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Original Article

16S metabarcoding analysis reveals the influence of organic and conventional farming practices on bacterial communities from the rhizospheric of *Coffea arabica* L.

A análise de metabarcode 16S revela a influência das práticas agrícolas orgânicas e convencionais nas comunidades bacterianas da rizosfera de *Coffea arabica* L.

P. H. M. Andrade[a,b], P. C. Machado[b,c], A. F. Paula[a,b], A. C. L. Paganin[d], G. S. Rezende[d], E. Matheucci Jr.[c,e], L. M. Carvalho[f], C. C. M. Freire[g], A. F. Cunha[c,d] and P. T. Lacava[b,c*]

[a]Universidade Federal de São Carlos – UFSCar, Programa de Pós-graduação em Genética Evolutiva e Biologia Molecular, São Carlos, SP, Brasil

[b]Universidade Federal de São Carlos – UFSCar, Centro de Ciências Biológicas e da Saúde, Departamento de Morfologia e Patologia, Laboratório de Microbiologia e Biomoléculas, São Carlos, SP, Brasil

[c]Universidade Federal de São Carlos – UFSCar, Programa de Pós-graduação em Biotecnologia, São Carlos, São Paulo, Brasil

[d]Universidade Federal de São Carlos – UFSCar, Departamento de Genética e Evolução, Laboratório de Bioquímica e Genética Aplicada, São Carlos, SP, Brasil

[e]DNA Consult, São Carlos, SP, Brasil

[f]Universidade Estadual de Campinas – Unicamp, Instituto de Biologia, Departamento de Genética e Evolução, Laboratório de Genômica e Expressão, Campinas, SP, Brasil

[g]Universidade Federal de São Carlos – UFSCar, Departamento de Genética e Evolução, Laboratório de Bioinformática Evolutiva, São Carlos, SP, Brasil

Abstract

Coffea sp. is cultivated in many tropical countries. Brazil has always adopted intensive agricultural practices, but organic coffee farming is an alternative system based on the non-use of agrochemicals and the rational management of soils. Metabarcoding 16S analysis using next-generation sequencing has been developed to identify and compare the diversity of the *Coffea arabica* L. rhizospheric bacterial community in two farming areas in São Paulo, Brazil. Dourado uses conventional farming, while Ribeirão Corrente uses organic. We found broad taxonomic composition, with sequences from 24 phyla, 55 classes, 61 orders, 146 families, and 337genus. The three most abundant phyla were *Proteobacteria* (38.27%), *Actinobacteria* (15.56%), and *Acidobacteria* (16.10%). In organic farming, the top 3 were the family *Sphingomonadaceae*, order *Rhizobiales,* genus *Nocardioides*, and *Gp6*. The genus *Gp2* and the phylum *Candidatus Saccharibacteria* were the most abundant OTUs exclusively present in conventional farming. In the organic farming practice, *Proteobacteria*, *Actinobacteria,* and *Acidobacteria* were also present among the exclusive OTUs; we also found OTUs belonging to *Bacteroidetes*, *Firmicutes*, and *Verrucomicrobia*. Our study indicates a positive effect of organic farming on microbial communities. Fertilization may directly affect soil microbiota, suggesting that a large and active microbial community low in functional diversity might not adapt to new climatic conditions. A diverse community could provide better resilience to environmental changes, improving the productivity of this important crop.

Keywords: coffee, rhizospheric soil, microbiota, 16S rRNA, organic farming.

Resumo

Coffea sp. é cultivada em muitos países tropicais. O Brasil sempre adotou práticas agrícolas intensivas, mas a cafeicultura orgânica é um sistema alternativo baseado na não utilização de agrotóxicos e no manejo racional dos solos. A análise Metabarcode 16S utilizando o sequenciamento de última geração foi desenvolvida para identificar e comparar a diversidade da comunidade bacteriana rizosférica de *Coffea arabica* L. em duas áreas de cultivo em São Paulo, Brasil. Dourado usa agricultura convencional, enquanto Ribeirão Corrente usa agricultura orgânica. Encontramos ampla composição taxonômica, com sequências de 24 filos, 55 classes, 61 ordens, 146 famílias e 337 gêneros. Os três filos mais abundantes foram Proteobacteria (38,27%), Actinobacteria (15,56%) e Acidobacteria (16,10%). Na agricultura orgânica, os 3 primeiros foram a família Sphingomonadaceae, ordem Rhizobiales, gênero Nocardioides e *Gp6*. O gênero *Gp2* e o filo *Candidatus Saccaribacteria* foram as OTUs mais abundantes exclusivamente presentes na agricultura convencional. Na prática da agricultura orgânica, *Proteobacteria*, *Actinobacteria* e *Acidobacteria* também estiveram presentes entre as OTUs exclusivas; também encontramos OTUs pertencentes a *Bacteroidetes*, *Firmicutes* e *Verrucomicrobia*. Nosso estudo indica um efeito positivo da agricultura orgânica nas comunidades microbianas. A fertilização pode afetar diretamente a microbiota do solo, sugerindo que uma grande e ativa comunidade microbiana com baixa diversidade funcional pode não se adaptar às novas condições climáticas. Uma comunidade microbiana diversificada poderia proporcionar maior resiliência às mudanças ambientais, melhorando a produtividade desta importante cultura agrícola.

Palavras-chave: café, solo rizosférico, microbiota, 16S rRNA, agricultura orgânica.

*e-mail: ptlacava@ufscar.br
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1. Introduction

Coffee (*Coffea* sp.) is a perennial plant widely cultivated in many tropical countries. It belongs to the family *Rubiaceae*, which has about 500 genera and more than 6,000 species. It is the most important genus in economic terms, mainly due to the species *Coffea arabica* L. (Martins, 2012; Agler et al., 2016). Brazil has adopted intensive agricultural practices to meet the demand for coffee consumption in many countries; this practice includes heavy use of chemical fertilizers, a variety of chemical treatments to fight pests, and combined plants (herbicides), all with adverse effects and impact on the environment (Fernandes et al., 2020; Loftfield et al., 2018).

Organic agriculture is an alternative that can benefit consumers, farmers, and the environment by eliminating harmful chemicals (Ferdous et al., 2021). The legitimately organic coffee production concept is based on agricultural management similar to an organism's life, respecting the agricultural property's productive potential (Craheix et al., 2016; Fernandes et al., 2020).

Conventional and organic agricultural practices influence the bacterial community, and their relationship with coffee plantation soils has not been well elucidated (Caldwell et al., 2015). Bacterial diversity benefits sustainable practices, resistance to stress, disturbance, and changes in soil conditions (Bhat et al., 2020; Cavalcante et al., 2020; Fernandes et al., 2020; Parikh and James, 2012).

Most plant growth-promoting bacteria (PGPB) have been characterized based on the culture method (Bashan et al., 2014), including PGPB or associated with the coffee rhizosphere. PGPB in the coffee rhizosphere can increase agricultural production by acting as a plant growth promoter or by supplying plants with nutrients (Bhattacharyya and Jha, 2012; Emami et al., 2019; Gu et al., 2020; Liu et al., 2018); phosphate solubilizing bacteria (Rawat et al., 2020; Benoit et al., 2021), and nitrogen-fixing bacteria (Santoyo et al., 2016). Microbial community assembly in the rhizosphere is also determined by abiotic and biotic factors influencing both natural and agricultural ecosystems (Fernandes et al., 2020; Philippot et al., 2013). The complex soil microbiome responses before organic and conventional management are determinants for production and ecosystem maintenance (Bill et al., 2021).

