited positive correlations with plant parameters (30 positives and 13 negatives; Fig. 6A). Among these, the phyla demonstrating the highest number of correlations with plant parameters were Patescibacteria (3 positives and 1 negative), Gemmatomonadetes (4 positives), and Tenericutes (2 positives and 2 negatives). Conversely, the plant parameters that displayed the highest number of correlations with microbial phyla were the content of magnesium (4 positives and 3 negatives), iron (5 positives), and copper (3 positives and 1 negative) in the leaf, along with total dry mass (4 positives and 1 negative), and stem diameter (3 positives and 1 negative). Also, given that inoculation impacted both the diversity and complexity of interactions within the rhizosphere microbiome, the correlation of these parameters with plant nutrients and biometrics was verified (Fig. 6B). The results revealed a positive correlation between diversity and shoot dry mass, total dry mass, and iron, while an increase in complexity demonstrated a positive correlation with stem dry mass and magnesium leaf content.

4. Discussion

In this study, the impact of rhizobacteria inoculation on soil properties, plant growth parameters, and the rhizosphere microbiome was assessed. The introduction of bacterial species notably influenced soil pH, leading to observed reductions, especially pronounced with SynCom inoculation, resulting in increased soil acidity. Various species of PGPR secrete chelators such as organic acids and siderophores in the rhizosphere, contributing to the lowering of soil pH (Virk et al., 2022). Additionally, certain bacterial groups release protons during metabolic processes such as respiration or fermentation. It was also observed that both inoculated and non-inoculated rhizospheres displayed decreased base saturation and increased aluminum saturation in the soil. Plant roots play a pivotal role in modulating rhizosphere properties by releasing H^+ ions or organic acids, thus lowering pH and directly affecting microbial communities and nutrient accessibility (Melo, 2006). Similarly, bacterial inoculation induces changes in the soil's chemical and biochemical parameters by promoting acidification, thereby influencing soil buffering capacity and cation exchange capacity (Zhang et al., 2009; Miransari, 2011). As expected, the most significant

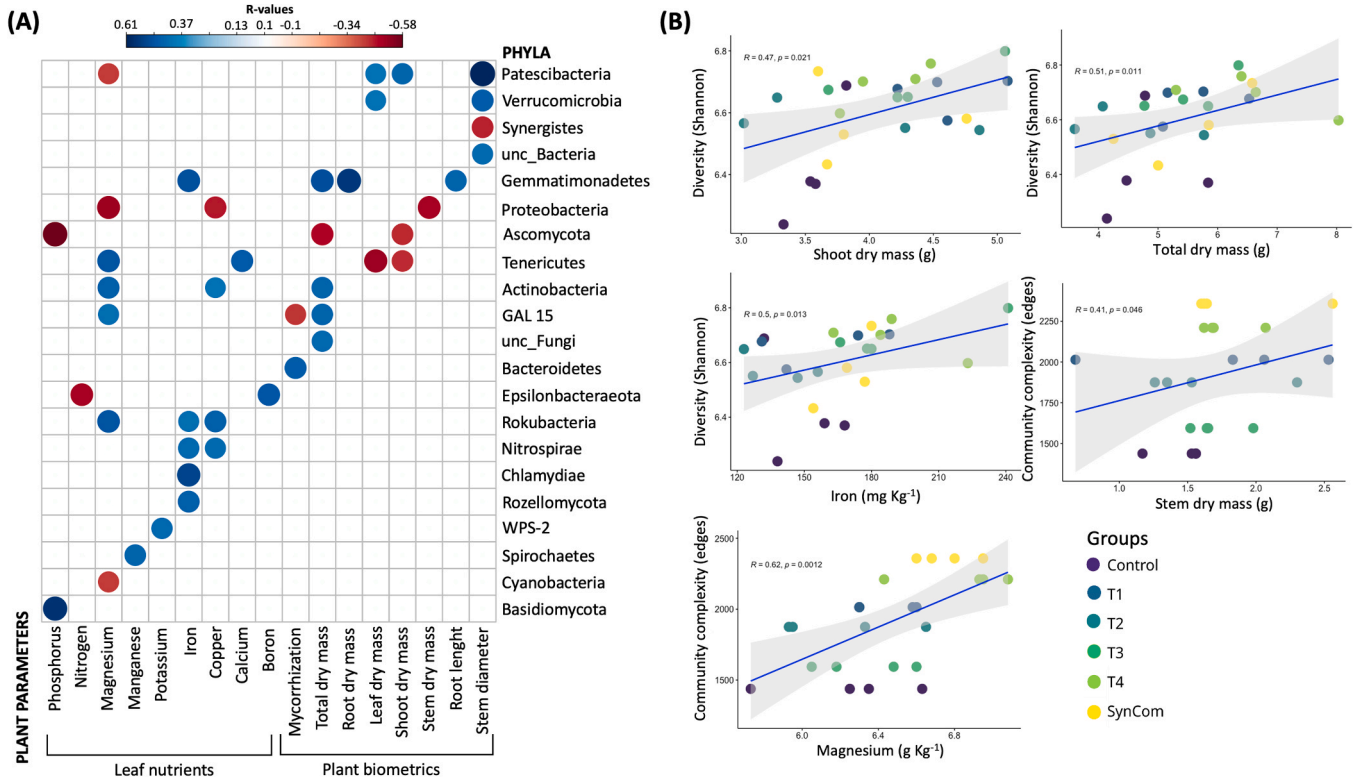

Fig. 6. Correlation between microbial community traits and plant parameters. **(A)** Heatmap showing the correlations between microbial groups at phyla level and leaf nutrients and plant biometric traits. **(B)** Linear regression graphs showing the correlation between community diversity (Shannon's index) and complexity (number of interactions from network analysis) and leaf nutrients and plant biometric traits. The correlation is based on Spearman's rank coefficients with corrections using the Benjamini-Hochberg FDR method ($P < 0.05$).

