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Biological inoculants and chemical fertilizers application produce differential effects on rhizobacterial community structure associated to peanut (*Arachis hypogaea* L.) and maize (*Zea mays* L.) plants

María Soledad Anzuay[1] · Natalia Pin Viso[2] · Liliana Mercedes Ludueña[1] · Federico Daniel Morla[1] · Romina Yanet Dalmasso[1] · Jorge Guillermo Angelini[1] · Tania Taurian[1]

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Abstract
The use of biological inoculants in replacement of the application of chemical fertilizers is a desirable strategy taking into account it is more sustainable and economically less costly. Considering that agricultural practices can produce effects on soil microbial communities associated to the plant crops, the objective of this study was to analyze and compare the effect of these two practices on the structure of the rhizobacterial community of peanut and maize plants. For this purpose, microcosm assays were performed in which peanut and maize plants were inoculated individually with native peanut phosphate solubilizing strains or chemical fertilized with phosphorus, nitrogen, zinc and sulphur. At the beginning and at the end of the assays, samples of rhizospheric soil DNA were obtained and the structure of the rhizospheric bacterial community was analyzed by high-throughput sequencing of the 16S rRNA gene by using Illumina MiSeq platform. The results obtained indicated that the structures of the rhizospheric bacterial communities were different depending on plant type. It was possible to observe changes with respect to the initial bacterial structure in all taxonomic levels analyzed of all treatments. The more notorious structural changes of bacterial community were observed in those rhizospheres exposed to chemical fertilizers, mainly in soil samples associated to maize plants. The rhizospheric bacterial community of peanut showed to change mainly with plant growth. In conclusion, the rhizobacterial community structure is highly dynamic and influenced by different factors such as type of plant, the fertilizer input and bio-inoculant applied.

Keywords Rhizobacterial community · Bacterial inoculation · Fertilizers · Peanut · Maize · Illumina MiSeq platform

1 Introduction

The constant increase in world population generates a challenge at the level of food production. Early agricultural revolutions in industrialized countries primarily involved expansion of an agricultural area to increase aggregate food production. Such extensification was later followed by intensified use of resources on the same land causing deterioration of soil health (McBratney et al. 2014; Pretty and Bharucha 2014). Agricultural intensification has greatly increased soil nutrient demand of crops which has led to an increase in the application of

✉ Tania Taurian
ttaurian@exa.unrc.edu.ar

María Soledad Anzuay
manzuay@exa.unrc.edu.ar

Natalia Pin Viso
pinviso.natalia@inta.gob.ar

Liliana Mercedes Ludueña
lluduena@exa.unrc.edu.ar

Federico Daniel Morla
fmorla@ayv.unrc.edu.ar

Romina Yanet Dalmasso
rdalmasso@exa.unrc.edu.ar

Jorge Guillermo Angelini
jangelini@exa.unrc.edu.ar

[1] Instituto de Investigaciones Agrobiotecnológicas (CONICET-UNRC), Río Cuarto, Argentina

[2] Instituto de Agrobiotecnología y Biología Molecular, IABiMo, INTA-CONICET, Hurlingham, Buenos Aires, Argentina

synthetic fertilizers (Fertilizar 2020). Nitrogen (N), phosphorus (P) and potassium (K) fertilization replenishes some of the nutrients removed by intensive production (Jones et al. 2013). Nevertheless, the use of fertilizers and other agrochemicals is not always the most effective and healthier strategy. A percentage of the fertilizers applied is precipitated and is converted in potential soil pollutants (Sainju et al. 2019). A healthier strategy in replacement of this agricultural practice is the use of soil bacteria with plant beneficial traits designated as plant growth promoting bacteria (PGPB) (Olanrewaju et al. 2017). The plant growth promotion by this group of microorganisms may be attributed by several mechanisms such as nitrogen-fixing, phosphate-solubilizing, hormone production and biological control (Santoyo et al. 2016). Along with the enhancement of plant productivity and yield, the application of biological inoculants with PGPB can allow for significant reductions in the application of chemical fertilizers and pesticides (Singh et al. 2016; Vicario et al. 2016; Timmusk et al. 2017). When selecting PGP bacteria, those that are native of the agricultural soil in which they are going to be applied is an additional benefit since they are better adapted to the ecological soil environment (Trabelsi et al. 2011).

The microbial soil community structure can be affected by a wide range of factors including environmental factors, soil types, plant species and phenological stage, agricultural management regime, inorganic fertilizer application, bacterial inoculation (Sessitsch et al. 2012; Trabelsi and Mhamdi 2013; Ma et al. 2018; Anzuay et al. 2021). The most significant studies that reported the effect of biological inoculants and fertilization on soil microbial communities has been assessed by a range of different techniques. The sequencing of amplicons in 16S rRNA gene hypervariable regions using next-generation sequencing technologies is currently considered to be one of the best approaches for describing microbial communities (Tringe and Hugenholtz 2008; Caporaso et al. 2010).

As mentioned, many soils of the planet have decreased their fertility due the intensive practices and those of the agricultural area of Argentina are not exempt (Sainz Rozas et al. 2013; Agrovoz 2015). One of the main crops of this country is peanut (*Arachis hypogaea* L.) being Argentina one of the world's leading peanut exporters and producers (National Institute of Agricultural Technology 2021). In the peanut cultivating area this legume is rotated mainly with maize (*Zea mays* L.) which is a crop of great economical importance of Argentina since this country is among the four main world maize producers (Food and Agriculture Organization of the United Nations 2018; Pedelini and Monetti 2021). In addition to the importance of these crops in Argentina, peanuts and maize are of great relevance in international food markets (Food and Agriculture Organization of the United Nations 2022).

In these Argentine agricultural soils, values below the critical levels of P were reported for peanut and maize (6.6–8.5 mg kg^{-1}) (Sainz Rozas et al. 2012; Anzuay et al. 2015, 2017). The critical values of P for plants peanut and maize plants are 10 and 12–20 mg kg^{-1}, respectively (Gudelj et al. 2016). Considering the importance of peanut and maize crops and the need to replace the high chemical fertilizer application, it is necessary to deeply analyze new healthier management practices. Within the alternative strategies, the use of native soil PGPB is a friendlier option, not only to increase productivity but also to reduce the disturbances generated by agrochemicals. Nevertheless, this exogenous bacterial application, although a more sustainable solution, would generate an impact on the microbial soil community. Thus, it is important to know the possible effect of the application of biological inoculants on the rhizobacterial community associated to these plants. The objective of this study was to analyze and compare the effect of bacterial inoculation and chemical fertilizer application on the structure of the rhizobacterial community of peanut and maize plants using next-generation sequencing technologies. For this, under microcosm conditions, peanut and maize plants were grown and treated with PGPB or chemical fertilizers and rhizospheric soil samples were taken at the beginning and at the end of the assays to explore the potential bacterial community shifts caused by these agricultural practices.

2 Materials and methods

2.1 Bacteria and culture media

Two native phosphate solubilizing bacteria isolated from nodules of peanut plants cultivated in central and southern region of Córdoba, Argentina (latitude, 32° to 34°, longitude, 63° to 65°) were employed in this study: *Enterobacter* sp. J49 and *Serratia* sp. J260 (Taurian et al. 2010; Anzuay et al. 2013). In addition to tricalcium phosphate solubilizing ability, these native strains presented also the ability to solubilize other P insoluble sources such as Fe- and Al-P, phosphatase activity and siderophore production (Anzuay et al. 2017). *Bradyrhizobium* sp. SEMIA 6144, recommended as peanut microsymbiont inoculant for Instituto de Pesquisas Agronómicas, IPAGRO, was used on peanut plants. Nodumax®Azo (Laboratorio López S.R.L.) is a commercial inoculant based on the diazotrophic microorganism *Azospirillum brasilense* and RIZOFOS® (RIZOBACTER) that contained the phosphate solubilizing bacteria *Pseudomonas fluorescens* PMT1 were the commercial inoculants used on maize plants. The phosphate solubilizing strains and *A. brasilense* were grown on LB (Luria–Bertani) (Miller 1972)

or TY (tryptone yeast) (Beringer 1974) media at 28 °C. *Bradyrhizobium* sp. SEMIA 6144 was grown in YEMA (yeast extract mannitol agar) (Vincent 1970) at 28 °C. All the bacteria were maintained in 20% glycerol (v/v) at − 80◦C in the respective medium.