Hence, the characterization of coffee-related bacterial rhizospheric microbiota is of the utmost importance, presenting agricultural and technological potential. The rhizosphere is characterized by high microorganism activity, and the produced enzymes are responsible for biogeochemical cycling, consequently affecting plant growth, health, and productivity (Cui et al., 2018). The interaction between plants and rhizospheric bacteria allows plants to withstand abiotic or biotic stress or disease (Taketani et al., 2015).

We investigated the microbiota associated with the *C. arabica* L. rhizosphere in response to conventional and organic farming practices. We sequenced 16S rRNA V3-V4 regions of rhizospheric samples of conventional and organic soils to account for bacterial diversity and operational taxonomic units (OTUs) so that we could identify differences and potential biomarkers related to increased coffee production.

2. Material and Methods

2.1. Sample collection

Samples of rhizospheric coffee soil from the conventional crop were obtained from Fazenda Monte Alto in Dourado, State of São Paulo; latitude 22°06'12.6"S longitude 48°15'49.6" and altitude of 546 m. This location has sand-textured soil. Sands consists of quartz that confers high susceptibility to erosion and excessive drainage, leading to nutrient leaching, high porosity, low water retention values, high permeability, and high infiltration rate (EMBRAPA, 1999). The climate is humid and temperate, with dry winters and hot summers (Cwa) (CEPAGRI, 2019).

Samples from organic cultivation were obtained from the Sítio Nova Aliança in Ribeirão Corrente, São Paulo State, Brazil; latitude 20°27'25.0" S, longitude 47°35'24.0", and altitude of 855 m. The soil is classified as Nitosol of red and dark red (EMBRAPA, 1999). It presents clay and a very clayey texture; it is structured in heavily developed blocks derived from basic and ultrabasic rocks, with remarkable horizon differentiation and high erosion risk. The climate is the humid temperate type with temperate summers (Cwa) (CEPAGRI, 2019).

The study sites were selected since one presents conventional and the other organic planting management. Rhizospheric soil from 9 healthy plants was randomly collected in the sampled area of conventional cultivation. In organic cultivation, the rhizospheric soil of 15 healthy plants was randomly collected. All soil samples were collected at a depth of 30 cm and adhered at most 3 mm from the roots. After collecting each plant, used tools were washed in running water and disinfected with 70% ethyl alcohol to avoid cross-contamination.

Samples were stored in sterilized plastic bags and transported to the Laboratory of Microbiology and Biomolecules - LaMiB, Department of Morphology and Pathology, Center for Biological and Health Sciences, Federal University of São Carlos, Via Washington Luís km 235, PO BOX 676, São Carlos, SP, Brazil.

2.2. DNA Isolation and 16S Sequencing

Total DNA from each sample was extracted using the PowerSoil DNA Isolation Kit (Catalog # 12888) according to the manufacturer's protocol (MoBio Laboratories, Inc.). Approximately 0.25g of rhizospheric soil was used for the extraction protocol.

The integrity of the extracted DNA was evaluated by agarose gel electrophoresis gel (0.7% w/v) at (3 volts.cm^{-1}) in 1X TEB buffer and stained with GelRed ™, using a molecular marker (1 kb DNA Ladder RTU – KASVI). Genomic DNA from each sample was purified using QIAamp Fast DNA Stool Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol. DNA quantification and quality were evaluated using the NanoVue Plus spectrophotometer (GE Healthcare, Marlborough, USA). Samples were diluted at 50 ng/µL and pooled using the same volume for each one (three samples were used to form one pool, resulting in four replicates from conventional management and six replicates from organic management).

Pooled samples were used to amplify approximately 460 bp of the 16S ribosomal RNA by PCR using specific primers V3 and V4. The PCR products were used to build the metagenomics library for sequencing using MiSeq Reagent kit v3 (600 cycles) (Illumina Inc.). Sequencing of partial 16S ribosomal RNA was performed by next-generation sequencing using the Illumina MiSeq platform that produced thousands of 300 bp paired-end reads (2 × 300 bp) for each library. The full-length primer sequences to follow the protocol targeting this region were 16S Amplicon PCR Forward Primer = 5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and 16S Amplicon PCR Reverse Primer = 5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC

2.3. Processing of the reads and data analyses

Sequencing data were analyzed on USEARCH (version 10.0.240) (Edgar, 2010). The paired-end reads from each management were filtered to receive high-quality reads. Phylogenetic analysis and taxonomic assignments of the V3-V4 portion of the 16S rRNA gene were used for constructing the Operational Taxonomic Units (OTUs) table with 97% of identity. This table was used to investigate the rarefaction curve, which evaluates whether the collected data represented the whole sample diversity. The ecological alpha diversity metrics (Richness, Chao 1, Shannon, Jost, Jost 1) and evenness (Simpson, Dominance, Equitability, Robbins, Berger Parker) were also evaluated. A Venn diagram was made considering the OTUs presented in all the samples by management (Lam et al., 2016). The heatmap and the principal component analysis (PCA) were done using ClustVis (Metsalu and Vilo, 2015). They were constructed with OTUs appearing in at least 200 reads per sample. The complete data sequence was registered at NCBI BioProject with the number PRJNA526486 (NCBI, 2019).

2.4. Statistical analysis

TDdata were analyzed using RStudio (version 3). Alpha diversity data was submitted to Wilcoxon-Mann-Whitney nonparametric test of significance level = 0.05. Based on the Shapiro-Wilk normality test, Phylum and OTUs frequency were analyzed by T-test or Wilcoxon-Mann-Whitney. Heatmap clusters were tested with pvclust (version 2.2-0) (Suzuki and Shimodaira, 2006), considering 1000 interactions.

3. Results

3.1. Evaluation and analysis of operational taxonomic units (OTUs)

The metagenomic analysis of microbiota from the coffee rhizosphere revealed 843,854 high-quality reads after filtering and 695,722 reads mapped with OTUs. For each management, 266,533 reads were revealed for conventional and 429,189 reads for organic. Based on 97% species similarity, 12,803 OTUs were obtained in conventional and organic.

Rarefaction curves suggested that enough sequence reads were collected per sample in each treatment, showing that sequenced samples were enough to uncover most OTUs (Figure 1A).

In the principal component analysis (PCA), values were grouped according to management type, i.e., organic and conventional (Figure 1B). Beta diversity analysis was performed by evaluating sample clustering, confirming the PCA analysis that separated conventional from organic coffee samples. None of the eleven alpha diversity metrics showed a significant difference (p-value < 0.05) in the Wilcoxon-Mann-Whitney test (Table 1).

3.2. Taxonomic diversity of microbial communities in different coffee management

We identified sequences from 24 phyla and 337 genera at the broad taxonomic level. We disregarded the unassigned taxa in this count, which accounted for 8.55% of the sequences.

Both types of management presented similar patterns at the phylum level. The three most abundant were *Proteobacteria* (38.27%), *Actinobacteria* (15.56%), and *Acidobacteria* (16.10%) (Figure 2). Other identified phyla were *Planctomycetes, Bacteroidetes, Firmicutes, Candidatus, Saccharibacteria, Verrucomicrobia, Candidate division WPS-, Parcubacteria, Gemmatimonadetes, Chloroflexi,* and *Nitrospirae* (Figure 3). There were significant differences in three phyla between managements *Acidobacteria, Gemmatimonadetes,* and *Candidate division WPS*.