differences were observed between Bulk soil and the rhizosphere, implying that chemical alterations are more closely linked to modifications induced by plant roots rather than solely the presence of an inoculant. Moreover, the rhizosphere samples, regardless of inoculation, notably impacted acid phosphatase activity. This enzyme increases when phosphorus is lacking in soil, boosting the solubilization and redistribution of phosphate. In the experiments conducted, inoculants had minimal influence on solubilization, with no differences observed between the Control and the treated samples. The acid phosphatase enzyme, produced by soil microorganisms and plant roots, hydrolyzes organic phosphates into inorganic forms, thereby enhancing plant nutrient accessibility. Its effectiveness is higher in acidic soils, and potentially more pronounced in those with lower pH (Caires et al., 2015). However, high aluminum saturation can hamper its function, limiting phosphorus availability to plants (Acosta-Martinez and Tabatabai, 2000). This saturation inhibits plant root activity, reducing nutrient absorption and affecting soil microorganism function, which confirms the findings.

The plant growth parameters did not exhibit significant differences among the treatments. However, the absence of statistical significance does not necessarily indicate that the isolates had no impact on plant growth but rather that this difference might not have been detectable. It is plausible that the indigenous soil microbiota may have outcompeted the introduced inoculants (Pereg and McMillan, 2015). Additionally, it's essential to note that the assessment occurred only 35 days after planting. It's possible that even though the inoculants remain effective, plant growth parameters might stabilize during further developmental stages (Berg et al., 2016; Pereg and McMillan, 2015). Further research conducted throughout the growth cycle is required to comprehend the influence of microbial inoculation on plant growth. Moreover, the recommended fertilization could also influence the growth parameters, especially for plants that do not solely rely on recruiting microorganisms to establish themselves in the soil (Hartman et al., 2018). The soil in the experiment was chemically amended to enhance basal fertility by adding the required macronutrients and raising the pH with lime, potentially reducing the plants' reliance on microbial interactions. Studies have indicated that bioinoculants can decrease dependency on chemical fertilizers (Etesami and Alikhan, 2016; Telles et al., 2023). Therefore, new experiments encompassing diverse soil fertility levels are necessary to assess the potential of these isolates in promoting plant growth.

Plant nutrition was also assessed, as the inoculation of different bacterial species can impact nutrient absorption by plants through the solubilization of these compounds. The findings revealed that the treatment inoculated with *P. polymyxa* (T4) exhibited better results for N and Fe in plants compared to other inoculated treatments and the Control. The solubilization process involves the production of organic acids and enzymes facilitating the release of nutrients from soil particles, making them readily available for plants (Yadav et al., 2017). Some diazotrophic bacteria, such as *Paenibacillus*, provide plants with a portion of the fixed nitrogen in the form of ammonia and nitrate, in addition to nitrogen fixation (Li et al., 2019). These bacteria are also capable of supplying Fe to plants when this element is limited in the soil by producing and secreting siderophores. These siderophores bind to soil-bound iron, thereby making it available to plants (Wang et al., 1993; Gray and Smith, 2005; Liu et al., 2019). Although Fe is a less mobile nutrient, the results obtained for this nutrient demonstrate the effect of the *P. polymyxa* strain, which synthesizes siderophores that chelate Fe_3^+ and make it available as Fe_2^+ for plant absorption (McRose et al., 2018). Interestingly, Fe was the leaf nutrient that correlated with the highest number of microbial phyla, namely Gemmatimonadetes, Rokubacteria, Nitrospirae, Chlamydiae, and Rozellomycota, suggesting that inoculation may have an indirect effect on plant nutrition. This finding emphasizes the importance of microbial inoculation for nutrient bioavailability in crops.

The findings indicated that the inoculation of bacterial strains affected the expression of CAB, GST, and WRKY genes in bean leaves, resulting in their down-regulation. Bacterial inoculation can impact gene expression in plants by activating signaling pathways, inducing defense responses, and employing other mechanisms (Andersen et al., 2018). Bacterial inoculation influences plant gene expression in diverse ways. This variability underscores the complexity of interactions, as documented in existing research (Borges, 2011; Zamioudis and Pieterse, 2012), reflecting the nuanced effects on different markers and metabolic processes. The GST gene plays a role in detoxifying toxic compounds within plant cells. Studies suggest that bacterial inoculation can down-regulate the GST gene in plants because certain bacteria enhance plant tolerance to toxic compounds without requiring the activation of this gene (Dixon et al., 2010). As the upregulation of GST genes in plants has been used as an indicator of oxidative stress and hypersensitive resistance (HR) type in plant-bacteria interactions (Alvarez et al., 1998; Desikan et al., 1998; Maleck et al., 2000), its downregulation here can indicate the beneficial effects of all treatments with rhizobacterial inoculation. The expression similar to the Control or the down-regulation of the CAB gene indicates no effects of the inoculation on the photosynthesis level or might occur because certain bacteria can improve photosynthetic efficiency without necessitating an increase in gene expression (Pickersky et al., 1989). WRKY genes oversee plant defense responses (Chi et al., 2013), and their down-regulation, according to expected, might indicate that the rhizobacterial inoculated were not harmful to the plant. The WRKY gene family is responsible for regulating the expression of PR genes in beans (Mayo et al., 2016) and Arabidopsis (Zheng et al., 2006). In summary, the down-regulation of CAB, GST, and WRKY genes could represent an adaptive response of plants to inoculation, potentially reallocating resources to other crucial functions. However, it's vital to note that the suppression of these genes could adversely affect plant resistance and growth, contingent on experimental conditions and the specific interaction between bacteria and plants. Understanding these mechanisms can aid in developing more effective strategies to boost plant resistance and growth through bacterial inoculation.