2.2 Microcosm assays

Seeds of peanut (cv. granoleico) or maize (NK 910 TD Max hybrid, Syngenta) were superficially disinfected according to Taurian et al. (2002) and Pereira et al. (2011), respectively and were inoculated with bacteria when required. Subsequently, 1 seed per pot was placed on plastic pots (35 cm diameter, 40 cm height) containing approximately 7 kg of unsterilized sieved soil per pot. The soil used as plant growth was obtained from the peanut cultivation area of Córdoba (organic matter: 1.92% (Walkley Black method), pH: 7.3 (Potenciometry 1:2.5), moisture: 14.71% (100–105 °C), N: 12.9 $\mu g g^{-1}$soil (phenolsulfonic acid) and P: 13.6 $\mu g g^{-1}$ (Kurtz and Bray I method)). The following treatments were used in peanut microcosm assays: (1) seeds inoculated with *Enterobacter* sp. J49 (P_IJ49); (2) seeds inoculated with *Serratia* sp. J260 (P_IJ260); (3) seeds inoculated with *Bradyrhizobium* sp. SEMIA 6144 (P_IB); (4) uninoculated seeds grown on fertilized soil (P_Fert); (5) uninoculated and unfertilized seeds (P_C). For maize microcosm assays the treatments used were: (I) seeds inoculated with *Enterobacter* sp. J49 (M_IJ49); (II) seeds inoculated with *Serratia* sp. J260 (M_IJ260); (III) seeds inoculated with Nodumax® Azo maíz and RIZOFOS® (M_IP.A); (IV) uninoculated seeds grown on fertilized soil (M_Fert); (V) uninoculated and unfertilized seeds (M_C). In fertilized treatments, MicroEssentials® SZ ™ (Mosaic) granules (12% Nitrogen, 40% Phosphorous Pentoxide, 10% Sulfur and 1% Zinc) was used in the recommended doses.

For preparation of bacterial inoculum, native bacteria were grown in TY liquid medium until stationary phase and stored at 4 °C until use. The number of viable cells, colony forming units (CFU) ml^{-1} was determined following the method described by Somasegaran and Hoben (1994). Bacterial inoculants prepared with the native strains were mixed with the disinfected seeds and a 20% sucrose solution was used as adherent. On the other hand, the commercial inoculants were prepared according to the technical specifications of the maker and disinfected seeds were mixed with the commercial adherent. The initial doses of the bacterial inoculum were 10^9 CFU ml^{-1} at the time of being placed in the seeds, being 10^8 CFU ml^{-1} the number of bacterial cells adhered to them.

Peanut and maize plants were maintained under controlled environmental conditions (light intensity of 200 $\mu R.m^{-2}\ s^{-1}$ 16 h day/8 h night cycle, at a constant temperature of 28 °C and a relative humidity of 50%) and watered regularly with tap water. Rhizospheric soil samples were obtained at the beginning of the assays (designated as ES-experimental soil) and at plants harvest. Peanut and maize were harvested at 120 and 100 days postinoculation, respectively. Approximately 5 g of rhizospheric soil was sampled of each pot and at harvest plants were removed. Two independent microcosm assays were performed (n = 8–10).

2.3 Analysis of rhizobacterial community structure of peanut and maize plants using next-generation sequencing technologies from soil samples

Rhizobacterial community structure, using Illumina MiSeq sequencing of 16S rRNA gene, of rhizospheric soil DNA associated to peanut and maize plants was analyzed. For this, four to five rhizospheric soil samples from each treatment were collected, pooled and homogenized. Two replicate DNA extractions were used per treatment on both microcosm assays.

2.3.1 Soil DNA extraction

Soil DNA was extracted using the commercial Ultra Clean soil DNA isolation kit (MoBio). DNA quantity and quality were verified by spectrophotometry (Nanodrop, ThermoScientific).

2.3.2 High-throughput sequencing of 16S rRNA gene and data processing

The V3-V4 hypervariable region of 16S rRNA gene was amplified from microbial genomic DNA using universal primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The following PCR conditions were used: 94 °C for 3 min followed by 35 cycles of 94 °C for 15 s, 55 °C for 45 s, and 72 °C for 1 min, followed by a final elongation step of 8 min at 72 °C. Library construction and high-throughput sequencing were performed at Macrogen Inc. (Seoul, South Korea) on an Illumina MiSeq platform and 2 bp/300 bp paired-end reads were generated. The datasets generated in this study are available under request.

The initial processing and quality control of the paired-end reads was performed using Trimmomatic v0.33 (Bolger et al. 2014), which remove all primers and adapters sequences and perform a quality filtering allowing a quality Phred cut-off score of at least Q20. FLASH was used to merge the pairs of reads from the original DNA fragments (Magoč and Salzberg 2011). Sequencing reads were assigned to each sample according to the unique barcode of each sample. Sequences were analyzed with the QIIME software package (Quantitative Insights Into Microbial Ecology)

(Caporaso et al. 2010). Briefly, chimeric sequences were filtered out using USERCH algorithm and those remaining sequences with ≥ 97% similarity were assigned to the same operational taxonomic units (OTUs). Singletons and OTUs with abundance below 0.005% were removed from the final OTU table, to avoid microbial diversity overestimation (Bokulich et al. 2013). A representative sequence for each OTU was picked and the SILVA v.128 database was used to obtain the taxonomic information.

Normalization of OTU counts was carried out by performing multiple rarefactions from 1000 to 47,300 sequences with steps of 1000 and 10 repetitions at each rarefaction depth. The distributions of the OTUs obtained were visualized using the average of the replicates in a hierarchically clustered heatmap implemented in R. Venn diagram was done to show the relationships between different communities associated to peanut and maize plants in all treatments analyzed. The observed species richness was defined as the number of OTUs present in each sample. Also, the Chao 1 (Chao 1984) and Shannon (Margelef 1958) indices, for analyzed richness and diversity of the communities respectively, were calculated using QIIME. The Good's coverage index, which is considered a relative measure of how well the sequences obtained represent the entire populations was calculated using QIIME.

2.4 Abundance of culturable rhizobacterial populations

The abundances of culturable heterotrophic bacteria (CHB), diazotrophic bacteria (DB) and phosphate solubilizing bacteria (PSB) at beginning and the end of the assays were determined. For this, soils and rhizospheric samples from all treatments were collected. Three replicates per treatment were used to analyze the culturable diversity. Each sample (0.5 g) was placed into flasks containing 4.5 ml of 0.1% sterile sodium pyrophosphate (NaPP) and mixed for 30 min at 180 rpm. For CHB, DB and PSB count, 0.1 ml aliquots of serial dilutions (up to 10^{-8}) were streaked onto Petri dishes containing tenfold diluted tryptic soy agar ($1{10}^{-1}$ TSA) (Smit et al. 2001), nitrogen-free bromothymol blue malate medium (NFb) (Döebereiner 1995) and National Botanical Research Institute's phosphate grown medium with bromophenol blue (NBRIP-BPB) (Mehta and Nautiyal 2001), respectively. Media were supplemented with 2.6-dicloro-4-nitroanilina (dicloran50 μg ml^{-1}) to prevent fungal growth. Plates were incubated at 28 °C during seven days and CFU ml^{-1} from rhizospheric soil were counted. Individual colonies grown on plates containing NFb solid medium were inoculated into assay tubes containing semisolid NFb medium. Pellicle formation under the medium surface was indicative of growth of free living nitrogen fixing bacteria. On the other hand, in NBRIP-BPB solid medium, the halo of clearance around the bacterial colony, evidenced by acidification of the medium (turn to yellow), indicated solubilizing ability.

2.5 Determination of growth parameters, chlorophyll, P and N content of peanut and maize plants

The growth, chlorophyll and P and N content of peanut and maize plants were analyzed at the end of the microcosm assays. The following growth parameters were determined: aerial length (cm), root length (cm), aerial dry weight (g $plant^{-1}$) and root dry weight (g $plant^{-1}$). Chlorophyll content was determined by following Cassol et al. (2008). P and N content of aerial tissues (mg g $plant^{-1}$) were determined following the procedure of Jackson with modifications (1973) and Nelson and Sommers with modifications (1973), respectively. The presence and number of nodules and boxes were determined in peanut plants.