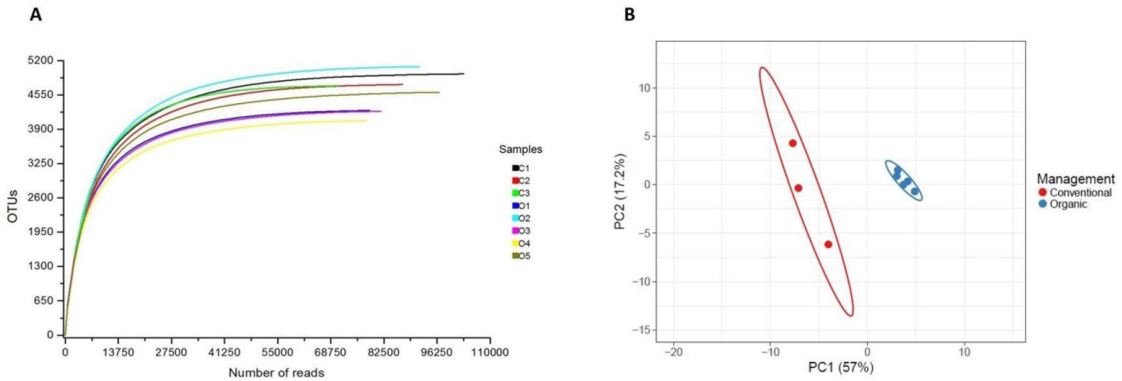

Figure 1. Rarefaction curve and Principal Component Analysis (PCA) of the rhizospheric bacteria associated with *Coffea arabica* from conventional and organic farming practices. (A) Rarefaction curve of rhizospheric bacteria of coffee from conventional (C) and organic (O) samples determined by the number of reads and OTUs. (B) PCA is based on the 50 most prevalent OTUs.

Table 1. Alpha diversity metrics value and statistical analysis by management.

Management	Conventional	Conventional	Conventional	Organic	Organic	Organic	Organic	Organic	Wilcoxon Mann Whitney
Sample	C1	C2	C3	O1	O2	O3	O4	O5	p-value
Berger_Parker	0.023	0.019	0.020	0.025	0.017	0.017	0.016	0.016	0.250
Buzas_Gibson	0.010	0.013	0.016	0.013	0.014	0.013	0.014	0.012	1.000
Chao1	4947.100	4747.200	4723.200	4255.300	5084.100	4238.200	4058.200	4598.200	0.250
Dominance	0.997	0.998	0.997	0.997	0.998	0.998	0.998	0.998	0.290
Equitability	0.817	0.835	0.835	0.832	0.841	0.838	0.840	0.832	0.365
Jost	541.600	635.800	601.500	566.400	726.700	632.600	619.500	644.500	0.393
Richness	4947.000	4747.000	4723.000	4255.000	5084.000	4238.000	4058.000	4598.000	0.250
Robbins	0.110	0.113	0.131	0.139	0.100	0.131	0.120	0.109	1.000
Simpson	0.003	0.002	0.003	0.003	0.002	0.002	0.002	0.002	0.143
Shannon	6.950	7.070	7.060	6.950	7.180	7.000	6.980	7.020	0.881

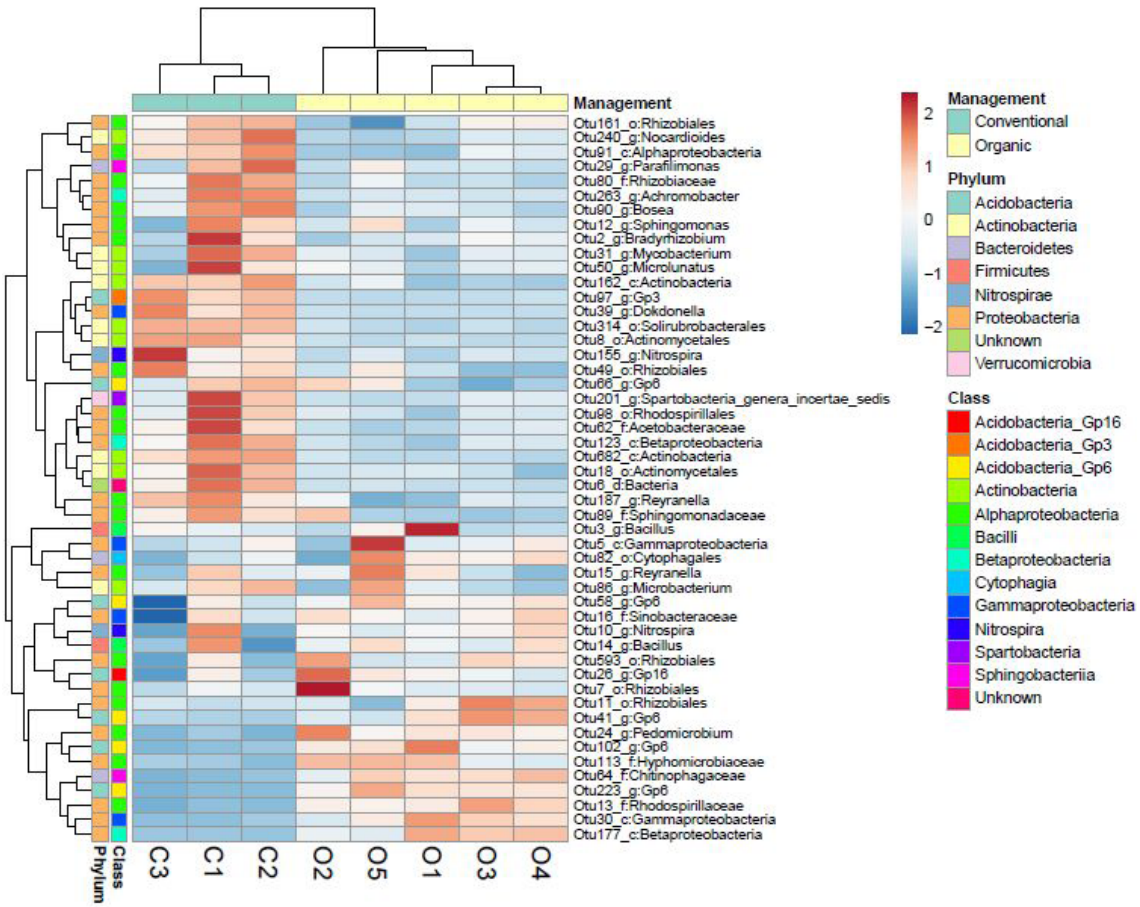

Figure 2. Relative abundance of operational taxonomic units (OTUs) for the 16S rRNA gene of rhizospheric bacteria associated with *Coffea arabica*. Heatmap showing the 50 most abundant OTUs in conventional (C) and organic (O) samples and their respective taxonomy identified following the RDP classifier. The taxonomy description corresponds to domain "d", phylum "p", class "c", order "o", family "f", and genus "g". The cluster was made to Euclidean distance and complete method in Clustvis. Cluster dendrogram support analysis was performed in pvclust (Suzuki and Shimodaira 2006).

