



SOIL SCIENCE

Structure and diversity of bacterial community in semiarid soils cultivated with prickly-pear cactus (*Opuntia ficus-indica* (L.) Mill.)

MARIA DO CARMO C.P. DE LYRA, RODRIGO G. TAKETANI, ANA DOLORES DE FREITAS, CAROLINA E.R.S. E SILVA, ADÁLIA C.E.S. MERGULHÃO, MARIA LUIZA R.B. DA SILVA, JADSON E.L.S. ANTUNES, ADEMIR S.F. DE ARAÚJO & POLIANA F. GIACHETTO

Abstract: Studies evaluating the structure and diversity of bacterial communities in arid environments including the rhizosphere of local and adapted plant species are important. Therefore, we used a sequencing of the 16S ribosomal RNA gene for describing the structure and diversity of soil bacterial community in three zones: Agreste, Transition and Sertão. The bacterial community was clustered in 9,838 OTUs in Agreste, 8,388 OTUs in the transition, and 14,849 OTUs for Sertão. Among the most abundant phyla, Proteobacteria and Acidobacteria were abundant in Agreste and Sertão, respectively, while Actinobacteria were abundant in Transition and Sertão. Specific taxa of Proteobacteria, in Agreste, and Actinobacteria, in Sertão, exhibited differences according to biotic and abiotic conditions. Thus, the structure and diversity of bacterial community were different in these areas and were influenced by environmental and soil conditions.

Key words: Caatinga biome, metagenomics, microbial communities, diversity.

INTRODUCTION

Caatinga is an important biome in Brazil that presents high temperature, intense contrasts between seasons and differences in soil conditions. Also, the rainfall distribution is concentrated in three to four months per year (De BM Trovão et al. 2007). These characteristics associated with the improper use of the Caatinga soils can contribute for desertification (Kavamura et al. 2013, Silva et al. 2004).

Specifically, the caatinga region from Pernambuco state presents two distinct mesoregions known as Agreste and Sertão. Agreste presents annual average temperatures of 25°C and rainfall of 1,200 mm, while Sertão presents lowest rainfall (400 mm per year) and

highest temperature (annual average of 42°C). In these regions, the Prickly-Pear Cactus is a plant species adapted to these adverse weather conditions. Due to its photosynthetic process known as crassulacean acid metabolism (CAM) (Magalhães & Ferri 1979). Also, this plant is important as the main source of animal feed during the dry period.

On the other hand, different environmental conditions found in Agreste and Sertão found in both regions could influence the microbial community found in soils. The knowledge of how microbial communities interact with this plant is important for understanding of its responses to environmental shifts. Specially, bacterial community plays important roles in the

soil ecosystem and is responsible for processes such as organic matter decomposition, being influenced by biotic and abiotic factors, such as soil temperature and moisture. The relationship between bacterial species may be influenced by the plant species present in the soil (Klindworth et al. 2013).

Plants are essential for soil microbial function and structure (Bardgett 2005, Bais et al. 2006, Horner-Devine et al. 2004) and the rhizosphere characteristics influence the composition of bacterial community in soil (Philippot et al. 2013). Natural ecosystems such as the Caatinga, plant species and their capacity are influenced by the availability of nutrients in the soil. Mechanisms that differentially affect the responses of plants as well as the microbiome of rhizosphere (Philippot et al. 2013). Thus, the structure and diversity of microbial community is important for indicating changes in soil quality in many ecosystems, such as Caatinga. On the other hand, the bacterial community associated with Prickly-Pear Cactus in Caatinga biome has not been studied yet. We hypothesized that there may be differences in the structure and diversity of bacterial community in rhizosphere of Prickly-Pear Cactus found in Agreste and Sertão, including the transition between regions, since these regions present different environmental conditions. Thus, this study assessed the structure and diversity of bacterial community in the rhizosphere of Prickly-Pear Cactus in three different regions: Agreste, Transition, and Sertão.