2.6 Data analysis

The statistical analyses were performed using Infostat software (Balzarini et al. 2008). Principal component analysis (PCA) was performed and the results of this analysis are presented as biplot graphs. Data obtained from the determination of abundance of culturable rhizobacterial populations and plants growth and content of chlorophyll, P and N were subjected to one-way analysis of variance (ANOVA) and differences among treatments were detected by LSD test ($P < 0.05$).

3 Results

3.1 Rhizospheric bacterial communities associated to peanut and maize show the most structural changes during plant growth and with fertilized treatment

The high-throughput sequencing analysis indicated a total of 6,803,162 reads obtained from a total of 22 samples, with an average of 309,235 ± 26,926.05 reads per sample, and a minimum and maximum range of 240,560 and 363,926 reads, respectively. After quality filtering, merge, and removal of chimeric reads, the number of bacterial OTUs detected ranged from 2723 to 2968 (Table 1). In all communities analyzed it was possible to observe a Good's coverage index greater than 99%, indicating that the analyzed sequences covered almost the entire diversity of bacterial populations (Fig. S1-supplementary material). Alpha diversity indexes of soil samples are shown in Table 1. The highest richness value (Chao 1 index and observed OTUs) was detected in the ES sample and the lowest corresponded to M_Fert

Table 1 Alpha diversity indices of rhizobacterial communities associated with peanut and maize plants with different treatments analyzed

Treatments	OTU_S	Good's coverage	Chao 1	Shannon
ES	2968 ± 9^a	0.9940 ± 0.0003^a	3079 ± 13^a	10.350 ± 0.020^a
P_IJ49	2954 ± 1^a	0.9960 ± 0.0010^a	3020 ± 19^a	10.370 ± 0.020^a
P_IJ260	2937 ± 15^a	0.9950 ± 0.0010^a	3029 ± 52^a	10.280 ± 0.003^a
P_IB	2902 ± 30^a	0.9950 ± 0.0010^a	2982 ± 28^a	10.300 ± 0.084^a
P_FERT	2931 ± 13^a	0.9950 ± 0.0010^a	3011 ± 7^a	10.328 ± 0.062^a
P_C	2928 ± 16^a	0.9960 ± 0.0010^a	2997 ± 37^a	10.293 ± 0.036^a
M_IJ49	2857 ± 37^a	0.9970 ± 0.0010^a	2930 ± 21^a	10.176 ± 0.057^a
M_IJ260	2918 ± 11^a	0.9980 ± 0.0001^a	2958 ± 8^a	10.275 ± 0.018^a
M_P.A	2893 ± 2^a	0.9970 ± 0.0010^a	2950 ± 20^a	10.369 ± 0.052^a
M_FERT	2723 ± 153^a	0.9960 ± 0.0010^a	2837 ± 100^a	9.927 ± 0.237^a
M_C	2864 ± 57^a	0.9970 ± 0.0010^a	2922 ± 72^a	10.078 ± 0.106^a

Mean ± SD are showing. Different letters indicate significant differences among samples according to Kruskal Wallis with Mann–Whitney post-hoc test ($P < 0.05$) and Bonferroni correction. ES experimental soil, P_IJ49 seeds of peanut inoculated with *Enterobacter* sp. J49, P_IJ260 seeds of peanut inoculated with *Serratia* sp. J260, P_IB seeds of peanut inoculated with *Bradyrhizobium* sp. SEMIA 6144, P_Fert uninoculated seeds of peanut grown on fertilized soil, P_C uninoculated and unfertilized seeds of peanut, M_IJ49 seeds of maize inoculated with *Enterobacter* sp. J49, M_IJ260 seeds of maize inoculated with *Serratia* sp. J260, M_IP.A seeds of maize inoculated with Nodumax® Azo maíz and RIZOFOS®, M_Fert uninoculated seeds of maize grown on fertilized soil, M_C uninoculated and unfertilized seeds of maize

treatment. Shannon diversity index showed the highest value on the samples of bacterial rhizospheric community of treatment P_IJ49 and the lowest corresponded to that of treatment M_Fert.

The community structure showed the presence of 10 phyla with more than 1% of relative abundance in all the samples, comprising 96 classes (18 > 1%), 161 orders (33 > 1%), 248 families (41 > 1%) and 351 genera (38 > 1%) (data not shown). The most abundant bacterial phyla across all treatments were Proteobacteria, Acidobacteria and Actinobacteria (Fig. 1A, B). No statistically significant differences could be found in the relative abundance of phyla present between treatments. Notable trends were observed in the rhizobacterial communities of both plants with respect to ES (Fig. S2 and S3-supplementary material). In the rhizobacterial communities associated to peanut, all treatments and control samples showed an increase in the relative abundances of Actinobacteria and Chloroflexi, and a decrease in the relative abundances of Bacteroidetes, Gemmatimonadetes, Nitrospirae, β-, δ-, and γ-Proteobacteria (Fig. S2-supplementary material).

On the other hand, the rhizobacterial communities of treatment M_Fert and M_IP.A showed variations in the relative abundances in several of the analyzed phyla with respect to ES (Fig. S3-supplementary material). M_Fert treatment produced an increase in the relative abundances of Planctomycetes and α-Proteobacteria, and a decrease in Acidobacteria, Gemmatimonadetes, Nitrospirae, β-, δ-, and γ-Proteobacteria, respect to ES. In rhizospheric samples of M_IP.A treatment, an increase in Bacteroidetes was observed with respect to ES, while a decrease in Gemmatimonadetes, Nitrospirae, Planctomycetes, and α-Proteobacteria was detected.

A cluster analysis, based on the family level, showed that the relative abundance of the families analyzed could be split in two clusters (Fig. 2). One cluster showed all treatments associated to maize plant and ES, and the other cluster contained all treatments associated to peanut plant. Both fertilized treatments, P_Fert and M_Fert, showed the greatest differences within each group. In addition, the most abundant families found in peanut and maize rhizosphere were Sphingomonadacea, Chitinophagaceae, Hyphomicrobiaceae, and one unclassified family belonging to the Acidimicrobiales order.

The communities associated to peanut and the M_Fert treatment showed an increase in the relative abundance of the families Microccacea, Gaiellacea, Rhizobiaceae and Bacillaceae, with respect to ES. On the contrary, a decrease in the bacterial families Xanthomonadacea and Comamonadaceae was detected in these treatments respect to the initial sample ES. Furthermore, all peanut samples showed an increase in the relative abundance of Bradyrhizobiaceae and Streptomitaceae families. Moreover, these rhizobacterial communities presented a decrease in the relative abundance of the Chitinophagaceae and Sphingomonadacea families. Within maize samples, it was possible to observe that M_IJ260 treatment produced an increase in the relative abundance of the Xanthomonadacea family, with respect to ES and M_IJ49 treatment an increase on the relative abundance of members of the Pseudomonadacea and Sphingomonadacea families.

