

## Bacterial community in biological soil crusts from a Brazilian semiarid region under desertification process

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**ABSTRACT:** Biological soil crusts (BSC) are commonly found in soils in the drylands regions, which can influence stabilization, water retention, nutrient cycling (particularly carbon (C) and nitrogen (N) dynamics), and several ecological processes. However, the composition of BSC in Brazilian soils undergoing the desertification process remains poorly understood. This study aimed to characterize the bacterial community in BSC formed in a Brazilian semiarid region under the desertification process. Thus, a highly desertified region was selected from which 34 BSC samples were collected. The total DNA of the BSC was extracted from 0.5 g samples, and the bacterial community was sequenced by a Next Generation Sequencing (NGS) platform (Miseq – Illumina®) using universal primers (515F and 806R). Bioinformatic analysis was carried out in QIIME (v.1.9), and the Operational Taxonomic Units (OTU) table was constructed following the Sumacust methodology. The pH of BSC, C, N, and phosphorus contents was analyzed. Our study identified a diverse bacterial community in the BSCs. Cyanobacteria, Chloroflexi, and Proteobacteria phyla presented the greatest relative abundance (%) across the samples. Cyanobacteria were dominated by the orders Nostocales and Leptolyngbyales. The prediction of the putative functions found that most OTU were related to phototrophy, photosynthetic cyanobacteria, and photoautotrophy. The study found correlations between bacterial phyla and BSC properties, with Cyanobacteria positively related to C. Chloroflexi, Armatimonadetes, and WPS-2 were negatively correlated with C and N contents. These results suggest the critical roles bacteria communities play in BSCs from the Caatinga biome and highlight the potential impact of environmental factors on their diversity and functions.

**Keywords:** Caatinga, cyanobacteria, drylands, microbial ecology, biofilms

### Introduction

Biological soil crusts (BSC) are well-known structures found in soils which can be formed by the association of soil particles and photo and heterotrophic organisms such as cyanobacteria, microalgae, bacteria, fungi, lichens, mosses, and other organisms (Deng et al., 2020; Weber et al., 2022). BSC is usually found in arid and semiarid regions (Maestre et al., 2011) and acts on several soil processes, such as inputs of carbon and nitrogen dynamics (Belnap and Lange, 2003). Indeed, as primary producers, BSC can fix ~0.6 Tg C and ~24 Tg N per year (Rodríguez-Caballero et al., 2018).

As regards semiarid ecosystems, Brazil has ~20 % of its territory covered by semiarid conditions representing 1.8 million km<sup>2</sup> (Alvalá et al., 2019). This semiarid ecosystem is known as Caatinga and presents low pluviometry and high evaporation (Alvares et al., 2013). The overgrazing of native vegetation has accelerated soil desertification (Pereira et al., 2021), which contributes to the formation of BSC (Szyja et al., 2019) since cyanobacteria can increase their growth and bring about photosynthesis in this condition (Belnap and Lange, 2003).

Biological soil crust can stabilize soils and reduce soil losses (Deng et al., 2020). In addition, although BSC is mainly composed of cyanobacteria, eukaryotic algae, lichens, and bryophytes (Belnap and Lange, 2003), they can be highly biodiverse (Hernandez and Knudsen, 2012). It includes archaeal, bacterial, and fungal communities, which play essential biological processes, such as biomass degradation and nutrient cycling (Zhang et al., 2018a; Glaser et al., 2022).

In Brazilian semiarid regions previous studies have assessed the microbial community in BSC reporting cyanobacteria, algae, lichens, and bryophytes as the most abundant groups (Lima et al., 2019; Szyja et al., 2019; Lima and Branco, 2020). Although these studies on Brazilian semiarid regions assessed groups of organisms related to BSC the composition of bacteria community in BSC formed under an intense desertification process (i.e., such as Caatinga biome) still needs to be better understood.

Therefore, this study aimed to characterize the bacteria community in BSC formed in a Brazilian semiarid ecosystem under the desertification process (Caatinga biome). This study hypothesized that BSC formed under the desertification process reveals important microbial groups with potential functions.