Using the 50 most abundant OTUs of each sample, we could observe the clustering of each treatment with several differences that further reinforce differences between management (Figure 3).

The groups were statistically different in conventional phyla *Proteobacteria*, including class *Alphaproteobacteria* (OTU91), order *Rhizobiales* (OTUs 161 and 49), family *Sphingomonadaceae* (OTU89), genus *Reyranella* (OTU187), and genus *Dokdonella* (OTU39). Another phylum that was prevalent in conventional coffee was *Actinobacteria*, including class *Actinobacteria* (OTUs 682 and 162), order *Actinomycetales* (OTU8), order *Solirubrobacterales* (OTU314), genus *Nocardioides* (OTU240). One bacterium classified in the phylum Acidobacteria, genus Gp3 (OTU97), was also prevalent in conventional.

The groups from organic management that had statistical differences when compared to conventional included the phylum *Proteobacteria*, class *Gammaproteobacteria* (OTU30), class *Betaproteobacteria* (OTU177), family *Rhodospirillaceae* (OTU13), and genus *Pedomicrobium* (OTU24). Another two phyla that were significantly different in abundance were *Acidobacteria*, including genus Gp6 (OTUs 223,102 and 41), and *Bacteroidetes*, including family *Chitinophagaceae* (OTU64).

3.3. Exclusive OTUs to each management and taxonomic analyses

We selected the ten most abundant OTUs in all management samples from the Venn diagram, with at least two OTUs present in each specific sample (Figure 4).

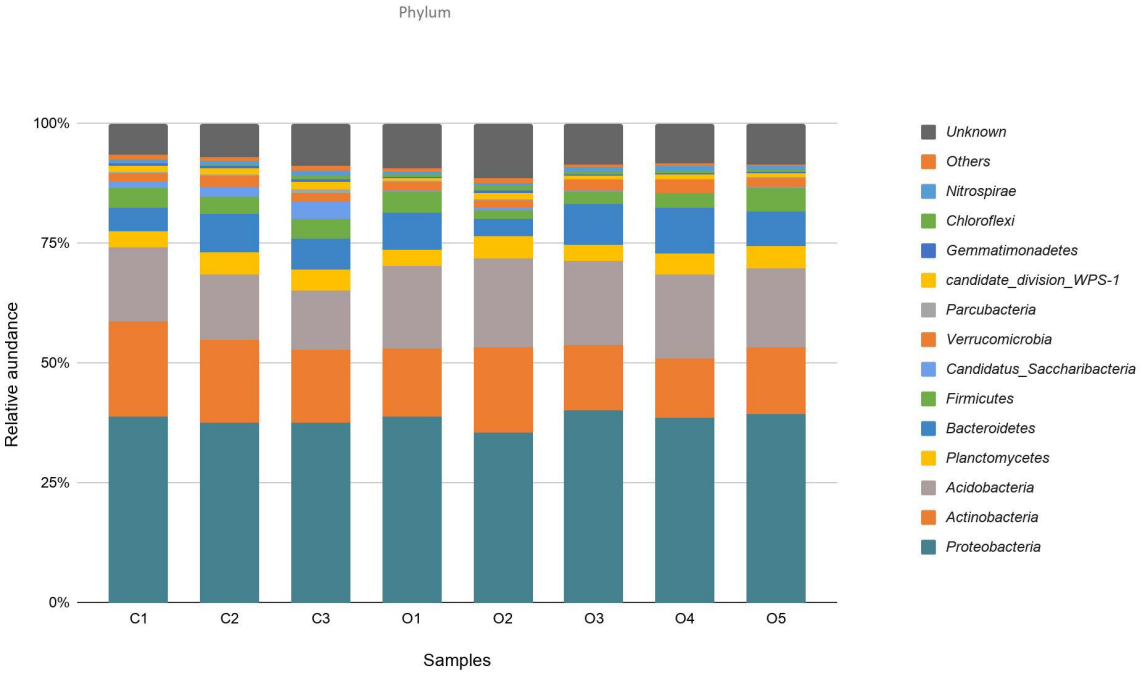

Figure 3. Microbial taxonomic composition of the coffee rhizosphere. Relative abundance at phylum levels in conventional (C) and organic (O) farming practices.

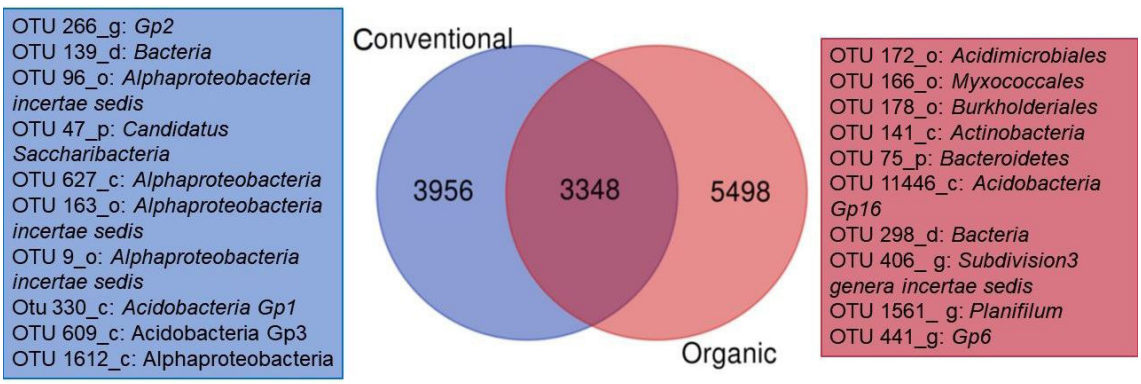

Figure 4. Shared and exclusive OTUs in rhizosphere samples associated with *Coffea arabica*. Venn diagrams the OTUs in all samples analyzed, shared, and exclusives conventional and organic farming. The maximum taxonomic level (domain "d", phylum "p", class "c", order "o", family "f", and genus "g") identified to each ten most abundant OTUs were described to the respective farming practices.

Several of the taxonomic levels identified were shown in the most abundant OTUs selected to make the heatmap. At the phylum level, five *Proteobacteria*, three *Acidobacteria,* and one *Candidatus saccharibacteria* were identified in conventional, while two *Proteobacteria*, *Acidobacteria, and Actinobacteria*, one *Bacteroidetes*, *Firmicutes,* and *Verrucomicrobia* classified in organic farming.

4. Discussions

Agriculture intensification considerably impacts the diversity of plants, animals, and microbial communities (Gabriel and Al, 2006; Jonason et al., 2011). The complexity and technical constraints limit our understanding of the relationship between soil microbiota and agricultural management. Differences in the microbiota relationship between conventional and organic management can be better understood using high-throughput analysis (Lupatini et al., 2017).

Alpha diversity metrics did not show statistical differences in the rhizosphere microbiome, indicating similarity (Table 1), as Pershina et al. (2015) observed. Although not significantly different, some differences between management were more evident when samples clustered in the PCA according to the agricultural management and when the most frequent OTUs were analyzed. This method allows a more in-depth study of the relationship between the microbiota and management type.