Venn diagram indicated that the rhizospheric bacterial communities of each treatment share 236 bacterial families

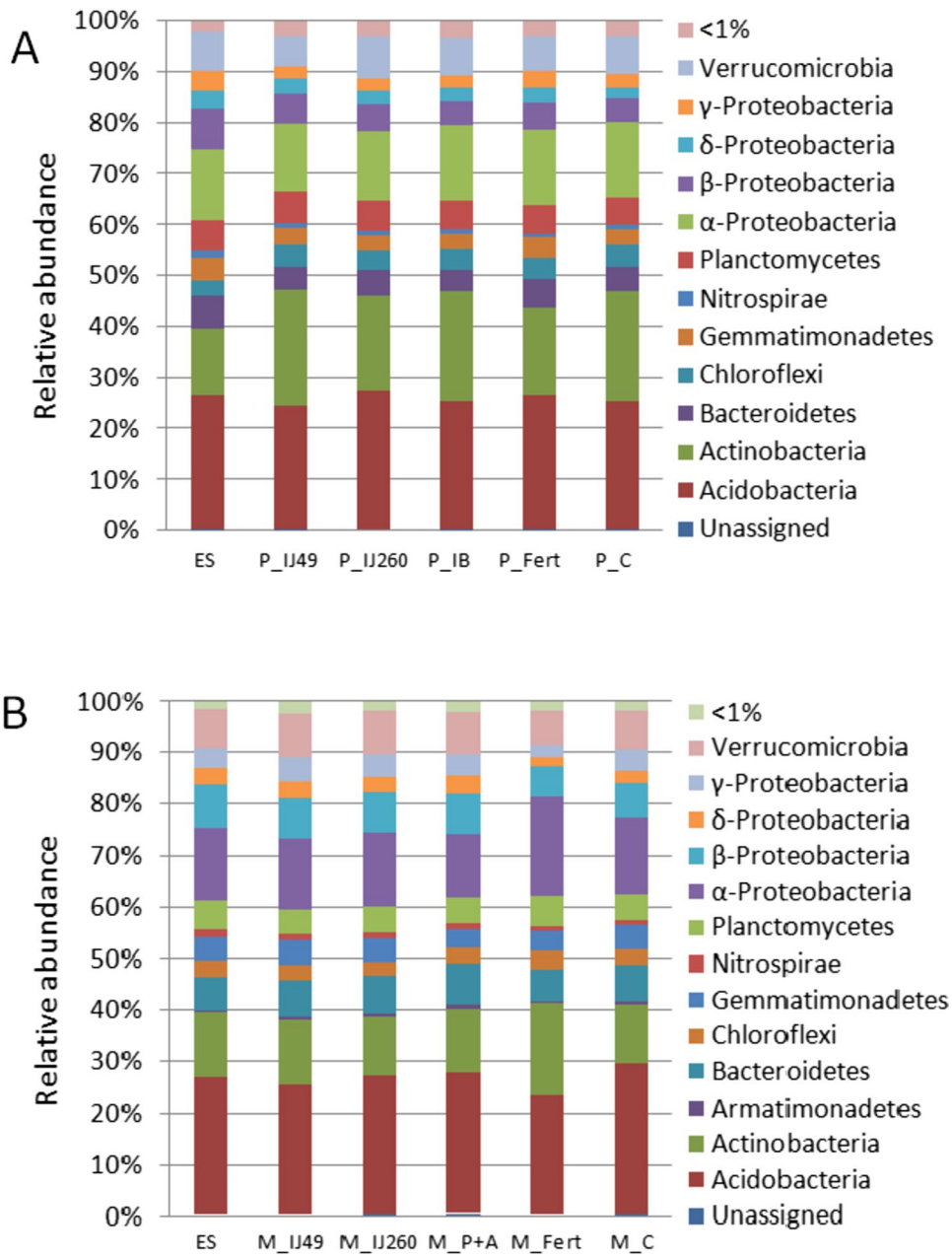


Fig. 1 Rhizobacterial communities structure associated to peanut and maize plants from different treatments. Stacked bar graph represents the relative abundance of the most abundant phyla. ES: experimental soil, P_IJ49: seeds of peanut inoculated with *Enterobacter* sp. J49, P_IJ260: seeds of peanut inoculated with *Serratia* sp. J260, P_IB: seeds of peanut inoculated with *Bradyrhizobium* sp. SEMIA 6144, P_Fert: uninoculated seeds of peanut grown on fertilized soil, P_C: uninoculated and unfertilized seeds of peanut, M_IJ49: seeds of maize inoculated with *Enterobacter* sp. J49, M_IJ260: seeds of maize inoculated with *Serratia* sp. J260, M_IP.A: seeds of maize inoculated with Nodumax® Azo maíz and RIZOFOS®, M_Fert: uninoculated seeds of maize grown on fertilized soil, M_C: uninoculated and unfertilized seeds of maize

in peanut rhizospheric samples (Fig. 3). In these communities, the P_C and P_IJ49 treatments presented one unshared OTUs for each treatment, been classified as a family of the Bacteroidetes phylum and a member of the Clostridiaceae family, respectively. In maize rhizosphere samples, Venn diagram showed that all samples shared 235 bacterial families. The M_Fert treatment community was the only one that presented bacteria belonging to the Brevibacterium family (Fig. 3).

When the analysis of the rhizospheric bacterial communities of peanut and maize plants were analyzed at genus level, the results indicated that samples of all treatments of both plants presented many genera associated with beneficial mechanisms of plant growth promotion (Fig. 4). The most abundant genera detected in all rhizobacterial communities of both plants were *Rhodoplanes* and *Kaistobacter*. The results indicate that the P_IJ260 treatment showed a slight increase in the genera *Bacillus, Rhizobium,* and *Bradyrhizobium*, with respect to ES. On the other hand, the members of this genus presented an increase in all samples associated with peanuts samples. On the other hand, the results indicated an increase in the abundance of the *Pseudomonas* genus in maize rhizospheric samples, mainly in the M_IJ49 treatment, and a decrease in samples associated with the M_Fert treatment.