To test this hypothesis, this study employed universal primers targeting the 16S rRNA gene, and the FAPROTAX approach was applied to assign ecological functions associated with BSC effectively.

## Materials and Methods

### Site description

The study was carried out in the municipality of Irauçuba, CE, Brazil, situated in the northern hinterland region of the state of Ceará, 150 km from Fortaleza (state capital), at geographic coordinates 3°44'46" S, 39°47'00" W, altitude 164 m. The region is part of the Caatinga biome and is one of the most affected desertification nuclei in the Brazilian semiarid region. The geographic location of the city, along with the sampling region, is visually depicted in Figure 1.

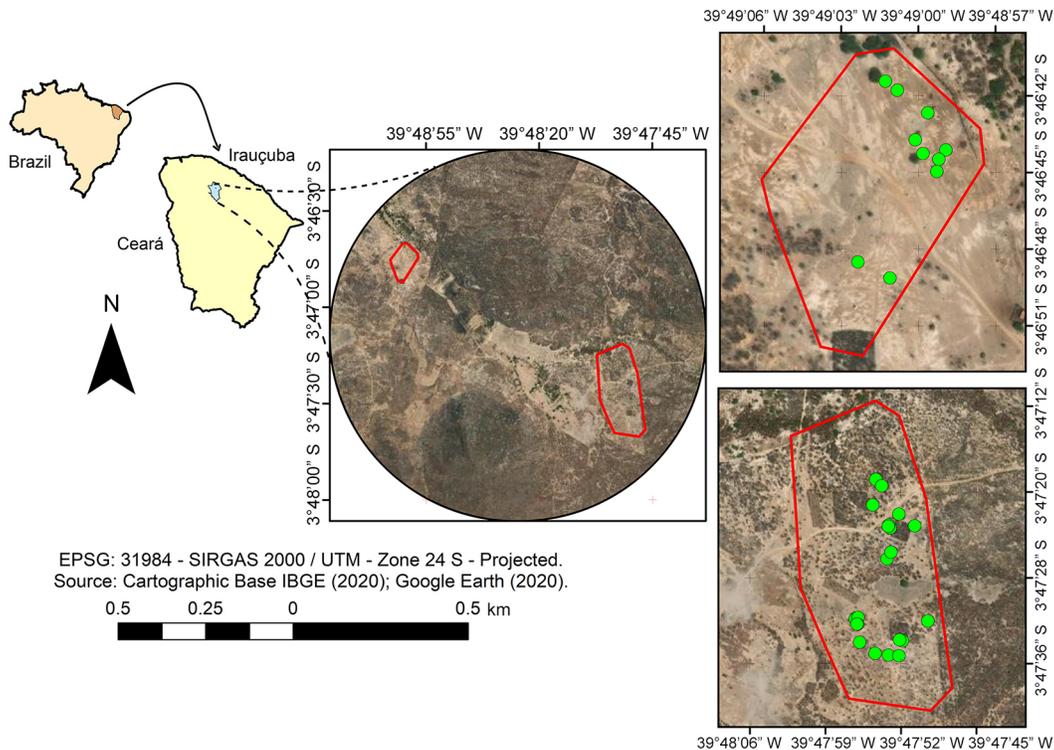
The local climate is semiarid, with an average annual rainfall of ~500 mm, concentrated from Jan to May. The climate defined by the Köppen-Geiger classification is BSw'h (hot semi-arid tropical), with average annual temperatures ranging from 26 to 28 °C (Alvares et al., 2013). The soil was classified as Eutrophic Haplic Planosol (EMBRAPA, 2020). The local economic activities are extensive livestock farming (e.g., sheep, goats, and cattle), as well as subsistence agriculture, which is often harmful to the soil as it does not consider the limitations of the

environment and employs low-level technological knowledge of agricultural management (Oliveira-Filho et al., 2019). These activities, which have experienced a significant escalation over the past three decades, are concurrently accelerating soil desertification in the region, mainly promoted by the detrimental effects of overgrazing (and a myriad of natural events such as low precipitation and high-temperature levels).