MATERIALS AND METHODS

Soils were sampled in three different areas with Prickly-Pear Cactus belonging to the Agronomic Institute of Pernambuco and are distributed throughout the state of Pernambuco: Agreste (8°14'18"S and 35°55'20"W); Transition

Agreste-Sertão (8°25'08"S and 37°03'00"W); and Sertão (7°59'00"S and 38°19'16"W). The Transition Area is located in the beginning of Sertão (an area well characterized as arid) and the end of Agreste (a less arid region.). Each area (400 m²) was divided in three transects (replication) where soil samples were collected at 0 - 5 cm depth around the rhizosphere (twenty plants per transect where the soil adhered to the roots were collected and mixed to form a composite sample per transect). All soil samples were immediately stored in sealed plastic bags and transported in an icebox to the laboratory. A portion of each soil sample was stored in bags and kept at -80°C for DNA analysis and another portion was air-dried, sieved through a 2-mm screen, and homogenized for chemical and Physical analyses. The soil samples were conducted at the Laboratory of Soil of Agronomic Institute of Pernambuco stored at -20 °C and estimated according to Embrapa (1997), are shown in Table I.

Total DNA was extracted from 0.5 g (total humid weight) of soil using the HiPurA Soil DNA Purification Kit™ (HIMEDIA), according to the manufacturer's instructions. The DNA Extraction was performed in triplicate for each soil sample. The quality and concentration of the extracted DNA was determined using spectrophotometry and measures in 260 and 280 nm. The ration 260/280 was calculated according to requirements of MacroGen Inc. (South Korea). Roche GS- FLX amplicon sequencing of bacterial diversity were characterized by high-throughput sequencing (454 GS FLX amplicon sequencing) (Binladen et al. 2007) by using the primer 27F/519R that targeted the variable region V1-V3 of 16S rRNA bacterial gene (Fagervold et al. 2014, Kumar et al. 2011).

DNA samples were shipped to MacroGen Inc, South Korea MacroGen. Available: <http://dna.macrogen.com/eng>. for high-throughput

Table I. Chemical and Physical characteristics of the soil sampled by each mesoregion the state of Pernambuco.

SOIL PROPERTIES	AGRESTE ZONE	TRANSITION ZONE	SERTÃO ZONE	SOIL PROPERTIES	AGRESTE ZONE	TRANSITION ZONE	SERTÃO ZONE
	Chemical characteristics			Physical characteristics			
<i>pH</i> (H ₂ O)	5.5	5.7	5.5	Course Sand (%)	48	30	54
P (mg dm ⁻³)	228	178	42	Thin Sand (%)	19	36	20
Ca³⁺ (cmol _c dm ⁻³ %)	6.90	5.60	3.35	Silt (%)	22	26	18
Mg²⁺ (cmol _c dm ⁻³ %)	0.75	1.00	1.05	Clay (%)	11 SL	8 SL	8 SL
Na²⁺ (cmol _c dm ⁻³ %)	0.07	0.08	0.06	Available water ~Inches of water/foot of soil			
K' (cmol _c dm ⁻³ %)	0.25	0.19	0.25	Field Capacity (in/ft)	8.36	6.15	6.22
AL³⁺ (cmol _c dm ⁻³ %)	0.10	0.05	0.10	Oven Dry (in/ft)	1.12	0.95	0.90
H' (cmol _c dm ⁻³ %)	4.93	3.90	3.20	Ds (Kg dm ⁻³)	1.34	1.54	1.45
SB (cmol _c dm ⁻³ %)	8.0	6.9	4.7	Dp (Kg dm ⁻³)	2.56	2.56	2.56
T (cmol _c dm ⁻³ %)	13.0	10.8	8.0				

T=Total Cation Exchange Capacity, SB- Sum of Bases, Ds-Soil Density, Dp- Particule Density; SL – sandy loam.

sequencing. GS FLX data processing was performed using Roche GS FLX software (v 2.9) in two stages, image processing and signal processing. Image processing involves normalization of raw images and generation of raw signals. In the signal processing stage, correction, filtering, and raw signal trimming were done prior to base calling with corresponding quality score of reads. Sequence reads from each sample were segregated with in-house script (Macrogen) using the tag (Barcode) sequences, and by matching the initial and final bases of the reads to the known tag sequences used in the preparation of the libraries (Lee et al. 2010). The sequences were processed using the Quantitative Insights Into Microbial Ecology toolkit (QIIME) (Caporaso et al. 2009, 2010). Sequences with quality > Q20 and homopolymers > 6 were aligned. Barcodes were removed and the sequences of the expected size (400 pb) were retained for further processing.