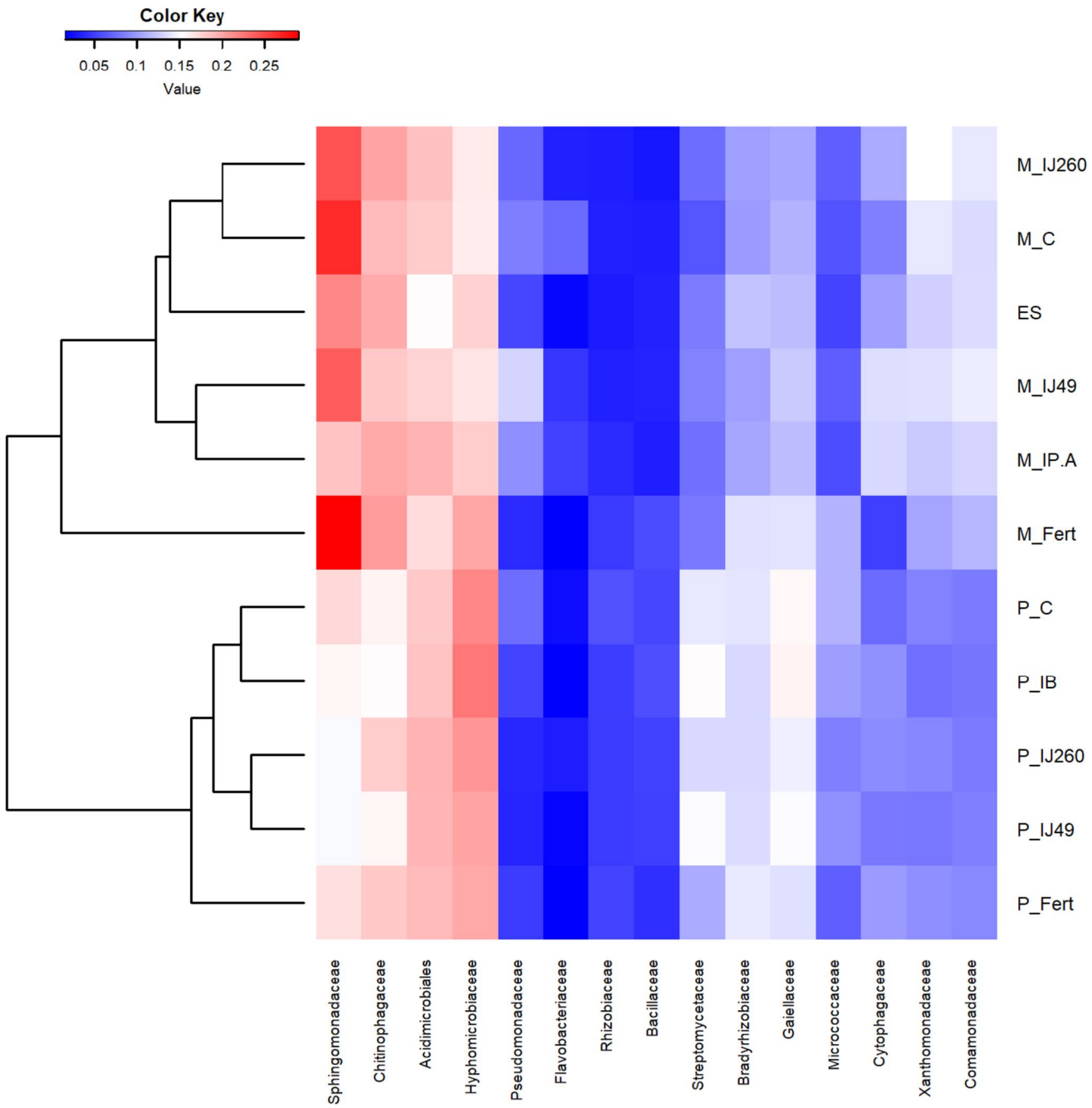


Fig. 2 Hierarchically clustered heatmap of bacterial distribution of different communities associated to peanut and maize plants from different treatments at the family level. Row represents different samples and column indicates the abundance of each bacterial group. The relative abundance for each bacterial family was depicted by color intensity with the legend indicated at the top of the figure. ES: experimental soil, P_IJ49: seeds of peanut inoculated with *Enterobacter* sp. J49, P_IJ260: seeds of peanut inoculated with *Serratia* sp. J260, P_IB: seeds of peanut inoculated with *Bradyrhizobium* sp. SEMIA 6144, P_Fert: uninoculated seeds of peanut grown on fertilized soil, P_C: uninoculated and unfertilized seeds of peanut, M_IJ49: seeds of maize inoculated with *Enterobacter* sp. J49, M_IJ260: seeds of maize inoculated with *Serratia* sp. J260, M_IP.A: seeds of maize inoculated with Nodumax® Azo maíz and RIZOFOS®, M_Fert: uninoculated seeds of maize grown on fertilized soil, M_C: uninoculated and unfertilized seeds of maize

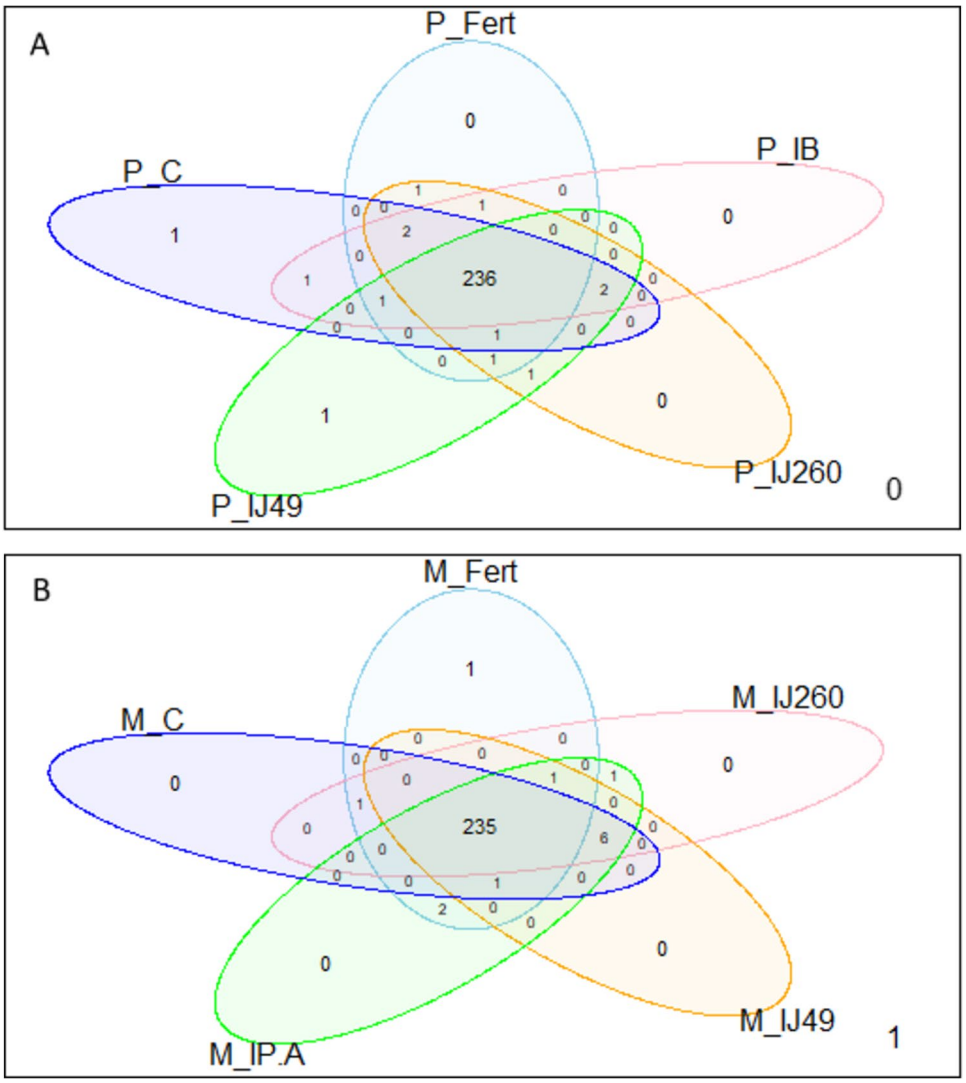


Fig. 3 Venn diagram showing relationships between different communities associated to peanut (**A**) and maize (**B**) plants from different treatments at family level. ES: experimental soil, P_IJ49: seeds of peanut inoculated with *Enterobacter* sp. J49, P_IJ260: seeds of peanut inoculated with *Serratia* sp. J260, P_IB:seeds of peanut inoculated with *Bradyrhizobium* sp. SEMIA 6144, P_Fert: uninoculated seeds of peanut grown on fertilized soil, P_C: uninoculated and unfertilized seeds of peanut, M_IJ49: seeds of maize inoculated with *Enterobacter* sp. J49, M_IJ260: seeds of maize inoculated with *Serratia* sp. J260, M_IP.A: seeds of maize inoculated with Nodumax® Azo maíz and RIZOFOS®, M_Fert: uninoculated seeds of maize grown on fertilized soil, M_C: uninoculated and unfertilized seeds of maize

3.2 Culturable rhizobacteria associated to peanut and maize plants are influenced by bacterial inoculation and fertilizer management

In general, the analysis of the abundance of culturable CHB, DB and PSB indicated an increase in the two former groups with the inoculation of native strain *Enterobacter* sp. J49 and the fertilization treatment in peanut plants. In particular, the results obtained from peanut microcosm assays indicated that the abundance of culturable heterotrophic bacteria (CHB) in rhizosphere presented a significant increase with the treatments P_IJ49 and P_Fert, with respect to the number detected in ES (Fig. S4A-supplementary material). These same treatments and P_C showed to produce a significant increase in the number of diazotrophic bacteria (DB) (Fig. S4B-supplementary material). All treatments significantly enhanced the abundance of phosphate solubilizing bacteria (PSB), respect to ES (Fig. S4C-supplementary material).

The analysis of the maize rhizospheric communities, indicated that the abundance of CHB and PSB showed a significant increase in all treatments, respect to ES (Fig. S4D and F-supplementary material). With exception of the control, M_C, the abundance of DB significantly enhanced in all treatments compared to ES (Fig. S4E-supplementary material).