The impact of inoculation on the association between plant roots and mycorrhizae was investigated. Results indicated that inoculation with *B. cereus* (T2) led to a higher mycorrhization rate, likely by promoting fungal colonization in plant roots. Studies suggest that bacteria from the genus *Bacillus* may assist in nutrient cycling, inorganic phosphorus solubilization, root growth enhancement, and the improvement of plant resistance to environmental stresses, facilitating interaction with arbuscular mycorrhizal fungi (Apaza-Castillo et al., 2022; Soares et al., 2023). Inoculants applied to the soil have the capability to assist the colonization of other microbial groups, potentially contributing to plant growth. Therefore, inoculation with the bacterium *B. cereus* could be a promising strategy to improve the mycorrhization rate in plants. However, it's essential to consider that different plant species and microorganisms may respond differently to inoculation. Hence, evaluating the effectiveness of inoculation under varying soil and climate conditions is crucial.

The analysis of the prokaryotic community composition revealed a differential abundance of ASVs between the Bulk soil and the rhizosphere. Interestingly, the results revealed that inoculating bacteria into the rhizosphere soil increased bacterial diversity to the same level as the Bulk soil, suggesting that inoculation might bolster functional resilience and positively impact plant health. The findings align with prior research indicating that bacterial inoculation enhances plant growth and augments bacterial diversity in the rhizosphere, thereby improving soil and plant health (Mendes et al., 2013; Trabelsi et al., 2013). Therefore, within the rhizosphere, significant variations in certain taxa were observed between the Control group and the groups treated with bacterial isolates. Notably, ASVs affiliated with the Burkholderiaceae and Micrococcaceae families, as well as the genera *Dyella* and *Rhizobium*, were the most abundant in the rhizosphere of the Control group. These microorganisms likely adapted to the specific conditions of the rhizosphere and may have been influenced by nutritional and chemical

changes, alongside microbial competition post-inoculation (Philippot et al., 2013; Pii et al., 2015; Sasse et al., 2018). On the other hand, in treatments involving inoculation, we observed a higher abundance of ASVs associated with the Gaiellales order, Xanthobacteraceae family, and the genera *Nocardioides* and *Conexibacter*. Additionally, the genus *Bradyrhizobium* exhibited higher abundance in groups treated with *Paenibacillus polymyxa*. Prior studies have revealed that *Nocardioides* are nitrogen fixers, capable of degrading xenobiotics and antibiotics (Yoon et al., 1999; Kim et al., 2017; Nafis et al., 2019). The genus *Conexibacter* plays a vital role in nitrogen and carbon cycling, participating in organic matter decomposition and nutrient cycling. *Bradyrhizobium* is also associated with nitrogen fixation (VanInsberghe et al., 2015; Zhang et al., 2019; Pedrinho et al., 2020). These microorganisms are crucial for various processes within the nitrogen cycle, such as nitrification and denitrification. The findings obtained here suggest that inoculation influenced the abundance of microbial groups beneficial to plants, indicating the indirect effect of inoculation on soil conditions and the plant's response (Compant et al., 2019; Trivedi et al., 2020).

Regarding the fungal community structure, only the inoculation with *Paenibacillus polymyxa* had a significant effect on the fungal community structure, while the inoculation with other bacterial strains did not result in significant variations. These structural changes promoted by the *Paenibacillus* genus may be linked to the wide array of secondary substances produced by this genus, such as antibiotics and antifungals, suggesting the possibility of microbial community modulation (Yang et al., 2018; Padda et al., 2017; Carrión et al., 2019). Analyzing the diversity of fungi among the treatments in this study, lower diversity was observed in the Bulk soil and groups inoculated with *B. agri* and the SynCom, while the Control and the other treatments increased diversity. It was also interesting to note that the treatment with *P. polymyxa* resulted in higher diversity. In some cases, fungal diversity may increase after bacterial inoculation, with the main reasons being enhanced plant resistance, increased nutrient availability, soil property modification, and the positive interaction between bacteria and fungi (Carrión, 2019; Wu, 2022; Xu, 2012). In contrast to the results obtained for the prokaryotic community, the fungal community displayed a small number of ASVs with differential abundance after inoculation, suggesting minimal alteration in the community. This observation could be attributed to the prolonged life cycle of fungi and their interactions with bacteria. Due to these characteristics, potential impacts on their community may require an extended period to become noticeable (Bahram et al., 2018). Intriguingly, the findings indicate that different bacterial isolates can exert distinct influences on the differential abundance of fungi. Specifically, groups inoculated with *B. cereus* exhibited an increase in the abundance of certain taxa, including the genera *Saitozyma*, *Lycoperdon*, *Dimorphiseta*, and *Phialocephala*, while the treatment with *B. agri* showed a reduction in the genus *Saitozyma*. The differential presence of these fungal groups may be associated with increased organic matter decomposition, spore production, and the establishment of symbiotic relationships (Kjøller, 2012). However, a more pronounced effect was observed in treatments with *P. polymyxa*, with 2–5 times more ASVs responding to the inoculation. This heightened taxon diversity may reflect significant changes in soil ecological functions, such as nutrient cycling, functional resilience, secondary compound production, and plant-microorganism interactions (Jing et al., 2015). Overall, these findings indicate that the introduction of a single bacterial species as an inoculant can lead to changes in the entire community structure, with implications for functional traits and plant interactions. It is worth noting that the prokaryotic communities were more sensible to inoculation compared to the fungal community.