### Soil biological crust sampling and chemical characterization

Samples were collected from a highly desertified region, and 34 BSC samples were collected (Figure 1). The location of each sample was georeferenced by GNSS (Global Navigation Satellite System) (Figure 1). In some cases, due to the higher morphological heterogeneity of BSC, more than one BSC per spot was sampled. The BSCs (~10 - 15 g) were removed from the soil surface using spatulas and placed in glass Petri dishes (15 mm high × 100 mm diameter) to preserve them for molecular analysis.

In this study, we analyzed the pH, C, N, and P contents of BSC. BSC samples were first dried in a forced-air oven at 65 °C for 48 h. A potentiometer measured the pH using a soil solution suspension (1:2.5 - H<sub>2</sub>O) to ensure accurate results. Nitrogen content was determined using the semi-micro Kjeldahl method, a widely accepted technique for N



**Figure 1** – Irauçuba is a municipality geographically located in the state of Ceará, Brazil. The biological soil crusts were collected from highly desertified hotspots. The green circles on the maps represent the specific spot from which each crust was collected.

analysis. For P content, the sulfuric extraction method ( $\text{H}_2\text{SO}_4 - 1:1$ ) was adopted, and later, the molybdenum blue colorimetry method with a spectrophotometer was used to precisely quantify P contents. To assess total organic C, the Walkley-Black method was used, which involves oxidizing the organic carbon in potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) with sulfuric acid ( $\text{H}_2\text{SO}_4$ ) and then quantifying the excess dichromate through titration with ammonium ferrous sulfate. All procedures were carried out according to Teixeira et al. (2017).

### DNA extraction, amplification, and sequencing

The total DNA of the BSC was extracted from 0.5 g of each sample using the DNeasy® PowerLyzer® PowerSoil® Kit (Qiagen), according to the manufacturer's instructions. The DNA was quantified using the Nanodrop ND 1000 (Thermo Scientific) and verified by agarose gel electrophoresis (0.8 %).

The 16S rRNA gene was amplified in the V4 region using the primer set 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVHHHTWTCTAAT-3') (Caporaso et al., 2011). The amplification occurred in reactions consisting of 95 °C for 4 min, 60 °C for 1 min, 72 °C for 2 min, followed by 25 cycles at 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 2 min. For these primers a fragment size of 254 pb was expected.

The polymerase reaction products were purified using calibrated Agencourt AMPure XP beads (Beckman Coulter), paired, and sequenced using an Illumina MiSeq Reagent kit v2 (300 cycles, 2 × 150 bp) on an Illumina MiSeq sequencer (Illumina) at the Central de Genômica e Bioinformática (CeGenBio) at the Universidade Federal do Ceará, Brazil.

### Bioinformatic processing

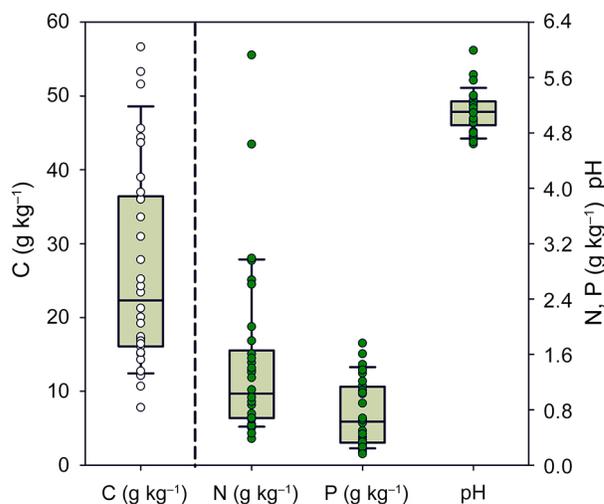
Raw sequences were analyzed using QIIME (Quantitative Insights Into Microbial Ecology) (v. 1.9) (Caporaso et al., 2010), following the instructions available on the QIIME website (qiime.org). The reads were filtered for quality, and chimeric sequences were detected and removed. The files were grouped into Operational Taxonomic Units (OTUs) using the Sumacust algorithm with a 97 % similarity threshold (Kopylova et al., 2016). Each OTU was taxonomically classified based on the SILVA (132) database (Quast et al., 2013). Relative abundance (%) was determined by dividing the counts (frequencies) of individual features (OTU) in each sample by the total count (frequency) of all features in that sample. In general, a total of ~2.5 million high-quality sequences were obtained, and the OTU table was rarified to 28,287 sequences, following the number of sequences present in the sample with the lowest value. The putative functions of BSC were predicted by accessing the FAPROTAX

database v. 1.2.3 (Louca et al., 2016). A Spearman correlation analysis was carried out to evaluate the correlations between the microbial groups and the chemical parameters of the BSC.