3.3 Bacterial inoculation produced the most significative increases on growth, chlorophyll, P and N content of peanut plants

Growth parameters of peanut plants inoculated with either native strains or commercial strains increased at least one of the plant growth parameters analyzed with respect to uninoculated plants (Fig. S5-supplementary material). In general, the results indicated that plants inoculated with the native PSB *Enterobacter* sp. J49 showed the best grow parameters chlorophyll and N and P content. The aerial and

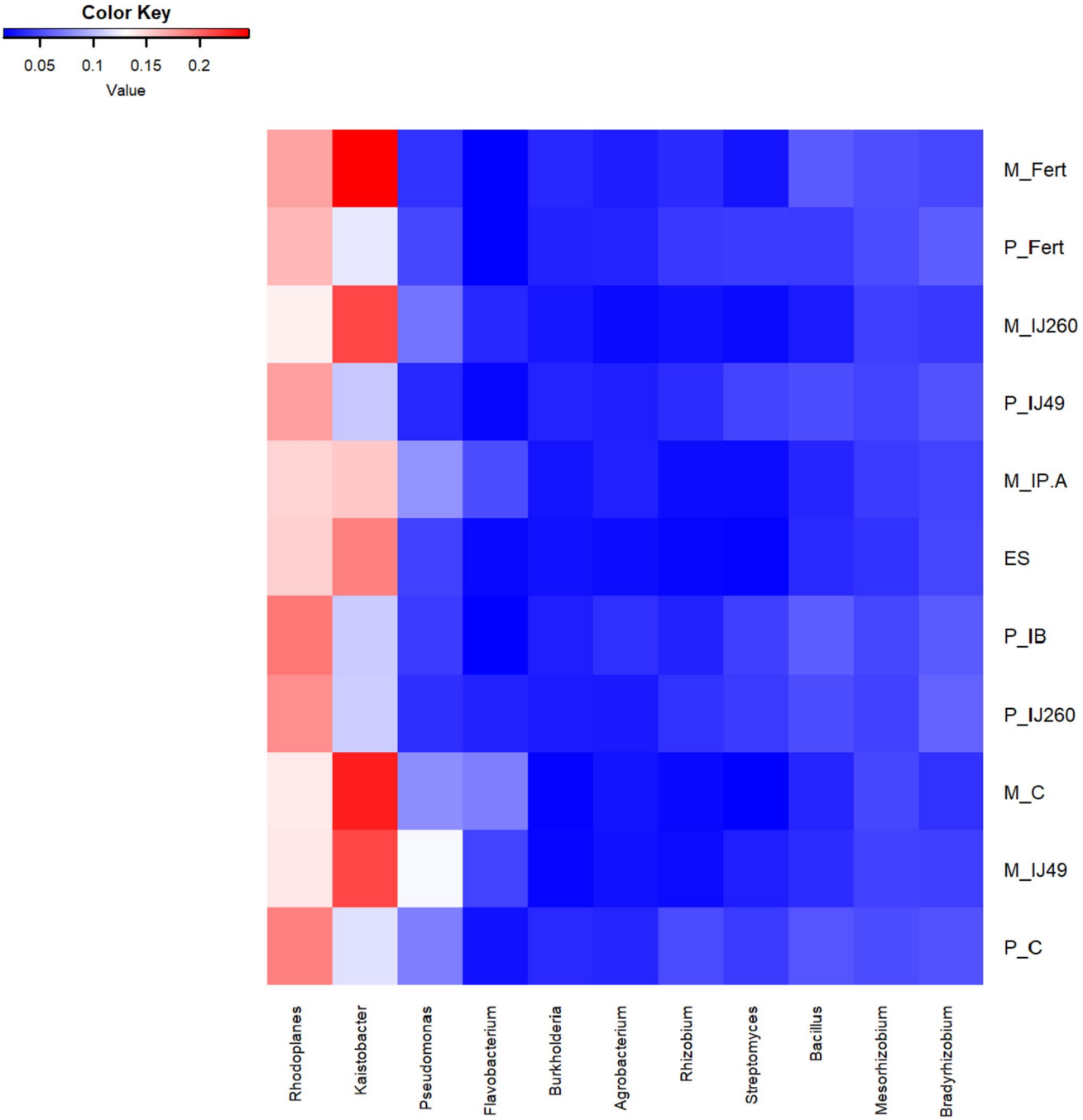
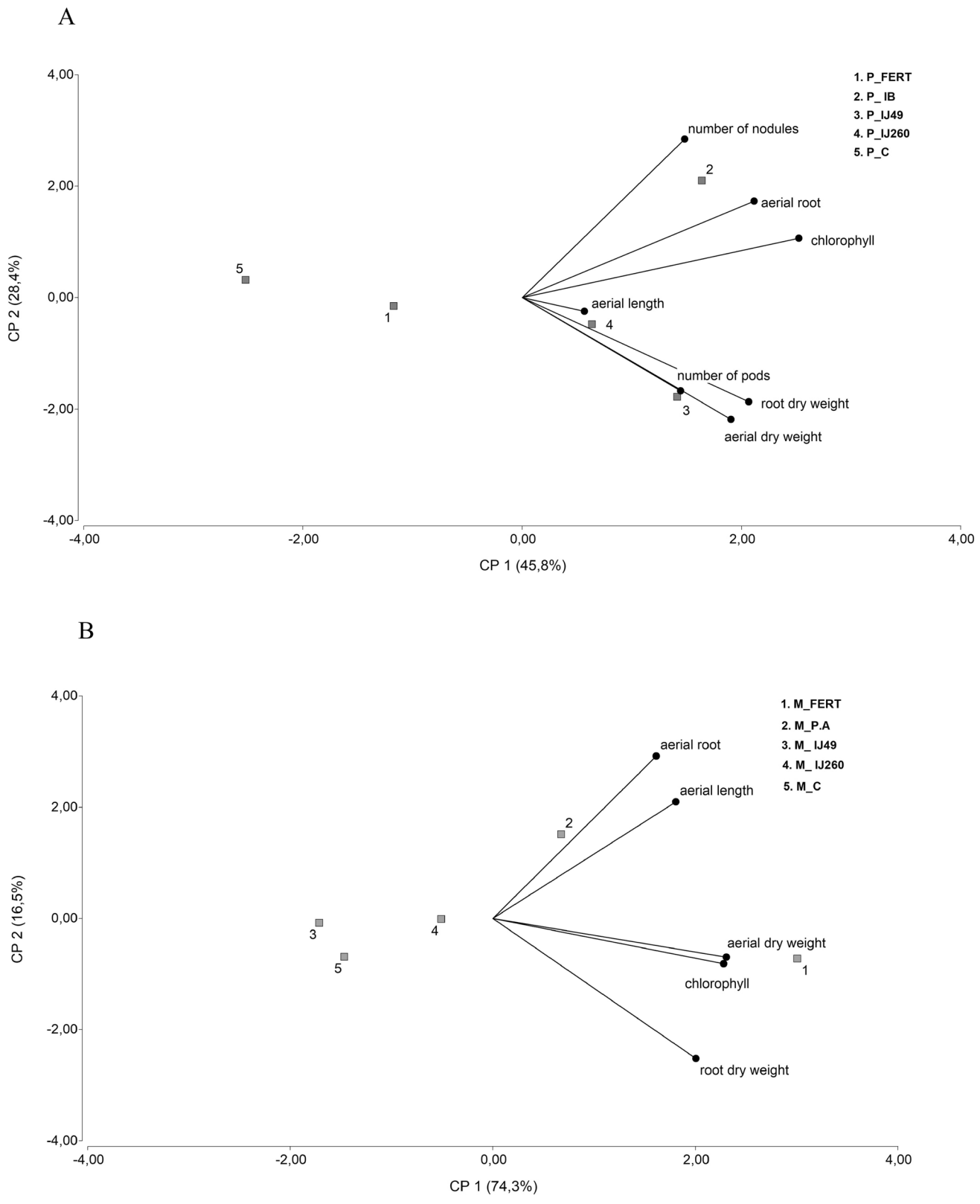


Fig. 4 Hierarchically clustered heatmap of bacterial distribution of different communities associated to peanut and maize plants from different treatments at the genera level. Row represents different soil samples and column indicates the abundance of each bacterial group. The relative abundance for each bacterial genus was depicted by color intensity with the legend indicated at the top of the figure.ES: experimental soil, P_IJ49: seeds of peanut inoculated with *Enterobacter* sp. J49, P_IJ260: seeds of peanut inoculated with *Serratia* sp. J260, P_IB: seeds of peanut inoculated with *Bradyrhizobium* sp. SEMIA 6144, P_Fert: uninoculated seeds of peanut grown on fertilized soil, P_C: uninoculated and unfertilized seeds of peanut, M_IJ49: seeds of maize inoculated with *Enterobacter* sp. J49, M_IJ260: seeds of maize inoculated with *Serratia* sp. J260, M_IP.A: seeds of maize inoculated with Nodumax® Azo maíz and RIZOFOS®, M_Fert: uninoculated seeds of maize grown on fertilized soil, M_C: uninoculated and unfertilized seeds of maize

root dry weight of plants inoculated with the native PSB *Enterobacter* sp. J49 showed a significant increase respect to control plants (Fig. S5C and D-supplementary material). Also, the inoculation with *Enterobacter* sp. J49 and *Serratia* sp. J260 and peanut reference strain SEMIA 6144 significantly increased chlorophyll content of peanut plants with



respect to uninoculated plants, while fertilized treatment showed no differences with the control treatment (Fig. S5E-supplementary material). It was possible to observe that number of peanut boxes was enhanced in plants inoculated with native strains *Enterobacter* sp. J49 and *Serratia* sp. J260 and with the treatment P_Fert (Fig. 5F-supplementary