Finally, co-occurrence and niche occupancy analyses were performed to assess the effect of inoculation on the microbial interactions in the rhizosphere. The analysis of ecological niche occupancy revealed that all treatments exhibited an increase in specialists compared to the Control. However, the treatments with *B. agri*, *P. polymyxa*, and SynCom showed the most substantial increase in these groups. Inoculation potentially enhances the recruitment of rhizosphere-specific bacteria, which might assist in processes such as nutrient acquisition, strengthening the plant's immune system, and even improving soil conditions (Compant et al., 2019). Previous studies have demonstrated that specialist organisms have a limited niche but exhibit higher fitness in their ideal habitat (Kneitel; Chase, 2004; Monard et al., 2016). Moreover, according to Pandit et al. (2009), specialists are highly responsive to environmental disturbances, including changes in soil chemical properties. Therefore, alterations in pH and nutrient availability in the rhizosphere might have created highly specific conditions that favored the growth and diversity of specialists.

The network analysis revealed that the rhizosphere exhibits less complex interactions compared to the Bulk soil, suggesting the specialization of specialist taxa and potentially specific metabolic pathways. A study of the soybean rhizosphere in Amazonian soils showed similar results, demonstrating that the rhizosphere exhibits a selection of specialists, leading to less complex relationships (Mendes et al., 2014). However, in the rhizosphere, the inoculation with different bacterial strains appears to increase this complexity compared to the Control, indicating a change in microbial relationships after the addition of an inoculant. Bacterial inoculants may distinctly modulate the rhizosphere microbiome, altering both positive and negative relationships and the complexity of interactions (Compant et al., 2019). Particularly, treatments with *B. agri*, *P. polymyxa*, and SynCom stood out for presenting considerably higher interaction complexity, demonstrating a broader diversity and complexity of relationships compared to the Control treatment. This suggests that inoculation may stimulate a more complex network of interactions in the rhizosphere, which could have significant implications for plant health and performance (Banerjee et al., 2018). In this study, enhanced microbial diversity, together with higher community complexity, showed a positive correlation with overall plant dry mass (total, shoot, and stem), indicating the benefits of these traits for plant growth. Similar outcomes are observed when applying agricultural residues to soils, as a more complete and complex community can significantly alter community interactions (Bell et al., 2019). Additionally, in a study manipulating soil microbial diversity, higher diversity was associated with increased plant productivity (Barros et al., 2022). The treatment with *P. polymyxa* CENA-BCM009 was the only one that substantially altered interactions between fungi and bacteria. These results highlight that inoculation with different strains affects not only the structure and complexity of interaction networks but also the types of interactions among different taxonomic domains (Tipton et al., 2018; Hartman et al., 2018). In summary, the network analysis revealed that bacterial inoculation in the rhizosphere can distinctly influence the complexity of microbial interactions and the balance between different taxonomic domains, with significant implications for the functionality of the microbial community, and consequently, for the health and growth of plants.

5. Conclusion

In conclusion, the comprehensive investigation into the impact of bacterial inoculation on soil properties, plant growth parameters, rhizosphere microbiome, and microbial interactions yields valuable insights into the complexity within the soil-plant-microbe continuum. Overall, bacterial inoculation significantly influenced the rhizosphere microbiome, leading to alterations in microbial community composition and interactions, which in turn affected soil chemistry, plant nutrition, and plant gene expression. Inoculation resulted in increased bacterial diversity and complexity of interactions within the rhizosphere, potentially enhancing plant health and performance. Notably, different bacterial strains exhibited varied effects on microbial communities, emphasizing the importance of selecting appropriate inoculants tailored to specific soil and plant conditions. Moreover, bacterial inoculation, particularly with *Paenibacillus polymyxa*, positively influenced plant nutrition by enhancing nutrient availability through solubilization

processes and potentially modulating gene expression in plants to reallocate resources for optimal growth and defense mechanisms. This study highlights the diverse impacts of microbial inoculation on soil, plants, and microbial communities. However, further research across different stages and conditions is crucial for a comprehensive understanding. Evaluating functional impacts using omics approaches will shed light on community resilience despite changes induced by inoculants, thereby advancing the understanding of plant-microbe relationships in the rhizosphere.

CRediT authorship contribution statement

Ana Vitória Reina da Silva: Methodology, Investigation. **Camila Patreze:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Siu Mui Tsai:** Resources, Writing – review & editing. **Lucas William Mendes:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Izadora de Cássia Mesquita da Cunha:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Luis Felipe Guandalin Zagatto:** Methodology, Investigation. **Solange dos Santos Silva Zagatto:** Methodology, Investigation. **Miriam Chaves:** Conceptualization, Data curation, Investigation, Methodology. **Rodrigo Mendes:** Conceptualization, Methodology, Writing – review & editing. **Eduardo Henrique Marcandalli Boleta:** Methodology, Investigation. **Thierry Alexandre Pellegrinetti:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation.