Regarding the relative abundance of bacteria, the most prevalent phyla were *Proteobacteria, Actinobacteria,* and *Acidobacteria*, as previously observed (Zheng et al., 2019). *Proteobacteria* include plant growth promoter genera and can replace chemicals in agriculture, horticulture, silviculture, and environmental clean-up (Malisorn et al., 2020). A study comparing the prokaryotic diversity of the rhizosphere of intensive, transitional, and organic coffee farms showed that *Actinobacteria* was among the most abundant, five times more abundant in organic farms (Caldwell et al., 2015). This phylum is commonly identified in the Cerrado biome of eastern Brazil (Dini-Andreote et al., 2010) and on Brazilian soils with sugarcane crops (Rampelotto et al., 2013), suggesting it may play an important role in diverse soils of Brazilian crops. *Acidobacteria* was the third most prevalent group in rhizosphere coffee. This group is important for its ability to use nitrite as a nitrogen source and respond to soil macro, micronutrients, and soil acidity, among other abilities (Kielak et al., 2016). Lupatini et al. (2017) reported that conventional and organic farming systems had a higher influence on soil microbial composition, with *Acidobacteria* among the most predominant phyla in the conventional rhizosphere, corroborating our results.

Bacteroidetes abundance was significantly different between managements. *Bacteroidetes* are dominant members of plant/soil (rhizosphere, endosphere, and phyllosphere) (Lidbury et al., 2021; Thomas et al., 2011). Rhizosphere soil acid phosphatase activity significantly increases with higher lead (Pb) concentration, and it has been positively correlated with *Bacteroidetes* abundance (Hou et al., 2021). A study found that plant-associated *Bacteroidetes* expressed many previously characterized proteins targeting organic phosphorus in response to phosphate depletion (Lidbury et al., 2021), indicating that these traits may enable their success in the rhizosphere. Thus, characteristics related to the niche of the phylum *Bacteroidetes* may explain its greater abundance in the rhizosphere of organic coffee.

When comparing areas by bacterial group, the family *Sphingomonadaceae* (OTU89), order *Rhizobiales* (OTU161), and genus *Nocardioides* (OTU240) were within the statistically different OTU between managements. The *Sphingomonadaceae* family can use many carbon sources, including recalcitrant xenobiotic molecules (Pinyakong et al., 2003). A comparative metagenomic analysis of the rhizosphere microbial community composition of the *Rehmannia glutinosa* crop showed significantly increased relative abundances of *Sphingomonadaceae* (Wu et al., 2018), corroborating our data. This family also presented the highest relative abundance of *Brassica napus* in the rhizosphere by adding N-fertilizer (Monreal et al., 2018). The abundance of bacteria in this group in conventional coffee farming compared to conventional farming may also be favored using N-fertilizer.

Rhizobiales include associations with plant nodules, such as *Bradyrhizobium*, *Agrobacterium,* and *Methylobacterium* (Wang et al., 2020). Most bacterial species within *Rhizobiales* are consistently enriched in the roots and leaves of leguminous and non-legume plant species (Garrido-Oter et al., 2018). Unlike our results, a previous study found a significant increase in *Rhizobiales* abundance in organic farming compared to conventional farming when investigating the response of bacteria communities of different crops (rice, tea, and vegetable) (Wang et al., 2016). A greater abundance of *Rhizobiales* was also found in organic farming when comparing the soil microbiota of three Brazilian coffee farms with different managements (Caldwell et al., 2015).

Nocardioides from the cucumber rhizosphere exhibited biocontrol activity on soil-borne pathogens and the best plant growth potential under greenhouse conditions due to higher exudate production (Chen et al., 2013). The direct relationship between conventional management and *Nocardioides* has not been elucidated, but further studies will be carried out to clarify this relationship.

Considering organic management, the genus Gp6 (OTU41) also had statistical differences compared to conventional. In a study of root-associated (rhizosphere and endosphere) microbiomes of the *Miscanthus sinensis*, *Acidobacteria* Gp6 was identified as a member of the core root endosphere microbiome. However, its abundance was higher in the soil matrix (Sun et al., 2021). Therefore, biological interactions of Gp6 with other microbial populations decreased from the bulk soil to the endosphere, indicating it might be more important within the rhizosphere. As we saw in organic management, the Gp6 group is widely present in soil environments. Several studies (Randall et al., 2019; Risueño et al., 2020) have also shown enrichment in Gp6, which is significantly related to environmental parameters, including soluble organic content, nitrogen, and temperature. However, Gp6 has been reported in the rhizosphere and endosphere in plants (Poudel et al., 2019; Martinez-Rodriguez et al., 2019), but its ecological role on plant growth and implications on organic management remains poorly understood.

Our results corroborate that, for all three crops studied (rice, tea, and vegetable), organic farming has a more stable (Wang et al., 2016) microflora. The bacterial community structure uniform of organic farming significantly increased the abundance of nutrition-related bacteria while reducing some abundance of acid and alkali-resistant bacteria (Lori et al., 2017).

From the most abundant OTUs exclusively present in all the samples in common for both managements presented on the Venn diagram, we can highlight the genus Gp2 (OTU266) and the phylum *Candidatus saccharibacteria* (OTU47) present in conventional farming.

The genus Gp2 has been previously detected in Brazilian forest soils (Navarrete et al., 2013; Catão et al., 2014). It is related to aluminum-rich soils, which indicates a possible metabolic mechanism developed by this genus (Chaves et al., 2019). The pH difference between conventional and organic management could explain the presence of Gp2 exclusively in conventional systems (Lori et al., 2017). This hypothesis on OTUs exclusivity could be tested in a future study to elucidate the pH and nutrient influence on microbiota composition.

Candidatus saccharibacteria was exclusively present in the rhizosphere of conventional cultivation. The same result has been previously shown (Krishnamoorthy et al., 2021). Although this bacterium is abundant and widespread, little is known about its ecophysiology. The genus *Candidatus* plays a role in the degradation of various organic and sugar compounds in aerobic conditions and nitrate reduction in anaerobic conditions (Kindaichi et al., 2016). This group is present in the rhizosphere, but little is known about its metabolism and how it differs from related organisms growing in other environments (Beckers et al., 2017; Correa-Galeote et al., 2018).

5. Conclusion

This study demonstrated how multiple management aspects alter coffee agroecosystems' soil microbial communities. While dealing with conventional and organic management, we found that each management has its diversity of bacteria and specific functions. Fertilization can alter the rhizosphere microbial composition and affect plant growth. Combining practice with other factors can affect enzyme activity in the rhizosphere, directly affecting associated microbiota. This study identified bacteria associated with the coffee rhizosphere of organic and conventional cultivation, which can be used in future studies aiming to use bacterial strains in plant growth promotion assays to develop biofertilizers. Such studies will be fundamental for developing strategies to improve the management of this important crop for the world economy.

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References

AGLER, M.T., RUHE, J., KROLL, S., MORHENN, C., KIM, S.-T., WEIGEL, D. and KEME, E.M., 2016. Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biology*, vol. 14, no. 1, e1002352. http://dx.doi.org/10.1371/journal.pbio.1002352. PMid:26788878.

BASHAN, Y., DE-BASHAN, L.E., PRABHU, S.R. and HERNANDEZ, J.P., 2014. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998-2013). *Plant and Soil*, vol. 378, no. 1-2, pp. 1-33. http://dx.doi.org/10.1007/s11104-013-1956-x.

BECKERS, B., DE BEECK, M.O., WEYENS, N., BOERJAN, W. and VANGRONSVELD, J., 2017. Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. *Microbiome*, vol. 5, no. 1, pp. 25. http://dx.doi.org/10.1186/s40168-017-0241-2. PMid:28231859.