◂**Fig. 5** Biplotdisplay of PCA of the parameters analyzed with the different treatments in peanut plants (**A**) and in maize plants (**B**). Variables analyzed in peanut plants: aerial length, aerial dry weight, root length, root dry weight, chlorophyll content, number of nodules and number of pods. Variables analyzed in maize plants: aerial length, aerial dry weight, root length, root dry weight and chlorophyll content. The sense and size of the vectors indicate the way and weight of each variable respectively to separate the different treatments studied. ES: experimental soil, P_IJ49: seeds of peanut inoculated with *Enterobacter* sp. J49, P_IJ260: seeds of peanut inoculated with *Serratia* sp. J260, P_IB: seeds of peanut inoculated with *Bradyrhizobium* sp. SEMIA 6144, P_Fert: uninoculated seeds of peanut grown on fertilized soil, P_C: uninoculated and unfertilized seeds of peanut, M_IJ49: seeds of maize inoculated with *Enterobacter* sp. J49, M_IJ260: seeds of maize inoculated with *Serratia* sp. J260, M_IP.A: seeds of maize inoculated with Nodumax® Azo maíz and RIZOFOS®, M_Fert: uninoculated seeds of maize grown on fertilized soil, M_C: uninoculated and unfertilized seeds of maize

material) respect to control plants. Aerial P content values of plants indicated that the treatments P_IJ49 and P_Fert significantly increased this nutrient respect to P_C (Table 2). Soil P content increased with the treatment P_Fert and N soil content was significantly enhanced in peanut plants inoculated with the native strain *Serratia* sp. J260 respect to the soil of uninoculated plants. The Principal Component Analysis (PCA) of the 7 variables evaluated on peanut plants indicated that PC1 explained 45.8% of total variance of the assay and PC2 explained 28.4% (Fig. 5A). PC1 showed that the bacterial inoculation with strains *Enterobacter* sp. J49 (treatment P_IJ49), *Serratia* sp. J260 (treatment P_IJ260) and *Bradyrhizobium* sp. SEMIA 6144 (treatment P_IB) clustered together and were separated from treatment P_FERT and control plants (P_C). The treatment P_IB was the most associated with the variables nodule number and root length. Also, the treatment P_IJ49 showed association with the variables pod number and root and aerial dry weight.

3.4 Fertilizer management produced the most significative increases on growth, chlorophyll, P and N content on maize plants

In general, maize plant treated with chemical fertilizer (treatment M_Fert) showed to significantly increase growth and nutrient content. Results obtained from these maize assays indicated that treatments M_IJ49, M_IP.A and M_Fert significantly increased plant root length, with respect to uninoculated plants (Fig. S6B-supplementary material). Also, the results obtained indicated a significant increase in aerial and root dry weight and chlorophyll content at the end of microcosm assays in fertilized maize plants, respect to M_C (Fig. S6C, D, E-supplementary material). Aerial N content of maize plants indicated that the treatments M_IJ49, M_IJ260 and M_Fert significantly increased this nutrient respect to M_C (Table 2). An increase of P content was observed in pots of maize plants with the treatment M_Fert and N content was higher in maize plants inoculated with the native strain *Serratia* sp. J260 respect to uninoculated plants. PCA of the maize plant assays indicated that PC1 explained 74.3% and PC2 the 16.5% of the variance. In general, the treatment M_Fert was associated with the variables aerial and root dry weight and chlorophyll content (Fig. 5B).

4 Discussion

The application of PGPB as a bio-inoculant allows replacing the use of synthesis fertilizers, being this an environmentally sustainable alternative (Khan et al. 2021). Regardless of the agricultural practice employed, it is necessary to analyze the effect of the selected strategy on the structure and functionality of soil microbiota. In this study, we aimed to conduct a comparative analysis between the effect of bacterial inoculation and chemical fertilizer on the composition of the rhizospheric bacterial community associated to peanut and maize plants. We also aimed to compare the effect of the inoculation of native bacteria with respect to commercial bacterial strains, commonly used for these crops. Although there are many studies that analyze the effect of fertilizer application on bacterial community composition of several plants (Gu et al. 2017; Zhou et al. 2017; Ma et al. 2018; Wang et al. 2019), there are scarce reports regarding peanut and maize rhizospheric bacterial communities (Peiffer et al. 2013; Liu et al. 2015). The results obtained in this study show that the analysis of the structure of rhizobacterial communities associated to peanut and maize plants exhibits the characteristic distribution pattern described for agricultural soils (Gu et al. 2017; Anzuay et al. 2021), with the presence of Proteobacteria, Acidobacteria, Actinobacteria, Verrucomicrobia, Bacteroidetes, Chloroflexi, Planctomycetes, Gemmatimonadetes, and Firmicutes as the more abundant phyla (Schulz et al. 2013; Hong et al. 2015). The changes observed in the rhizobacterial community of the samples of peanut plants were similar to those observed in the control peanuts sample, indicating that they are more attributed to the phenological stage of plants rather that the different treatments. On the other hand, in the communities associated with maize, a differential effect associated with the different treatments was observed with respect to ES. Particularly, a notable change in the bacterial structure associated with the chemical fertilization treatment was observed, suggesting that this treatment causes more significant disturbances in the structure of the rhizosphere community associated with maize. Similarly, Li et al. (2017) found differential modulation of the soil microbial community under inorganic fertilization management or under combined organic–inorganic fertilization.

Table 2 Soil characteristics of microcosm assay and aerial P and N content of peanut and maize plants of the treatments analyzed at the end of the assay

Treatments	Soil properties					Plants aerial tissues	
	Available N[1] (µg g suelo^{-1})	Available P[2] (µg g suelo^{-1})	Organic Matter[3] (%)	pH[4]	Moisture[5] (%)	P (mg plant^{-1})	N (mg plant^{-1})
P_IJ49	11.50	12.20	1.95	6.70	6.61	2.68 ± 0.13*	23.5 ± 0.7
P_IJ260	33.51	10.75	1.88	6.80	6.38	2.51 ± 0.12	22.4 ± 1.9
P_IB	24.09	17.50	1.91	6.72	5.96	2.22 ± 0.28	25.8 ± 4.2
P_Fert	18.53	22.30	1.68	6.73	7.97	2.82 ± 0.22*	26.1 ± 1.0
P_C	26.40	15.55	1.72	6.58	7.25	1.98 ± 0.13	20.9 ± 5.1
M_IJ49	12.88	11.20	1.91	6.79	6.61	1.37 ± 0.12	5.48 ± 0.7*
M_IJ260	23.65	10.90	1.76	6.65	6.38	1.29 ± 0.08	6.03 ± 0.5*
M_P.A	14.66	11.60	2.03	6.54	5.96	1.11 ± 0.11	3.92 ± 0.3
M_Fert	20.10	38.45	1.92	6.57	7.97	1.27 ± 0.13	7.77 ± 0.5*
M_C	18.46	10.10	1.76	6.75	7.25	1.48 ± 0.22	3.53 ± 0.5

Data of aerial P and N content are means ± S.E. of 8 replicates, according to LSD-Fisher test ($P < 0.05$). *: indicates statistically significant difference compared to uninoculated plants (control) in each type of plant evaluated

[1]: phenolsulfonic acid; [2]: Kurtz and Bray I method; [3]: Walkley–Black method; [4]: Potenciometry 1:2.5 and [5]: 100–105 ºC