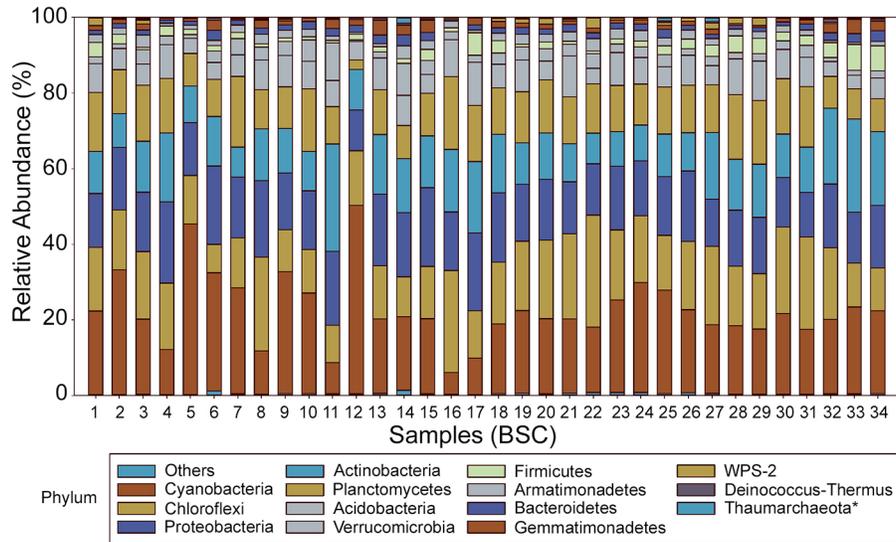
## Results

The pH value was acidic (4.5-6.0). Carbon, N, and P content were, on average, 23 g kg<sup>-1</sup>, 1.0 g kg<sup>-1</sup>, and 0.7 g kg<sup>-1</sup>, respectively (Figure 2). The results showed 53 phyla, 145 classes, 416 orders, 838 families, and 1895 genera. The most abundant phyla were Cyanobacteria (22 %), Chloroflexi (17 %), Proteobacteria (16 %), Actinobacteria (14 %), Planctomycetes (13 %), and Acidobacteria (7 %) (Figure 3). As regards the top three most abundant phyla, Cyanobacteria was dominated by the Nostocales (17 %) and Leptolyngbyales (8 %) (Figures 4A and B) orders. Chloroflexi consisted basically of Ktedonobacteria and Chloroflexia, while Proteobacteria was dominated by Alphaproteobacteria and Gammaproteobacteria. Furthermore, the class Alphaproteobacteria, which presented three orders (Rhizobiales, Acetobacterales, and Sphingomonadales) with high abundance in BSC samples stood out from the others.