Chimeras were identified using ChimeraSlayer and removed. Organelles and archaeal contaminations were also excluded within QIIME (Version 1.9.0) The sequences were grouped into OTUs with 97% similarity using UCLUST (Edgar 2010). A representative sequence of each OTU was aligned again in GreenGenes core set using PyNAST (Caporaso et al. 2010), with sequences classified in the database GreenGenes via UCLUST (Edgar 2010). The profiles of alpha and beta diversity were obtained according to the Qiime's 454 tutorial (<http://qiime.org/tutorials/tutorial.html>). The analyses PCoA (Principal Coordinate Analyses) was used with QIIME The transformations used taxonomic (Bray–Curtis Distances) measures of the bacterial community composition considered all dimensions relevant between bacterial composition and zones (Fierer et al. 2012). We have applied the rarefaction procedure indicated in the qiime tutorial was applied (<http://qiime.org/scripts/>

core_diversity_analyses.html?highlight=core). After rarefaction, the libraries covered from 75.7-88.4% of the original sample based on Good's coverage.

RESULTS AND DISCUSSION

A total of 137,291 reads were obtained from soil samples and were clustered in 9,838 OTUs in Agreste, 8,388 OTUs in the Transition zone, and 14,849 OTUs for Sertão (Figure 1a). Although sertão zone has shown highest number of OTUs, the Shannon's diversity index was lowest in this area as compared with Agreste and Transition (Figure 1b). The PCoA of OTUs stands for significant differences between areas according to their bacterial community (Figure 2). The soils have a texture class of sandy loam, however, have differences in their physical and chemical characteristics, where the soils of the transition zone behave in a very characteristic way of a changing zone. The transition zone has a field capacity very similar to the Sertão region and

35% smaller than the Agreste zone. The results showed that soil microbial communities in the transition zone have fewer OTUs than in the Agreste and Sertão zones. It is known that biotic and abiotic factors may be responsible for the composition and structures of communities, but a rhizospheric effect of soil microorganisms may increase or decrease the occurrence of OTUs. What we can infer is that the composition and structures of these communities in the transition zone may not be in equilibrium and therefore present a lower OTU value or present no similarities between collection points and periods. Andrew et al. (2012), studying abiotic factors in desert soil, observed that genetic diversity can be influenced by abiotic factors such as extreme temperature fluctuation, high UV radiation, low nutrient content and low soil moisture.

The results of OTUs richness found in this study contrast with those Shange et al. (2012) who observed increased richness in native and cultivated soil. However, the regions studied here were all cultivated with Prickly-Pear Cactus,