Data Availability

The sequences are submitted to the NCBI Sequence Read Archive under the identification PRJNA1054175

Acknowledgments

This study was supported by a grant from Fundação de Amparo à pesquisa do Estado de São Paulo (FAPESP 2014/03217-3, 2015/00251-9, 2019/16043-7, 2020/12890-4), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES 88887.185941/2018-00, 88887.636994/2021-00), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 408191/2018-0, 402050/2023-1), and PRPI-USP (22.1.08498.01.0). LWM also thank CNPq for the Productivity Research Grant (CNPq 307670/2021-0).

Author agreement statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the Corresponding Author is the sole contact for the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.micres.2024.127706.

References

Acosta-Martinez, V., Tabatabai, M.A., 2000. Enzyme activities in a limed agricultural soil. Biol. Fertil. Soils 31, 85–91.

Alvarez, M.E., Pennell, R., Meijer, P.J., Ishikawa, A., Dixon, R.A., Lamb, C., 1998. Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell 92, 773–784. https://doi.org/10.1016/S0092-8674(00)81405-1.

Andersen, E.J., et al., 2018. Disease resistance mechanisms in plants. Genes 9, 339. https://doi.org/10.3390/genes9070339.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.

Apaza-Castillo, G.A., Hosaka, G.K., Quecine, M.C., 2022. Genome insights from the Amazonian rhizobacterium Bacillus paramycoides RZ3MS14 reveal plant growth-promoting multi-traits and bioprotection against phytopathogens and environmental stresses. Research Square. https://doi.org/10.21203/rs.3.rs-2379212/v1.

Araujo, A.S.F., et al., 2019. Bacterial community associated with the rhizosphere of maize and cowpea in a subsequent cultivation. App. Soil Ecol. 143, 26–34.

Bahram, M., et al., 2018. Structure and function of the global topsoil microbiome. Nature 560, 233–237. https://doi.org/10.1038/s41586-018-0386-6.

Banerjee, S., Schlaeppi, K., van der Heijden, M.G.A., 2018. Keystone taxa as drivers of microbiome structure and functioning. Nat. Rev. Microbiol. 16 (9), 567–576. https://doi.org/10.1038/s41579-018-0024-1.

Barros, F.M.R., Pedrinho, A., Mendes, L.W., Freitas, C.C.G., Andreote, F.D., 2022. Interactions between Soil Bacterial Diversity and Plant-Parasitic Nematodes in Soybean Plants. Appl. Environ. Microbiol. 88, e00963-22 https://doi.org/10.1128/aem.00963-22.

Bastian, M., Jacomy, M., 2009. Gephi: an open source software for exploring and manipulating networks. Int AAAI Conf. Weblogs Soc. Media San. Jose, CA, Usa.

Bell, T.H., et al., 2019. Changes in the structure of bacterial and fungal communities in agricultural soil resulting from the application of dairy manure and commercially available microbial products. Appl. Soil Ecol. 136, 125–133.

Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P., Kauserud, H., 2010. ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC Microbiol 10, 189. https://doi.org/10.1186/1471-2180-10-189.

Berg, G., et al., 2016. The plant microbiome explored: implications for experimental botany. J. Exp. Bot. 67 (4), 995–1002. https://doi.org/10.1093/jxb/erv466.

Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 68, 1–13.

Bolyen, E., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37 (8), 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Bomfim, C.A., Coelho, L.G.F., Vale, H.M.M., Mendes, I.C., Megías, M., Ollero, F.J., Reis Junior, F.B., 2021. Brief history of biofertilizers in Brazil: from conventional approaches to new biotechnological solutions. Braz. J. Microbiol. 52, 2215–2232.

Borges, A., 2011. Análise da expressão de genes relacionados à interação incompatível Phaseolus vulgaris/Collestotrichum lindemuthianum. Orientadora: Danielle Gregório Gomes Caldas. 2011. 117 p. Dissertação (Mestrado em Ciências) – Centro de Energia Nuclear na Agricultura da Universidade de São Paulo, Piracicaba, 2011.

Caires, E.F., et al., 2015. Surface liming and nitrogen fertilization for crop grain production under no-till management in Brazil. Eur. J. Agron. 66, 41–53.

Callahan, B.J., McMudrie, P.J., Rosen, M.J., et al., 2016. DADA2: High resolution sample inference from Illumina amplicon data. Nat Methods 13 (7), 581–583.

Canarini, A., et al., 2019. Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. Front. Plant. Sci. 10, 157.

Carbonell, S.A.M., et al., 2014. IAC Milênio" - Common bean cultivar with high grain quality. Crop Breed. Appl. Biotechnol. 14, 273–276.

Carrión, V.J., et al., 2019. Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. Sci., N. Y. 366, 606–612. https://doi.org/10.1126/science.aaw9285.

Chapelle, E., Mendes, R., Bakker, P.A.H., Raaijmakers, J.M., 2016. Fungal invasion of the rhizosphere microbiome. ISME J. 10, 265–268.

Chi, Y., et al., 2013. Protein–protein interactions in the regulation of WRKY transcription factors. Mol. Plant 6, 287–300.

Compant, S., et al., 2019. A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. J. Adv. Res. v19, 29–37. https://doi.org/10.1016/j.jare.2019.03.004.

Desikan, R., Reynolds, A., Hancock, J.T., Neill, S.J., 1998. Harpin and hydrogen peroxide both initiate programmed cell death but have differential effects on defence gene expression in Arabidopsis suspension cultures. Biochem. J. 330, 115–120. https://doi.org/10.1042/bj3300115.