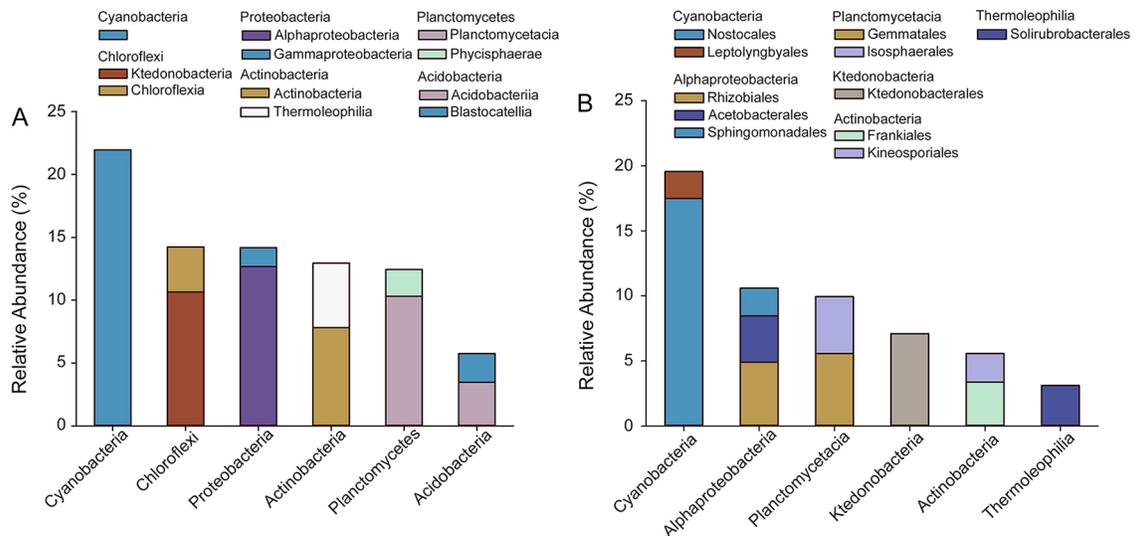
By using FAPROTAX as a model to predict putative functions of the bacterial community in BSC, a higher proportion of OTU was seen to be related to phototrophy, photosynthetic cyanobacteria, and Photoautotrophic organisms (Figure 5). A high number of OTU was also associated with chemoheterotrophy. The correlations between bacterial phyla and BSC properties showed Cyanobacteria positively related to C. However, Chloroflexi, Armatimonadetes, and WPS-2 correlated negatively with C, and N. In particular, Chloroflexi correlated negatively with pH (Figure 6).



**Figure 2** – Chemical characterization of biological soil crusts in a degraded hotspot of Brazilian semi-arid. C = soil organic carbon; N = total nitrogen; P = total phosphorus; and pH = hydrogen-ionic potential.



**Figure 3** – Relative abundance (%) of main phyla associated with biological soil crust in a hotspot of Brazilian semiarid under desertification. Other = low abundant (< 3 %) and unclassified. BSC = Biological soil crusts. \*Archaea.



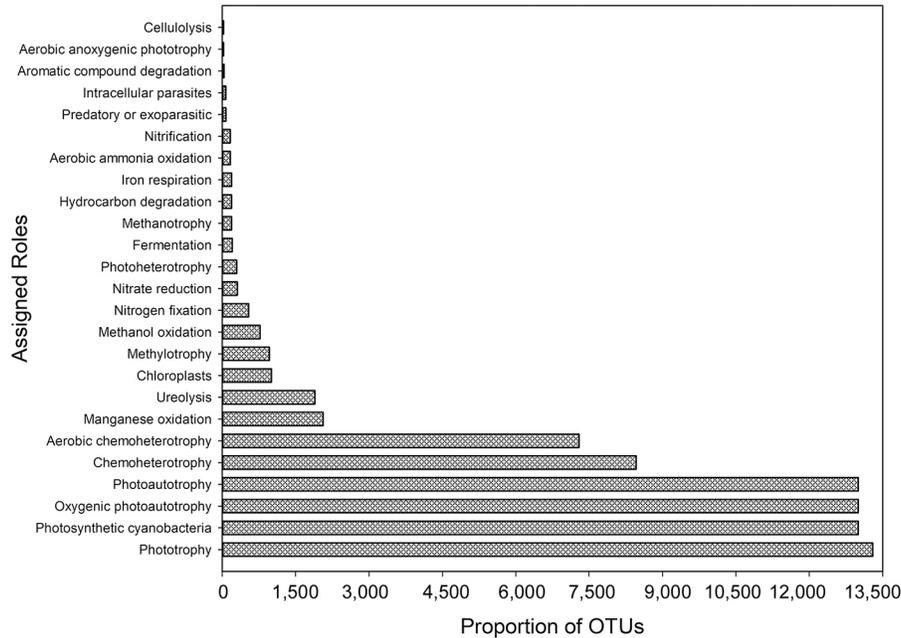
**Figure 4** – Absolute abundance (%) of bacterial community groups associated with biological soil crust in a degraded hotspot of Brazilian semiarid. A) phyla and classes, and B) classes and orders.