BENOIT, D., HOA, N.X., HA, P.V., STEFANO, C., PHAP, T.Q., GIANG, H.T., TUYET, T.N., PIERRE, M., MICHEL, L. and ROBIN, D., 2021. Identification and characterization of Vietnamese coffee bacterial endophytes displaying in vitro antifungal and nematicidal activities. *Microbiological Research*, vol. 242, pp. 126613. http://dx.doi.org/10.1016/j.micres.2020.126613. PMid:33070050.

BHAT, M.A., KUMAR, V., BHAT, M.A., WANI, I.A., DAR, F.L., FAROOQ, I., BHATTI, F., KOSER, R., RAHMAN, S. and JAN, A.T., 2020. Mechanistic insights of the interaction of plant growth-promoting rhizobacteria (PGPR) with plant roots toward enhancing plant productivity by alleviating salinity stress. *Frontiers in Microbiology*, vol. 11, pp. 1952. http://dx.doi.org/10.3389/fmicb.2020.01952. PMid:32973708.

BHATTACHARYYA, P.N. and JHA, D.K., 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology & Biotechnology*, vol. 28, no. 4, pp. 1327-1350. http://dx.doi.org/10.1007/s11274-011-0979-9. PMid:22805914.

BILL, M., CHIDAMBA, L., GOKUL, J.K., LABUSCHAGNE, N. and KORSTEN, L., 2021. Bacterial community dynamics and functional profiling of soils from conventional and organic cropping systems. *Applied Soil Ecology*, vol. 157, pp. 103734. http://dx.doi.org/10.1016/j.apsoil.2020.103734.

CALDWELL, A.C., SILVA, L.C.F., DA SILVA, C.C. and OUVERNEY, C.C., 2015. Prokaryotic diversity in the rhizosphere of organic, intensive, and transitional coffee farms in Brazil. *PLoS One*, vol. 10, no. 6, pp. 1-17. http://dx.doi.org/10.1371/journal.pone.0106355. PMid:26083033.

CATÃO, E.C., LOPES, F.A., ARAÚJO, J.F., DE CASTRO, A.P., BARRETO, C.C., BUSTAMANTE, M.M., QUIRINO, B.F. and KRÜGER, R.H., 2014. Soil acidobacterial 16S rRNA gene sequences reveal subgroup level differences between savanna-like cerrado and atlantic forest Brazilian biomes. *International Journal of Microbiology*, vol. 2014, pp. 156341. http://dx.doi.org/10.1155/2014/156341. PMid:25309599.

CAVALCANTE, V.S., BORGES, L.S., COSTA, T.L., MOURA, W.M., JACOB, L.L. and FREITAS, M.A.S., 2020. Adubação organomineral na nutrição e produtividade de café arábica. *Cadernos de Agroecologia*, vol. 15, no. 1, pp. 1-5.

CENTRO DE PESQUISAS METEOROLÓGICAS E CLIMÁTICAS APLICADAS À AGRICULTURA – CEPAGRI, 2019 [viewed 22 April 2019]. *A classificação climática de koeppen para o estado de São Paulo* [online]. Campinas: CEPAGRI. Available from: https://www.cpa.unicamp.br/

CHAVES, M.G., SILVA, G.G., ROSSETTO, R., EDWARDS, R.A., TSAI, S.M. and NAVARRETE, A.A., 2019. Acidobacteria subgroups and their metabolic potential for carbon degradation in sugarcane soil amended with vinasse and nitrogen fertilizers. *Frontiers in Microbiology*, vol. 10, pp. 1680. http://dx.doi.org/10.3389/fmicb.2019.01680. PMid:31417506.

CHEN, F., WANG, M., ZHENG, Y., LI, S., WANG, H., HAN, D. and GUO, S., 2013. The effect of biocontrol bacteria on rhizosphere bacterial communities analyzed by plating and PCR-DGGE. *Current Microbiology*, vol. 67, no. 2, pp. 177-182. http://dx.doi.org/10.1007/s00284-013-0347-0. PMid:23483308.

CORREA-GALEOTE, D., BEDMAR, E.J. and ARONE, G.J., 2018. Maize endophytic bacterial diversity as affected by soil cultivation history. *Frontiers in Microbiology*, vol. 9, pp. 484. http://dx.doi.org/10.3389/fmicb.2018.00484. PMid:29662471.

CRAHEIX, D., ANGEVIN, F., DORÉ, T. and DE TOURDONNET, S., 2016. Using a multicriteria assessment model to evaluate the sustainability of conservation agriculture at the cropping system level in France. *European Journal of Agronomy*, vol. 76, pp. 75-86. http://dx.doi.org/10.1016/j.eja.2016.02.002.

CUI, Y., FANG, L., GUO, X., WANG, X., ZHANG, Y., LI, P. and ZHANG, X., 2018. Ecoenzymatic stoichiometry and microbial nutrient limitation in rhizosphere soil in the arid area of the northern Loess Plateau, China. *Soil Biology & Biochemistry*, vol. 116, pp. 11-21. http://dx.doi.org/10.1016/j.soilbio.2017.09.025.

DINI-ANDREOTE, F., ANDREOTE, F.D., COSTA, R., TAKETANI, R.G., VAN ELSAS, J.D. and ARAÚJO, W.L., 2010. Bacterial soil community in a Brazilian sugarcane field. *Plant and Soil*, vol. 336, no. 1-2, pp. 337-349. http://dx.doi.org/10.1007/s11104-010-0486-z.

EDGAR, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, vol. 26, no. 19, pp. 2460-2461. http://dx.doi.org/10.1093/bioinformatics/btq461. PMid:20709691.

EMAMI, S., ALIKHANI, H.A., POURBABAEI, A.A., ETESAMI, H., SARMADIAN, F. and MOTESSHAREZADEH, B., 2019. Effect of rhizospheric and endophytic bacteria with multiple plant growth promoting traits on wheat growth. *Environmental Science and Pollution Research International*, vol. 26, no. 19, pp. 19804-19813. http://dx.doi.org/10.1007/s11356-019-05284-x. PMid:31090003.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA – EMBRAPA, 1999. *Sistema Brasileiro de Classificação de Solos*. Rio de Janeiro: EMBRAPA.

FERDOUS, Z., ZULFIQAR, F., DATTA, A., HASAN, A.K. and SARKER, A., 2021. Potential and challenges of organic agriculture in Bangladesh: a review. *Journal of Crop Improvement*, vol. 35, no. 3, pp. 403-426. http://dx.doi.org/10.1080/15427528.2020.1824951.

FERNANDES, A.L.T., FRAGA JÚNIOR, E.F., DE SANTANA, M.J., SILVA, R.O. and DIAS, M.M., 2020. Use of organic fertilization with irrigation in coffee production in Brazilian cerrado. *Revista Ambiente e Água*, vol. 15, no. 5, pp. 1-13. http://dx.doi.org/10.4136/ambi-agua.2578.

GABRIEL, D., ROSCHEWITZ, I., TSCHARNTKE, T. and THIES, C., 2006. Beta diversity at different spatial scales: plant communities in organic and conventional agriculture. *Ecological Applications*, vol. 16, no. 5, pp. 2011-2021. http://dx.doi.org/10.1890/1051-0761(2006)016[2011:BDADSS]2.0.CO;2. PMid:17069391.