ES experimental soil, P_IJ49 seeds of peanut inoculated with *Enterobacter* sp. J49, P_IJ260 seeds of peanut inoculated with *Serratia* sp. J260, P_IB seeds of peanut inoculated with *Bradyrhizobium* sp. SEMIA 6144, P_Fert uninoculated seeds of peanut grown on fertilized soil, P_C uninoculated and unfertilized seeds of peanut, M_IJ49 seeds of maize inoculated with *Enterobacter* sp. J49, M_IJ260 seeds of maize inoculated with *Serratia* sp. J260, M_IP.A seeds of maize inoculated with Nodumax® Azo maíz and RIZOFOS®, M_Fert uninoculated seeds of maize grown on fertilized soil, M_C uninoculated and unfertilized seeds of maize

Regarding the application of fertilizers, in the present study it was possible to detect that this treatment produced notable effects on the bacterial communities associated to maize and peanut plants at the family level. In particular, it was interesting to note that this treatment increased the growth of the maize plants while showing lower values of richness and diversity compared to the rest of the treatments. In this sense, Celestina et al. (2019) reported that differences in microbial community structure, reduced microbial community richness, and soil diversity should be associated with the direct or indirect effect of fertilization. Additionally, in the rhizosphere of maize plants inoculated with commercial strains (M_IP.A), differential structural changes were found. These changes were also previously described by Saadouli et al. (2021), who reported that inoculation with *P. agglomerans* showed modifications in the overall edaphic bacterial community, significantly impacting its structure and composition. Other studies showed that these effects depend on the strain used, for example Trabelsi et al. (2011) analyzed the effect of field inoculation of rhizobia strains on the soil bacterial structure associated to common bean plants and found that this treatment improved the populations of alpha- and gamma-proteobacteria, along with Firmicutes and Actinobacteria. Conversely, Herschkovitz et al. (2005) observed that the inoculation of *Azospirillum* strains did not show outstanding effects on the population structure of rhizobacterial communities of maize.

The most abundant genera detected in all rhizospheric soil samples belong to the Proteobacteria phylum and within them, those associated with plant growth promoting mechanisms were detected. It was interesting to note that the abundance of bacterial species belonging to the genera *Bacillus* and *Pseudomonas*, which contain species with several and well characterized PGP properties (Gutierrez-Manero et al. 2001; Richardson et al. 2009; Abbasdokht and Gholami 2010; Zachow et al.2017), showed to be affected with some of the analyzed treatments. Thus, an increase in the abundance of *Bacillus* was observed in the rhizospheric samples of both plants when they were inoculated with the native strain *Serratia* sp. J260. On the other hand, a decrease in the abundance of *Pseudomonas* was observed in the communities of the M_Fert treatment.

The effect of these agricultural practices was also analyzed by culturable approaches from which it was possible to detect and increase of CHB and DB cells in peanut rhizospheric samples inoculated with the native strain *Enterobacter* sp. J49 and chemical fertilized. On the other hand, in the maize samples, the treatments did not show a significant difference in abundance compared to control samples in none of the populations analyzed. An increase in the abundance of PSB associated with the rhizosphere of both peanut and maize was detected in all treatments and in control samples. This could be attributed to a selection by the plant of that group of microorganisms associated with phosphorus found at low levels in the agricultural soils employed in this study. Alternatively, it could be suggested that PSB bacteria would be a must robust population or either a more diverse group

which could change its structure with treatments applied but maintain the pool of bacterial cells.

Peanut plants inoculated with the native phosphate solubilizing *Enterobacter* sp. J49 strain presented the highest diversity values compared to the rest of the rhizospheric bacterial communities analyzed. This strain is also a free-living nitrogen fixer and consequently the inoculation of *Enterobacter* sp. J49 could influence the abundance of certain microbial populations; either because they increase the availability of nutrients or because they indirectly benefit their growth. The competitiveness of a phosphate-solubilizing microorganism in natural environments will depend on its ability to survive and multiply in the soil. However, understanding the dynamics of PSB in soils is the most limiting factor and it is difficult to predict the behavior and efficacy of this inoculation in a particular location (Gyaneshwar et al. 2002).

An increase in the abundances of CHB, DB, and PSB was detected in the rhizospheric communities of peanut and maize, with respect to ES, in the fertilized treatments. Similarly, Mandic et al. (2011) reported that the fertilizers had a significant effect on the soil microbial characteristics, noting that the treatments with the *Klebsiella planticola* SL09-based biofertilizer and nitrogen fertilizers induced the greatest increase in the total microbial count. These authors suggested that rhizospheric microorganisms could use the fertilizer components as a source of macro and micronutrients, thus increasing the growth of certain microbial populations.

The two native phosphate solubilizing bacteria employed in this study (*Enterobacter* sp. J49 and *Serratia* sp. J260) present N_2 fixing ability and production of indole acetic acid (IAA). Also, their inoculation in peanut and maize plants have shown positive results in promoting the plant growth parameters and P content in previous microcosm assays (Taurian et al. 2010, 2013; Anzuay et al. 2015, 2017). Field assays in the peanut growing region of Córdoba Province (Argentina) of peanut and maize plants inoculated with *Enterobacter* sp. J49 showed a growth promoting effect on the yield of these crops (Anzuay et al. 2023). In this study, peanut plants inoculated with native strains of *Enterobacter* sp. J49 increased a large number of peanut plant growth parameters evaluated. The inoculation of peanut with BSP has shown encouraging results in several studies (Mudalagiriyappa et al. 1997; Dey et al. 2004; Taurian et al. 2010; Anzuay et al. 2015, 2017). Anzuay et al. (2023) reported a growth promoting effect on peanut crop yield, mainly under drought stress, in peanut plants inoculated with BSP *Enterobacter* sp. J49. In line with this, Jiang et al. (2018) analyzed inoculations with BSPs (*Bacillus megaterium*, *Enterobacter* sp., *Providencia rettgeri* and *Ensifer adhaeren*) under saline stress conditions and observed growth promotion in peanut plants.

In this study, plants inoculated with *Enterobacter* sp. J49 presented a better performance than the commercial nitrogen-fixing strain *Bradyrhizobium* sp. SEMIA 6144. The efficiency of phosphate solubilization can be expressed by the accumulation of P in plant tissues. In this sense, the increases in P in plant tissues could be due to the efficiency in the solubilization of phosphates or to an increase in the P content in the soil. In line with this, other authors reported growth promotion in maize and peanut plants inoculated with native BSP in field and microcosm assays (Viruel 2014; Pradhan et al. 2017). On the other hand, although peanut plants fertilized (P_Fert) also showed a significant increase in aerial P content and plant growth, these parameters were not statistically significant. This could be attributed to the fact that peanut plants have been described as not responding to the direct application of fertilizers (Pedelini and Monetti 2021).

Regarding maize plants, Anzuay et al. (2017) and Ludueña et al. (2017) reported that inoculation with BSP in microcosm assays produced increases in plant growth parameters. However, the results of this paper indicate that chemical fertilization presented the best results to promote maize growth.

The results in peanut plants indicate that inoculation with native bacteria significantly promotes plant growth, producing minimal disturbances in soil communities.These results encourage the use of this practice as an alternative to the application of chemical fertilizers. In addition to this, although chemical fertilizers increase the growth parameters of maize plants, they notably alter the structure of soil microbial communities, since diversity and specific richness decreased with this treatment.

5 Conclusion

From the results obtained it is possible to conclude that rhizobacterial community structure is highly dynamic and influenced by different factors such as type of plant, the fertilizer input and bio-inoculant applied. In particular, chemical fertilizer application is a practice that exert a more significative impact on bacterial community associated to peanut and maize plants and thus its replacement with biological inoculants based on PGPB would be a better ecological strategy.

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Author contributions María Soledad Anzuay: Investigation, formal analysis, writing. Natalia Pin Viso: Investigation, formal analysis, writing. Liliana Mercedes Ludueña: Writing. Federico Daniel Morla: Formal analysis. Romina Yanet Dalmasso: Writing. Jorge Guillermo Angelini: Writing. Tania Taurian: Investigation, Supervision.

Data availability Data will be made available on request.

Declarations

Conflict of interest The authors have no conficts of interest to declare that are relevant to the content of this article.

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