### Discussion

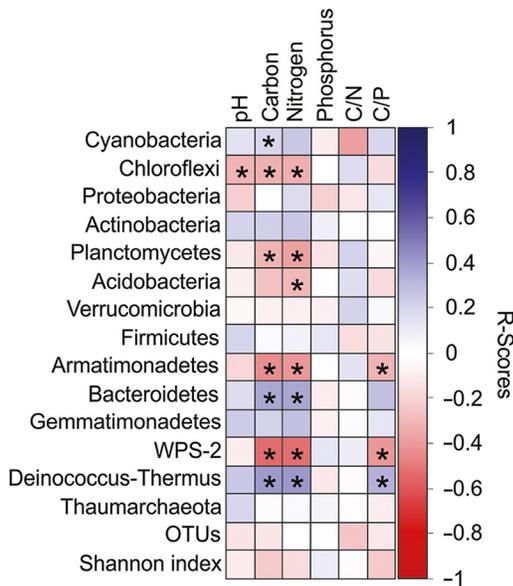
This pioneering study presents a comprehensive analysis of the bacteria community within BSC from highly degraded soil located in the Brazilian semiarid region. While previous studies have explored several groups of organisms of BSC in the Brazilian semiarid region (Lima et al., 2019; Szyja et al., 2019; Lima and Branco, 2020), this research study stands out as being the first to focus specifically on BSC samples from a highly desertified location. Our results showed Cyanobacteria and Chloroflexi to be the most abundant phyla found in BSC samples. Interestingly, previous studies also

have reported Cyanobacteria and Chloroflexi as being the chief components of the community in BSC (Li et al., 2013; Zhang et al., 2016), including those found in Brazilian semiarid regions (Lima et al., 2019; Lima and Branco, 2020). Since certain species of these groups of microorganisms are C-fixing and live phototrophically (Zhang et al., 2016), they could survive in BSC in regions under desertification and contribute as a C source to heterotrophs (Zhang et al., 2015).

With regard to the high relative abundance of cyanobacteria, they are key microorganisms in BSC (Warren et al., 2019; Samolov et al., 2020). In drylands, the soil surface is typically colonized by cyanobacteria,



**Figure 5** – Functional profile of biological soil crust in a degraded hotspot of Brazilian semiarid predicted by FAPROTAX database. OTU = Operational taxonomic unit.



**Figure 6** – Spearman ranking correlation test between main biological soil crust phyla and chemical characterization. \*Means significant correlation (%), blue and red are positive and negative, correlation respectively. OTU = Operational taxonomic unit.

which could subsequently initiate the formation of BSC (Weber et al., 2022). This may partially explain the higher relative abundance of Cyanobacteria in BSC samples from Brazilian semiarid regions under desertification. Additionally, Cyanobacteria release substances that bind the soil particles (Chaudhary et al., 2009), which is an essential strategy for soil restoration (Rocha et al.,

2020). It aligns with our hypothesis that the BSC from highly degraded regions can reveal important microbial groups. This bacterial group is a well-known primary producer, and certain groups would be able to fix N<sub>2</sub> from the atmosphere (Karlson et al., 2015). Therefore, this high abundance of cyanobacteria could contribute to soil restoration (by adding C and N).

Nostocales was the most abundant bacterial order, and it agrees with what has been previously reported by Nostocales as an abundant group in BSC worldwide (Yeager et al., 2004; Wang et al., 2020). These heterocystous cyanobacteria encode *ni*/H genes with diazotrophic ability found in crusts from desert ecosystems (Yeager et al., 2004), and this ability to fix N<sub>2</sub> is important to arid regions (Williams et al., 2016).