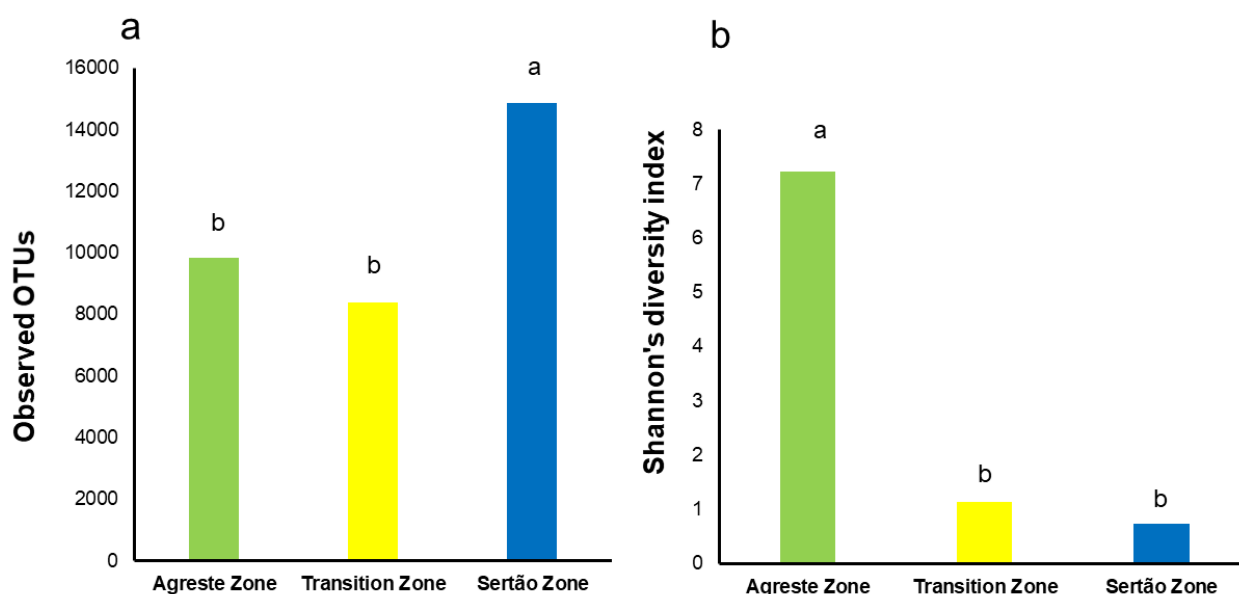


Figure 1. Observed OTUs (a) and Shannon's diversity index (b) in the mesoregions of the State of Pernambuco. Different lowercase letters refer to significant differences between treatments based on Tukey's HSD test ($P < 0.05$).

which would not explain the observed variation. However, according to Barros (2007), for the richness estimated in tropical regions, curve stabilization is very difficult to attain because more rare species appear, and increased sampling efforts are needed. Thus, although the number of samples for the areas is the same for each region, local samples may be subject to different influences because species richness and diversity depend on factors beyond the natural community of nature and the sampling effort. On the other hand, Agreste presented highest diversity of bacterial communities than Transition and Sertão. The species richness and diversity of different species exhibit variations due to the biotic and abiotic factors that influence the distribution and species selection (Magurran 1988). In the Sertão Zone, we observed an increased value of richness and reduced diversity, where a reduced Shannon index value can be attributed to the reduced richness of the high dominance taxonomic groups.

Eight most abundant phyla (Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Firmicutes, Bacteroidetes, Planctomycetes, and

the candidatus phyla TM7), with percentages of OTUs above 1%, represented more than 90% of the total OTUs (Figure 3). Three most abundant phyla were Proteobacteria, Actinobacteria, and Acidobacteria found in all areas. Proteobacteria and Acidobacteria were abundant in Agreste and Sertão, respectively, while Actinobacteria were abundant in Transition and Sertão.

Proteobacteria are the most abundant phylum in the soil bacterial community and exhibited a greater dominance of 70% in dry environmental samples (Xu et al. 2014, Taketani et al. 2015, Kavamura et al. 2013, Lançoni et al. 2013). The results showed that Agreste presented highest abundance of Proteobacteria, while Sertão presented highest abundance of Acidobacteria. These findings suggest that differences in soil conditions could regulate the abundance of these groups. Indeed, Sertão presents highest temperature and lowest soil moisture and fertility, and these conditions contribute for increasing the abundance of Acidobacteria which are adapted to poor soils and extreme environment (Rousk et al. 2010). On the other hand, Agreste zone presents better soil fertility