GARRIDO-OTER, R., NAKANO, R.T., DOMBROWSKI, N., MA, K.W., MCHARDY, A.C. and SCHULZE-LEFERT, P., 2018. Modular traits of the rhizobiales root microbiota and their evolutionary relationship with symbiotic rhizobia. *Cell Host & Microbe*, vol. 24, no. 1, pp. 155-167.e5. http://dx.doi.org/10.1016/j.chom.2018.06.006. PMid:30001518.

GU, Y., DONG, K., GEISEN, S., YANG, W., YAN, Y., GU, D., LIU, N., BORISJUK, N., LUO, Y. and FRIMAN, V.P., 2020. The effect of microbial inoculant origin on the rhizosphere bacterial community composition and plant growth-promotion. *Plant and Soil*, vol. 452, no. 1-2, pp. 105-117. http://dx.doi.org/10.1007/s11104-020-04545-w.

HOU, X.L., HAN, H., TIGABU, M., LI, Q.Y., LI, Z.X., ZHU, C.L., HUANG, S.Q., CAI, L.P. and LIU, A.Q., 2021. Lead contamination alters enzyme activities and microbial composition in the rhizosphere soil of the hyperaccumulator *Pogonatherum crinitum*. *Ecotoxicology and Environmental Safety*, vol. 207, pp. 111308. http://dx.doi.org/10.1016/j.ecoenv.2020.111308. PMid:32931972.

JONASON, D., ANDERSSON, G.K.S., ÖCKINGER, E., RUNDLÖF, M., SMITH, H.G. and BENGTSSON, J., 2011. Assessing the effect of the time since transition to organic farming on plants and butterflies. *Journal of Applied Ecology*, vol. 48, no. 3, pp. 543-550. http://dx.doi.org/10.1111/j.1365-2664.2011.01989.x. PMid:21731110.

KIELAK, A.M., BARRETO, C.C., KOWALCHUK, G.A., VAN VEEN, J.A. and KURAMAE, E.E., 2016. The ecology of *acidobacteria*: moving beyond genes and genomes. *Frontiers in Microbiology*, vol. 7, pp. 1-16. https://doi.org/10.3389/fmicb.2016.00744.

KINDAICHI, T., YAMAOKA, S., UEHARA, R., OZAKI, N., OHASHI, A., ALBERTSEN, M., NIELSEN, P.H. and NIELSEN, J.L., 2016. Phylogenetic diversity and ecophysiology of Candidate phylum Saccharibacteria in activated sludge. *FEMS Microbiology Ecology*, vol. 92, no. 6, pp. fiw078. http://dx.doi.org/10.1093/femsec/fiw078. PMid:27090759.

KRISHNAMOORTHY, R., CHOUDHURY, A.R., JOSE, P.A., SUGANYA, K., SENTHILKUMAR, M., PRABHAKARAN, J., GOPAL, N.O., CHOI, J., KIM, K., ANANDHAM, R. and SA, T., 2021. Long-term exposure to azo dyes from textile wastewater causes the abundance of saccharibacteria population. *Applied Sciences*, vol. 11, no. 1, pp. 379. http://dx.doi.org/10.3390/app11010379.

LAM, F., LALANSINGH, C.M., BABARAN, H.E., WANG, Z., PROKOPEC, S.D., FOX, N.S. and BOUTROS, P.C., 2016. VennDiagramWeb: a web application for the generation of highly customizable Venn and Euler diagrams. *BMC Bioinformatics*, vol. 17, no. 1, pp. 401. http://dx.doi.org/10.1186/s12859-016-1281-5. PMid:27716034.

LIDBURY, I.D.E.A., BORSETTO, C., MURPHY, A.R.J., BOTTRILL, A., JONES, A.M.E., BENDING, G.D., HAMMOND, J.P., CHEN, Y., WELLINGTON, E.M.H. and SCANLAN, D.J., 2021. Niche-adaptation in plant-associated *Bacteroidetes* favours specialisation in organic phosphorus mineralisation. *The ISME Journal*, vol. 15, no. 4, pp. 1040-1055. http://dx.doi.org/10.1038/s41396-020-00829-2. PMid:33257812.

LIU, K., MCINROY, J.A., HU, C.H. and KLOEPPER, J.W., 2018. Mixtures of plant-growth-promoting rhizobacteria enhance biological control of multiple plant diseases and plant-growth promotion in the presence of pathogens. *Plant Disease*, vol. 102, no. 1, pp. 67-72. http://dx.doi.org/10.1094/PDIS-04-17-0478-RE. PMid:30673446.

LOFTFIELD, E., CORNELIS, M.C., CAPORASO, N., YU, K., SINHA, R. and FREEDMAN, N., 2018. Association of coffee drinking with mortality by genetic variation in caffeine metabolism: findings from the UK Biobank. *JAMA Internal Medicine*, vol. 178, no. 8, pp. 1086-1097. http://dx.doi.org/10.1001/jamainternmed.2018.2425. PMid:29971434.

LORI, M., SYMNACZIK, S., MÄDER, P., DE DEYN, G. and GATTINGER, A., 2017. Organic farming enhances soil microbial abundance and activity-A meta-analysis and meta-regression. *PLoS One*, vol. 12, no. 7, e0180442. http://dx.doi.org/10.1371/journal.pone.0180442. PMid:28700609.

LUPATINI, M., KORTHALS, G.W., DE HOLLANDER, M., JANSSENS, T.K.S. and KURAMAE, E.E., 2017. Soil microbiome is more heterogeneous in organic than in conventional farming system. *Frontiers in Microbiology*, vol. 7, pp. 2064. http://dx.doi.org/10.3389/fmicb.2016.02064. PMid:28101080.

MALISORN, K., CHANCHAMPA, S., KANCHANASIN, P. and TANASUPAWAT, S., 2020. Identification and plant growth-promoting activities of proteobacteria isolated from root nodules and rhizospheric soils. *Current Applied Science and Technology*, vol. 20, no. 3, pp. 479-493. http://dx.doi.org/10.14456/cast.2020.32.

MARTINEZ-RODRIGUEZ, A., MACEDO-RAYGOZA, G., HUERTA-ROBLES, A.X., REYES-SEPULVEDA, I., LOZANO-LOPEZ, J., GARCÍA-OCHOA, E.Y., FIERRO-KONG, L., MEDEIROS, M.H.G., DI MASCIO, P., WHITE JUNIOR, J.F. and BELTRAN-GARCIA, M.J., 2019. Agave seed endophytes: ecology and impacts on root architecture, nutrient acquisition, and cold stress tolerance. In: S. VERMA and J. WHITE JUNIOR, eds. *Seed endophytes*. Cham: Springer, pp. 139-170. http://dx.doi.org/10.1007/978-3-030-10504-4_8.

MARTINS, A.L. (2012). *História do café*. 2. ed. São Paulo: Contexto.

METSALU, T. and VILO, J., 2015. ClustVis: A web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. *Nucleic Acids Research*, vol. 43, no. W1, pp. W566-W570. http://dx.doi.org/10.1093/nar/gkv468. PMid:25969447.

MONREAL, C.M., ZHANG, J., KOZIEL, S., VIDMAR, J., GONZÁLEZ, M., MATUS, F., BAXI, S., WU, S., DEROSA, M. and ETCHEVERRIA, P., 2018. Bacterial community structure associated with the addition of nitrogen and the dynamics of soluble carbon in the rhizosphere of canola (*Brassica napus*) grown in a Podzol. *Rhizosphere*, vol. 5, pp. 16-25. http://dx.doi.org/10.1016/j.rhisph.2017.11.004.