Dixon, D.P., Skipsey, M., Edwards, R., 2010. Roles for glutathione transferases in plant secondary metabolism. Phytochemistry 71 (4), 338–350. https://doi.org/10.1016/j.phytochem.2009.12.012.

Etesami, H., Alikhan, H.A., 2016. Co-inoculation with endophytic and rhizosphere bacteria allows reduced application rates of N-fertilizer for rice plant. Rhizosphere 2, 5–12.

Goswami, D., Thakker, J.N., Dhandhukia, P.C., 2016. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. Cogent Food Agric. 2 (1), 1127500. https://doi.org/10.1080/23311932.2015.1127500.

Gray, E.J., Smith, D.L., 2005. Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. Soil Biol. Biochem. 37, 395–412.

Hartman, K., et al., 2018. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. Microbiome 6, 14. https://doi.org/10.1186/s40168-017-0389-9.
Imam, J., Singh, P.K., Shukla, P., 2016. Plant microbe interactions in post genomic era: perspectives and applications. Front. Microbiol. 7, 1488.
Jing, X., et al., 2015. The links between ecosystem multifunctionality and above- and belowground biodiversity are mediated by climate. Nat. Commun. 6 https://doi.org/10.1038/ncomms9159.
Kim, H., et al., 2017. Syntrophic biodegradation of propoxur by Pseudaminobacter sp. SP1a and Nocardioides sp. SP1b isolated from agricultural soil. Int. Biodeterior. Biodegrad. v. 118, 1–9. https://doi.org/10.1016/j.ibiod.2017.01.024.
Kjøller, R., 2012. Dramatic changes in ectomycorrhizal community composition, root tip abundance and mycelial production along a stand-scale nitrogen deposition gradient. N. Phytol. 194 (1), 278–286. https://doi.org/10.1111/j.1469-8137.2011.04041.x.
Kneitel, J.M., Chase, J.M., 2004. Trade-offs in community ecology: Linking spatial scales and species coexistence. Ecol. Lett. 7, 69–80.
Li, Y., Li, Y., Zhang, H., Wang, M., Chen, S., 2019. Dizotrophic *Paenibacillus beijingensis* BJ-18 provides nitrogen for plant and promotes plant growth, nitrogen uptake and metabolism. Front. Microbiol. 10, 1119. https://doi.org/10.3389/fmicb.2019.01119.
Liu, X., et al., 2019. *Paenibacillus* strains with nitrogen fixation and multiple beneficial properties for promoting plant growth. PeerJ 7. https://doi.org/10.7717/peerj.7445.
Malavolta, E., Vitti, G.C., oliveira, A.S., 1997. Avaliação do estado nutricional das plantas: princípios e aplicações. Potafos, Piracicaba, p. 319.
Maleck, K., Levine, A., Eulgem, T., Morgan, A., Schmid, J., Lawton, K.A., et al., 2000. The transcriptome of Arabidopsis thaliana during systemic acquired resistance. Nat. Genet. 26, 403–410. https://doi.org/10.1038/82521.
Mayo, S., Cominelli, E., Sparvoli, F., González-López, O., Rodríguez-González, A., Gutiérrez, S., Casquero, P.A., 2016. Development of a qPCR strategy to select bean genes involved in plant defense response and regulated by the Trichoderma velutinum—Rhizoctonia solani interaction. Front. Plant Sci. 2016 (7), 1109. https://doi.org/10.3389/fpls.2016.01109.
McRose, D.L., Seyedsayamdost, M.R., Morel, F.M.M., 2018. Multiple siderophores: bug or feature? J. Biol. Inorg. Chem. 23, 983–993. https://doi.org/10.1007/s00775-018-1617-x.
Medina, A., et al., 2003. Interactions of arbuscular-mycorrhizal fungi and Bacillus strains and their effects on plant growth, microbial rhizosphere activity (thymidine and leucine incorporation) and fungal biomass (ergosterol and chitin). App Soil Ecol 22, 15–28.
Melo, I.S.Rizorremediação. EMBRAPA Documentos, 2006. Disponível em: https://www.agencia.cnptia.embrapa.br/Agencia23/AG01/arvore/AG01_22_299200692526.html. Acesso em: setembro 2022.
Mendes, L.W., et al., 2014. Taxonomical and functional microbial community selection in soybean rhizosphere. ISME J. 8, 1577–1587. https://doi.org/10.1038/ismej.2014.17.
Mendes, L.W., et al., 2019. Resistance Breeding of Common Bean Shapes the Physiology of the Rhizosphere Microbiome. Front. Microbiol. 10 https://doi.org/10.3389/fmicb.2019.02252.
Mendes, L.W., Raaijmakers, J.M., Hollander, M., Mendes, R., Tsai, S.M., 2018. Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. ISME J. 12, 212–224.
Mendes, L.W., Raaijmakers, J.M., Hollander, M., Sepo, E., Expósito, R.G., Chiorato, A.F., Mendes, R., Tsai, S.M., Carrión, V.J., 2023. Impact of the fungal pathogen Fusarium oxysporum on the taxonomic and functional diversity of the common bean root microbiome. Environ. Micro 18, 68.
Mendes, R., Garbeva, P., Raaijmakers, J.M., 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol. Rev. v. 37, n (5), 634–663. https://doi.org/10.1111/1574-6976.12028.
Miransari, M., 2011. Soil microbes and plant fertilization. Appl. Microbiol. Biotechnol. 92, 875–885. https://doi.org/10.1007/s00253-011-3521-y.
Monard, C., et al., 2016. Habitat generalists and specialists in microbial communities across a terrestrial-freshwater gradient. Sci. Rep. 6 https://doi.org/10.1038/srep37719.
Moroenyane, I., Mendes, L.W., Tremblay, J., Tripathi, B., Yergeau, É., 2021. Plant compartments and developmental stages modulate the balance between niche-based and neutral processes in soybean microbiome. Microb. Ecol. 82 (2), 416–428.
Nafis, A., et al., 2019. Actinobacteria from Extreme Niches in Morocco and Their Plant Growth-Promoting Potentials. Diversity 8. https://doi.org/10.3390/d11080139.
Nehra, V., Saharan, B.S., Choudhary, M., 2016. Evaluation of Brevibacillus brevis as a potential plant growth-promoting rhizobacteria for cotton (Gossypium hirsutum) crop. SpringerPlus 5, 948.
Nilsson, R.H., et al., 2018. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 47. D259-D264.
Novais, R.F. *et al.*, 1991. Ensaio em ambiente controlado. In: Oliveira, A. J.et al. (Coord.). Métodos de pesquisa em fertilidade do solo. Brasília: EMBRAPA-SEA. 190-253.