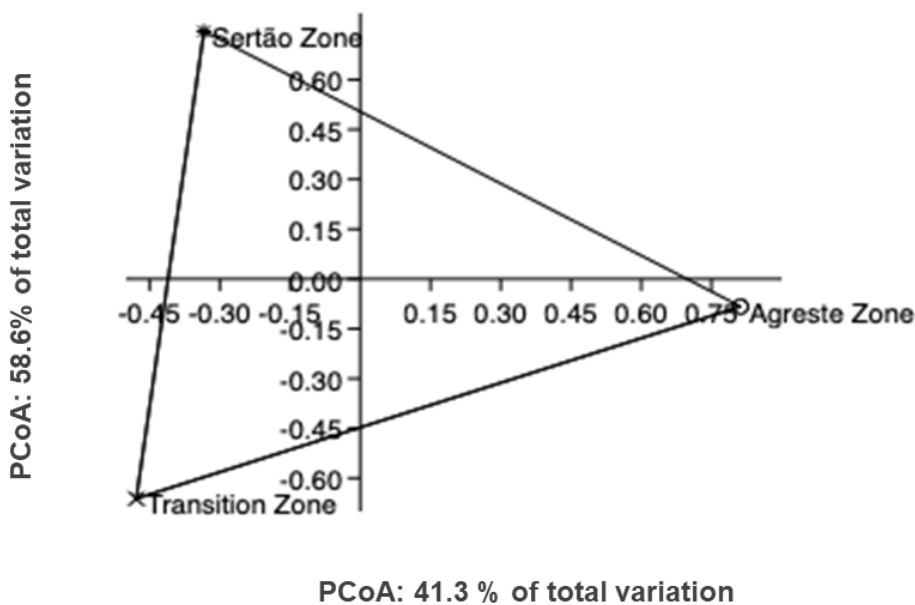


Figure 2. PCoA plots in the mesoregions of the State of Pernambuco.

than Sertão and it contributed for increasing the abundance of Proteobacteria. According to studies from Fierer et al. (2007) and Singh et al. (2010) Proteobacteria have a copiotroph (require high nutrients concentrations) lifestyle, whereas Acidobacteria are oligotrophic (require very low nutrients concentrations). This finding is interesting because in the case of the Prickly-Pear cactus crops, which underwent intensive farming, copiotroph bacteria should already be present. Actinobacteria were detected with higher abundance in Transition and Sertão zones. These bacteria play important role in the organic matter decomposition in soil (Kopecky et al. 2011) and, therefore, are important for these areas since they presented low soil fertility.

Bacteria from the group of α and γ -Proteobacteria were predominant in Agreste (37% and 13%, respectively) and the percentages were reduced in the Transition and Sertão zones. In Transition and Sertão zones, the largest predominance of Actinobacteria included Thermoleophilia (13 and 18%, respectively) and Actinobacteria (24 and 15%,

respectively) at the class level. Considering percentages of OTUs above 5%, we found the family Gaiellales with presence in the most arid region (Sertão). In contrast, the families Rhizobiales and Rhodospirillales exhibited an increased incidence in the Transition and Agreste zones. As the temperature decreases and the rainfall increases, the predominance of the orders Rhizobiales and Rhodospirillales is observed. Families with abundance above 5% included Caulobacteraceae, Bradyrhizobiaceae and Hyphomicrobiaceae, Acetobacteraceae and Rhodospirillaceae (Figure 4). The remaining families have a prevalence lower than 5%.

The results of this study clearly demonstrate that the different zones of Agreste, Transitions, and Sertão have distinct bacterial communities and it is influenced by environmental and soil conditions. Since that each zone presents the same plant species, the difference in bacterial community is associated with the environment which also influence the plant responses. Our study also demonstrate that semi-arid soils are environments where natural selection has