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION – NCBI, 2019 [viewed 22 April 2019]. *Coffea canephora* [online]. Available from: https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA526486

NAVARRETE, A.A., KURAMAE, E.E., DE HOLLANDER, M., PIJL, A.S., VAN VEEN, J.A. and TSAI, S.M., 2013. Acidobacterial community responses to agricultural management of soybean in Amazon forest soils. *FEMS Microbiology Ecology*, vol. 83, no. 3, pp. 607-621. http://dx.doi.org/10.1111/1574-6941.12018. PMid:23013447.

PARIKH, S.J. and JAMES, B.R., 2012. Soil: the foundation of agriculture. *Nature Education Knowledge*, vol. 3, no. 10, pp. 2.

PERSHINA, E., VALKONEN, J., KURKI, P., IVANOVA, E., CHIRAK, E., KORVIGO, I., PROVOROV, N. and ANDRONOV, E., 2015. Comparative analysis of prokaryotic communities associated with organic and conventional farming systems. *PLoS One*, vol. 10, no. 12, pp. 1-16. http://dx.doi.org/10.1371/journal.pone.0145072. PMid:26684619.

PHILIPPOT, L., RAAIJMAKERS, J.M., LEMANCEAU, P. and VAN DER PUTTEN, W.H., 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology*, vol. 11, no. 11, pp. 789-799. http://dx.doi.org/10.1038/nrmicro3109. PMid:24056930.

PINYAKONG, O., HABE, H. and OMORI, T., 2003. The unique aromatic catabolic genes in sphingomonads degrading polycyclic aromatic hydrocarbons (PAHs). *The Journal of General and Applied Microbiology*, vol. 49, no. 1, pp. 1-19. http://dx.doi.org/10.2323/jgam.49.1. PMid:12682862.

POUDEL, R., JUMPPONEN, A., KENNELLY, M.M., RIVARD, C.L., GOMEZ-MONTANO, L. and GARRETT, K.A., 2019. Rootstocks shape the rhizobiome: rhizosphere and endosphere bacterial communities in the grafted tomato system. *Applied and Environmental Microbiology*, vol. 85, no. 2, pp. e01765-e18. http://dx.doi.org/10.1128/AEM.01765-18. PMid:30413478.

RAMPELOTTO, P.H., DE SIQUEIRA FERREIRA, A., BARBOZA, A.D.M. and ROESCH, L.F.W., 2013. Changes in diversity, abundance, and structure of soil bacterial communities in Brazilian savanna under different land use systems. *Microbial Ecology*, vol. 66, no. 3, pp. 593-607. http://dx.doi.org/10.1007/s00248-013-0235-y. PMid:23624541.

RANDALL, K., BRENNAN, F., CLIPSON, N., CREAMER, R., GRIFFITHS, B., STOREY, S. and DOYLE, E., 2019. Soil bacterial community structure and functional responses across a long-term mineral phosphorus (Pi) fertilisation gradient differ in grazed and cut grasslands. *Applied Soil Ecology*, vol. 138, pp. 134-143. http://dx.doi.org/10.1016/j.apsoil.2019.02.002.

RAWAT, P., DAS, S., SHANKHDHAR, D. and SHANKHDHAR, S.C., 2020. Phosphate-solubilizing microorganisms: mechanism and their role in phosphate solubilization and uptake. *Journal of Soil Science and Plant Nutrition*. http://dx.doi.org/10.1007/s42729-020-00342-7.

RISUEÑO, Y., PETRI, C. and CONESA, H.M., 2020. The importance of edaphic niches functionality for the sustainability of phytomanagement in semiarid mining impacted ecosystems. *Journal of Environmental Management*, vol. 266, pp. 110613. http://dx.doi.org/10.1016/j.jenvman.2020.110613. PMid:32392146.

SANTOYO, G., MORENO-HAGELSIEB, G., DEL, M., OROZCO-MOSQUEDA, C. and GLICK, B.R., 2016. Plant growth-promoting bacterial endophytes. *Microbiological Research*, vol. 183, pp. 92-99. http://dx.doi.org/10.1016/j.micres.2015.11.008. PMid:26805622.

SUN, X., SONG, B., XU, R., ZHANG, M., GAO, P., LIN, H. and SUN, W., 2021. Root-associated (rhizosphere and endosphere) microbiomes of the *Miscanthus sinensis* and their response to the heavy metal contamination. *Journal of Environmental Sciences*, vol. 104, pp. 387-398. http://dx.doi.org/10.1016/j.jes.2020.12.019. PMid:33985741.

SUZUKI, R. and SHIMODAIRA, H., 2006. Pvclust: an R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics*, vol. 22, no. 12, pp. 1540-1542. http://dx.doi.org/10.1093/bioinformatics/btl117. PMid:16595560.

TAKETANI, R.G., KAVAMURA, V.N., MENDES, R. and MELO, I.S., 2015. Functional congruence of rhizosphere microbial communities associated to leguminous tree from Brazilian semiarid region. *Environmental Microbiology Reports*, vol. 7, no. 1, pp. 95-101. http://dx.doi.org/10.1111/1758-2229.12187. PMid:25870877.

THOMAS, F., HEHEMANN, J.H., REBUFFET, E., CZJZEK, M. and MICHEL, G., 2011. Environmental and gut bacteroidetes: the food connection. *Frontiers in Microbiology*, vol. 2, pp. 93. http://dx.doi.org/10.3389/fmicb.2011.00093. PMid:21747801.

WANG, S., MEADE, A., LAM, H.-M. and LUO, H., 2020. Evolutionary timeline and genomic plasticity underlying the lifestyle diversity in Rhizobiales. *mSystems*, vol. 5, no. 4, pp. e00438-e20. http://dx.doi.org/10.1128/mSystems.00438-20. PMid:32665328.

WANG, W., WANG, H., FENG, Y., WANG, L., XIAO, X., XI, Y., LUO, X., SUN, R., YE, X., HUANG, Y., ZHANG, Z. and CUI, Z., 2016. Consistent responses of the microbial community structure to organic farming along the middle and lower reaches of the Yangtze River. *Scientific Reports*, vol. 6, no. 1, pp. 35046. http://dx.doi.org/10.1038/srep35046. PMid:27725750.

WU, L., WANG, J., WU, H., CHEN, J., XIAO, Z., QIN, X., ZHANG, Z. and LIN, W., 2018. Comparative metagenomic analysis of rhizosphere microbial community composition and functional potentials under *Rehmannia glutinosa* consecutive monoculture. *International Journal of Molecular Sciences*, vol. 19, no. 8, pp. 2394. http://dx.doi.org/10.3390/ijms19082394. PMid:30110928.

ZHENG, Q., HU, Y., ZHANG, S., NOLL, L., BÖCKLE, T., DIETRICH, M., HERBOLD, C.W., EICHORST, S.A., WOEBKEN, D., RICHTER, A. and WANEK, W., 2019. Soil multifunctionality is affected by the soil environment and by microbial community composition and diversity. *Soil Biology & Biochemistry*, vol. 136, pp. 107521. http://dx.doi.org/10.1016/j.soilbio.2019.107521. PMid:31700196.