Padda, K.P., Puri, A., Chanway, C.P., 2017. Paenibacillus polymyxa: a prominent biofertilizer and biocontrol agent for sustainable agriculture. Agric. Important Microbes Sustain. Agric. v. 2, 165–191. https://doi.org/10.1007/978-981-10-5343-6_6.
Pandit, S.N., Kolasa, J., Cottenie, K., 2009. Contrasts between habitat generalists and specialists: an empirical extension to the basic metacommunity framework. Ecology 90, 2253–2262.
Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: Statistical analysis of taxonomic and functional profiles. Bioinformatics 30, 3123–3124.
Pedrinho, A., et al., 2020. The natural recovery of soil microbial community and nitrogen functions after pasture abandonment in the Amazon region. FEMS Microbiol. Ecol.
Pellegrinetti, T.A., Cunha, I.C.M., Chaves, M.G., Freitas, A.S., Silva, A.V.R., Tsai, S.M., Mendes, L.W., 2023. Draft genome sequences of representative *Paenibacillus polymyxa*, *Bacillus cereus*, *Fictibacillus sp.*, and *Brevibacillus agri* strains isolated from Amazonian dark earth. Microbiol. Resour. Announc. 12, 1–4.
Pellegrinetti, T.A., Cunha, I.C.M., Chaves, M.G., Freitas, A.S., Passos, G.S., Silva, A.V.R., Cotta, S.R., Tsai, S.M., Mendes, L.W., 2024. Genomic insights of *Fictibacillus terranigra* sp. nov., a versatile metabolic bacterium from Amazonian Dark Earths. Braz. J. Microbiol. https://doi.org/10.1007/s42770-024-01268-3.
Pereg, L., McMillan, M., 2015. Scoping the potential uses of beneficial microorganisms for increasing productivity in cotton cropping systems. Soil Biol. Biochem 80, 349–358.
Pfaffl, M.W., Horgan, G.W., Dempfle, L., 2002. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 30 (9), 36. https://doi.org/10.1093/nar/30.9.e36.
Philippot, L., et al., 2013. Going back to the roots: the microbial ecology of the rhizosphere, 2013 Nat. Rev. Microbiol. 11, 789–799. https://doi.org/10.1038/nrmicro3109.
Pickersky, E., et al., 1989. A new member of the CAB gene family: structure, expression and chromosomal location of Cab-8, the tomato gene encoding the Type III chlorophyll a/b-binding polypeptide of photosystem I. Plant Mol. Biol. 12 (3), 257–270. https://doi.org/10.1007/BF00043203.
Pii, Y., et al., 2015. Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. Biol. Fertil. Soils 51. https://doi.org/10.1007/s00374-015-0996-1.
Quast, C., et al., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 (D1), D590–D596.
Raij, B., van., Cantarella, H., Quaggio, J.A., Furlani, A.M.C., 1997. Recomendações de adubação e calagem para o Estado de São Paulo. 2.ed. Campinas. Inst. Agron. ômico De. Camp. 285.
Santoyo, G., et al., 2016. Plant growth-promoting bacterial endophytes. Microbiol. Res. 183, 92–99.
Sasse, J., Martinoia, E., Northen, T., 2018. Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? Trends Plant Sci. 23 (1), 25–41. https://doi.org/10.1016/j.tplants.2017.09.003.
Singh, I., 2018. Plant growth promoting rhizobacteria (PGPR) and their various mechanisms for plant growth enhancement in stressful conditions: a review. Eur. J. Biol. Res. 8, 191–213.
Soares, A.S., et al., 2023. Pseudomonas aeruginosa and Bacillus cereus Isolated from Brazilian Cerrado Soil Act as Phosphate-Solubilizing Bacteria. Curr. Microbiol 80 (5), 146.
Sousa, R.M.S., Mendes, L.W., Antunes, J.E.L., et al., 2020. Diversity and structure of bacterial community in rhizosphere of lima bean. App. Soil Ecol. 150, 103490.
Spencer, B., 1958. Studies on sulphatases: 20. Enzymic leavage of arylhydrogen sulphates in the presence of H2 18O. Biochem. J. 69, 155–159.
Suman, A., et al., 2022. Microbial community and function-based synthetic bioinoculants: a perspective for sustainable agriculture. Front. Microbiol. 12, 805498.
Tabatabai, M.A., 1994. Soil Enzymes. In: Weaver, R. (Ed.), Methods of Soil Analysis: Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, pp. 775–833.
Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307. https://doi.org/10.1016/0038-0717(69)90012-1.
Telles, T.S., Nogueira, M.A., Hungria, M., 2023. Economic value of biological nitrogen fixation in soybean crops in Brazil. Environ. Technol. Innov. 32, 103158 https://doi.org/10.1016/j.eti.2023.103158.
Tipton, L., et al., 2018. Fungi stabilize connectivity in the lung and skin microbial ecosystems. Microbiome 6, 12. https://doi.org/10.1186/s40168-017-0393-0.
Trabelsi, D., Mhamdi, R., 2013. Microbial inoculants and their impact on soil microbial communities: A Review. BioMed. Res. Int. https://doi.org/10.1155/2013/863240.
Trivedi, P., et al., 2020. Plant-microbiome interactions: from community assembly to plant health. Nat. Rev. Microbiol. 18 (11), 607–621. https://doi.org/10.1038/s41579-020-0412-1.
Tsotetsi, T., Nephali, L., Malebe, M., Tugizimana, F., 2022. *Bacillus* for plant growth promotion and stress resilience: what have we learned? Plants 11, 2482.
Untergasser, A., et al., 2021. Web-based LinRegPCR: application for the visualization and analysis of (RT)-qPCR amplification and melting data. BMC Bioinforma. 22, 398. https://doi.org/10.1186/s12859-021-04306-1.
VanInsberghe, D., et al., 2015. Non-symbiotic Bradyrhizobium ecotypes dominate North American forest soils. ISME J. 9, 2435–2441. https://doi.org/10.1038/ismej.2015.54.
Virk, Z.A., Farraj, D.A.A., Iqbal, M., Lewinska, K., Hussain, S., 2022. noculation with the pH Lowering Plant Growth Promoting Bacterium *Bacillus* sp. ZV6 Enhances Ni Phytoextraction by Salix alba from a Ni-Polluted Soil Receiving Effluents from Ni Electroplating Industry. Sustainability 14, 6975. https://doi.org/10.3390/su14126975.
Wang, Y., et al., 1993. Evidence for direct utilization of a siderophore, ferrioxamine B, in axenically grown cucumber. Plant, Cell Environ. 16, 579–585. https://doi.org/10.1111/j.1365-3040.1993.tb00906.x.
Wang, Y., et al., 2022. Evasion of plant immunity by microbial pathogens. Nat. Rev. Microbiol. 20, 449–464. https://doi.org/10.1038/s41579-022-00710-3.