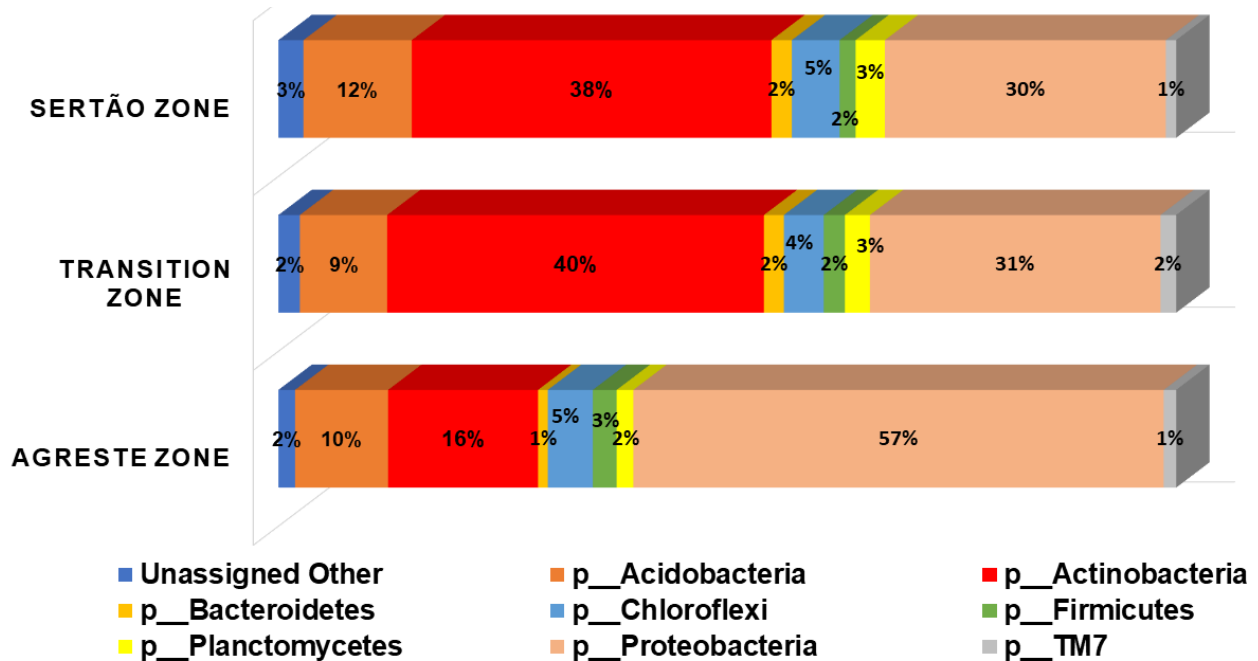


Figure 3. Relative abundance of the major taxonomic groups observed in the mesoregions of the State of Pernambuco.

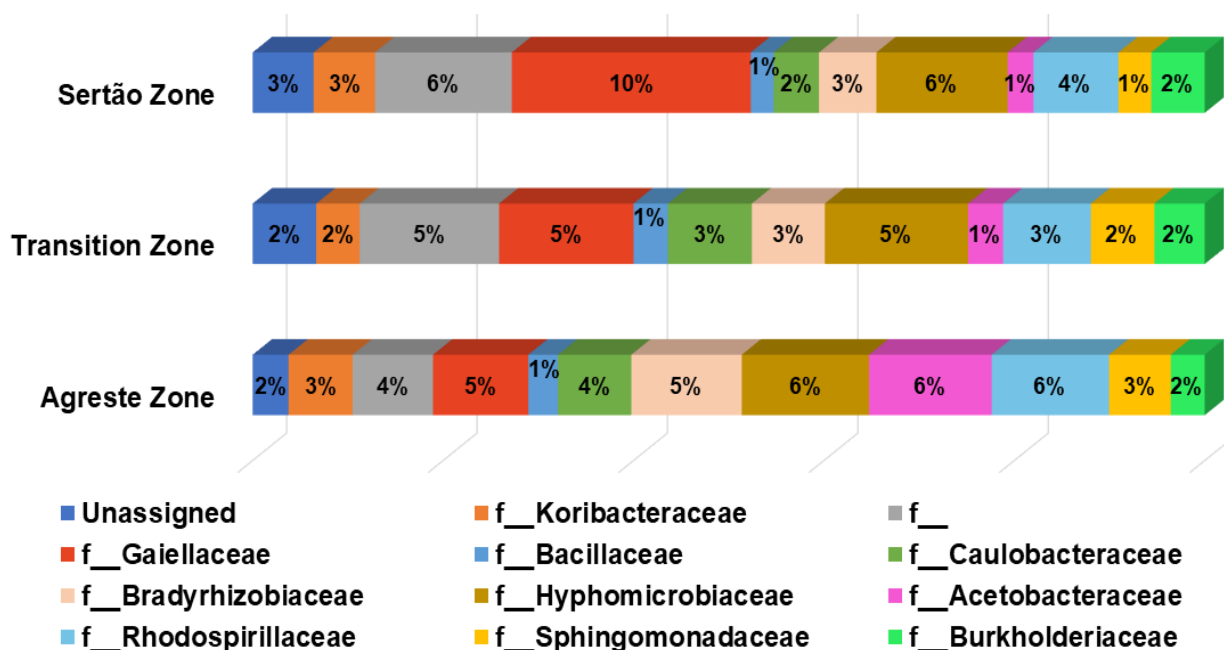


Figure 4. Taxonomic distribution of the microbial community of the soil at the level of family observed in mesoregions the state of Pernambuco.