Members of Chloroflexi were also found in high relative abundance in BSC samples, which can be explained as this phylum presents several oligotrophic microbes with high tolerance to stressful conditions (Costello and Schmidt, 2006), such as soil desertification. In this phylum, the class Ktedonobacteria, which comprises microorganisms with high adaptation to extreme oligotrophic conditions such as deserts (Lynch et al., 2012), dominated our BSC samples. The phylum Proteobacteria found in high relative abundance was dominated by Alphaproteobacteria and Gammaproteobacteria. A number of studies have also reported that among non-phototroph microbes, Proteobacteria are found in high abundance in biological soil crusts, which act as N<sub>2</sub>-fixers, ammonia-oxidizers, and denitrifiers (Garcia-Pichel et al., 2003; Nagy et al., 2005; Zhang et al., 2014). On a deeper level, in BSC samples, Rhizobiales, Acetobacterales, and Sphingomonadales

orders were in high abundance. These bacterial orders comprise  $N_2$ -fixers associated with roots that can provide N to mosses and vascular plants in BSC (Pepe-Ranney et al., 2016; Saravanan et al., 2008; Salazar et al., 2022). These bacteria can be potentially important to biological crusts when used for restoration strategies in the desertification process (Deng et al., 2020).

By using FAPROTAX, potential microbial functions were predicted, most of which are related to photoautotrophic microbes. Indeed, the bacterial composition of BSC in our study consists of Cyanobacteria and Chloroflexi. A previous study reported that photoautotrophs dominate BSC and control the abundance of other microbes mainly when the environmental conditions change (Maier et al., 2018). Therefore, in soil under desertification, the dominance of photoautotrophs could favor the abundance of important bacterial groups, such as Proteobacteria, which can explain the high abundance of this phylum. Another important predicted function was photosynthesis, which is related to chlorophyll-containing oxygenic photoautotrophs (Cyanobacteria) that are fundamental to BSC development (Tang et al., 2018). In addition, these organisms contribute to C and  $N_2$  fixation, and thereby improve the nutrient status in BSC (Hallenbeck, 2017). Ecologically, photoautotrophs like cyanobacteria stimulate the growth of other microbes and promote BSC development (Tang et al., 2018). This presence of chemoheterotrophs is significant since these organisms can store organic C and use it as an energy source, mainly when the environmental conditions are unfavorable (Hauschild et al., 2017), such as in regions under desertification (Pereira et al., 2022).

It was observed that Cyanobacteria was positively correlated to C content. This positive correlation is related to the ability of Cyanobacteria to fix C in biological soil crusts (Zhang et al., 2018a). This suggests that Cyanobacteria play a crucial role in carbon cycling and accumulation within this ecosystem (Zhang et al., 2018b). Furthermore, Chloroflexi, Armatimonadetes, and WPS-2 were negatively correlated with C, and N even though they are in the same crusts that cyanobacteria were (possibly) fixing C and N. Thus, additional studies are needed to explain and make meaningful conclusions or implications, including specific ecosystems and methods used to measure Cyanobacteria abundance and C and N contents.

This study provided a comprehensive overview of the bacterial diversity and putative functions in biological soil crusts (BSCs) in a highly degraded Brazilian semiarid region. The bacterial community in BSCs was dominated by Cyanobacteria, with Nostocales being the most abundant order. The putative functions of the bacterial community in BSCs were predicted to be mainly related to phototrophy, photosynthetic cyanobacteria, and chemoheterotrophy. Furthermore, there were significant correlations between bacterial

phyla and BSC properties, with Cyanobacteria being positively related to C content, and Chloroflexi, Armatimonadetes, and WPS-2 negatively correlated with C and N contents.

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## Authors' Contributions

**Conceptualization:** Pinheiro JI, Mendes Filho PF, Melo VMM, Pereira APA. **Data curation:** Pinheiro JI, Mendes Filho PF, Melo VMM, Pereira APA. **Formal analysis:** Pinheiro JI, Mendes Filho PF, Melo VMM, Pereira APA. **Funding acquisition:** Mendes Filho PF, Melo VMM, Pereira APA. **Methodology:** Pinheiro JI, Garcia KG, Moreira JV, Silva DF, Mendes Filho PF, Melo VMM, Pereira APA. **Project administration:** Mendes Filho PF. **Supervision:** Mendes Filho PF. **Writing-original draft:** Pinheiro JI, Mendes Filho PF, Araujo ASF, Verma JP, Melo VMM, Pereira APA. **Writing-review & editing:** Pinheiro JI, Mendes Filho PF, Araujo ASF, Verma JP, Melo VMM, Pereira APA.

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