Williams, A., et al., 2022. Root functional traits explain root exudation rate and composition across a range of grassland species. J. Ecol. 110, 21–33.
Wu, D., et al., 2022. Effect of Fenton pretreatment and bacterial inoculation on cellulose-degrading genes and fungal communities during rice straw composting. Sci. Total Environ. 806 https://doi.org/10.1016/j.scitotenv.2021.151376.
Xu, L., et al., 2012. Soil fungal community structure along a soil health gradient in pea fields examined using deep amplicon sequencing. Soil Biol. Biochem. 46, 26–32. https://doi.org/10.1016/j.soilbio.2011.11.010.
Yadav, A.N., et al., 2017. Plant growth promoting bacteria: Biodiversity and multifunctional attributes for sustainable agriculture. Adv. Biotechnol. Microbiol. https://doi.org/10.19080/AIBM.2017.05.555671.
Yang, A., et al., 2018. Characterization and antifungal activity against Pestalotiopsis of a fusaricidin-type compound produced by Paenibacillus polymyxa Y-1. Pestic. Biochem. Physiol. 147, 67–74. https://doi.org/10.1016/j.pestbp.2017.08.012.
Yang, J., Kloepper, J.W., Ryu, C.M., 2009. Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci. 14, 1–4.
Yoon, J.H., et al., 1999. Nocardioides nitrophenolicus sp. nov., a p-nitrophenol-degrading bacterium. Int. J. Syst. Evolut. Microbiol. 49 (2) https://doi.org/10.1099/00207713-49-2-675.
Yu, Y., Lee, C., Kim, J., Hwang, S., 2005. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol. Bioeng. 289, 670–679. https://doi.org/10.1002/bit.20347.
Zamioudis, C., Pieterse, C.M., 2012. Modulation of host immunity by beneficial microbes. Mol. Plant-Microbe Interact. 5 (2), 139–150. https://doi.org/10.1094/MPMI-06-11-0179.
Zhang, H., et al., 2009. A soil bacterium regulates plant acquisition of iron via deficiency-inducible mechanisms. Plant J. 58, 568–577. https://doi.org/10.1111/j.1365-313X.2009.03803.x.
Zhang, J., et al., 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinforma. (Oxf., Engl.) 30 (5), 614–620. https://doi.org/10.1093/bioinformatics/btt59.
Zhang, X., et al., 2019. Biochar-based organic fertilizer application rates for Tetrastigma hemsleyanum planted under Moso bamboo. J. For. Res. 31 (4) https://doi.org/10.1007/s11676-019-00965-2.
Zheng, Z., Qamar, S.A., Chen, Z., Mengiste, T., 2006. Arabidopsis WRKY33 transcription factor is required for resistance to necrotrophic fungal pathogens. Plant J. 2006 (48), 592–605. https://doi.org/10.1111/j.1365-313X.2006.02901.x.