resulted in the selection of organisms that resist extreme variation of climatic factors, such as the incidence of rain and high temperatures (Collins et al. 2008). Prickly-Pear Cactus are in the Cactaceae family, which is highly adapted to the semi-arid region. In the rhizosphere of these plants, it is possible that microbial communities interact with the Prickly-Pear Cactus roots.

Finally, in the Pernambuco semiarid region, the Caatinga is poorly studied, specifically on the structure of microbial communities and their roles in the environment. Thus, further studies are important understanding the role of these microorganisms and their adaptation to local plant species, such as the Prickly-Pear Cactus, which is important for this region.

CONCLUSIONS

This study showed the establishment of distinct bacterial communities in soils of different regions of Caatinga biome, i.e. Agreste, Transition, and Sertão. Therefore, the structure and diversity

of bacterial community were different in these areas and were influenced by environmental and soil conditions. In addition, the Prickly-Pear Cactus, as a highly adapted to semi-arid plant, can significantly modify the diversity of these micro-organisms. Future studies should be conducted to elucidate and validate the discrepancies identified in some taxa.

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MARIA DO CARMO C.P. DE LYRA¹

<https://orcid.org/0000-0003-1010-1692>

RODRIGO G. TAKETANI²

<https://orcid.org/0000-0002-5999-8704>

ANA DOLORES S. DE FREITAS³

<https://orcid.org/0000-0001-5808-097X>

CAROLINA E.R.S. E SILVA³

<https://orcid.org/0000-0001-8771-8055>

ADÁLIA C.E.S. MERGULHÃO¹

<https://orcid.org/0000-0003-1148-9967>

MARIA LUIZA R.B. DA SILVA¹

<https://orcid.org/0000-0002-8406-9472>

JADSON E.L.S. ANTUNES⁴

<https://orcid.org/0000-0001-7122-2506>

ADEMIR S.F. DE ARAÚJO⁴

<https://orcid.org/0000-0002-3212-3852>

POLIANA F. GIACHETTO⁵

<https://orcid.org/0000-0002-9991-6566>

¹Instituto Agrônomo de Pernambuco/IPA, Laboratório de Genômica, Avenida General San Martin, 1371, Bongi, 50761-000 Recife, PE, Brazil

²EMBRAPA Meio Ambiente, Rodovia SP-340, Km 127,5, Tanquinho Velho, Caixa Postal 69, 13820-000 Jaguariúna, SP, Brazil

³Universidade Federal Rural de Pernambuco/UFRPE, Laboratório de Microbiologia, Rua Dom Manoel de Medeiros, s/n, Dois irmãos, 52171-900 Recife, PE, Brazil

⁴Universidade Federal do Piauí, Centro de Ciências Agrárias, Rua Dirce de Oliveira, 3579, Campus da Soccopo, 64049-550 Teresina, PI, Brazil

⁵EMBRAPA Informática Agropecuária, Av. Dr. André Tosello, 209, Cidade Universitária, 13083-886 Campinas, SP, Brazil

Corresponding to: **Maria do Carmo C.P. de Lyra**

E-mail: mccatanho@gmail.com

Author contributions

The authors Maria do Carmo C. P. de Lyra and Poliana Gianchetto were the mentors of the work and the first author responsible for writing it, and both participated in all phases of the project. Ana Dolores S. Freitas and Carolina Santos collected all samples in the field. Adália Mergulhão, Maria L. Bastos and Jadson Antunes were responsible for the analyzes in the Laboratory and the researcher Ademir Araújo helped with the manuscript by making many contributions to it and the Researcher Rodrigo Taketani was responsible for all the bioinformatics analysis.

