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SOIL MICROBIOLOGY

Characterization of Soil Bacterial Assemblies in Brazilian Savanna-Like Vegetation Reveals Acidobacteria Dominance

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Abstract The Brazilian Cerrado is the second largest biome in Brazil and is considered a biodiversity hotspot. In this work, we compared the bacterial communities in Cerrado soil associated with four types of native vegetation (Cerrado Denso, Cerrado *sensu stricto*, Campo Sujo, and Mata de Galeria) by ribosomal RNA intergenic spacer analysis, terminal fragment restriction length polymorphism and pyrosequencing. The fingerprinting results were very similar. The bacterial communities of Cerrado Denso and Cerrado *sensu stricto* grouped together and were distinct from those in Campo Sujo and Mata de Galeria. Pyrosequencing generated approximately 40,000 16S rRNA gene sequences per sample and allowed the identification of 17 phyla in soil samples under Cerrado vegetation. Acidobacteria were dominant in all areas studied with a relative frequency of 40–47 %, followed closely by Proteobacteria accounting for 34–40 % of the sequences. Results from all molecular techniques used suggested that the bacterial communities of Cerrado *sensu stricto* and Cerrado Denso are very similar to each other, while Campo Sujo forms a separate group, and Mata de Galeria is the most distinct with higher species richness. This is the first extensive study of native Cerrado soil microbiota, an important but endangered biome.

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Introduction

The Brazilian Cerrado, a savanna-like region of central Brazil, is characterized by a continuous grass cover, usually with trees and shrubs [1]. Savanna vegetation is composed of communities of variable floristic composition, structurally ranging from open fields to woodlands [2]. The dynamics of these ecosystems is strongly influenced by the soil moisture, nutrient availability, and natural fire events that occur at various intensities [3]. The Cerrado biome is the second largest biome in Brazil and covers 24 % of the Brazilian territory, nearly 2,000,000 km^2. Some of the most distinctive vegetation types, which are the focus of this study, are in order of increasing woody cover: Campo Sujo, Cerrado *sensu stricto*, Cerrado Denso, and Mata de Galeria (see Fig.

S1). Campo Sujo is defined as grassland with scattered shrubs and small trees. Cerrado *sensu stricto* is the most abundant Cerrado vegetation type and is characterized as woodland savanna with a continuous grass layer and a woody layer of trees and shrubs varying in cover between10 and 60 %. Cerrado Denso has a dense tree layer ranging from 5 to 8 m of height. Finally, Mata de Galeria (gallery forest) is a forest formation that accompanies watercourses forming closed vegetation corridors [4, 5]. Total annual precipitation is around 1,500 mm with a strong seasonal distribution. Almost 90 % of the annual precipitation falls during the rainy season that begins in September/October and ends in April/May. Approximately 45 % of Cerrado total area presents clayey and dystrophic soils with low pH, low availability of calcium, magnesium, and phosphorus, and high aluminum content [6].

Studies of microbial diversity were historically hampered by the difficulty of cultivating the majority of microorganisms. The adoption of molecular techniques in which nucleic acids are studied directly bypass the need for cultivation of microorganisms to characterize microbial communities. These techniques have enabled investigation of the bacterial diversity in various soils [7–14]. Recently, pyrosequencing technology has been used to characterize microbial communities in detail since it produces a large number of DNA sequences, making it possible to detect low-abundance operational taxonomic units (OTUs) [15]. The development of bioinformatics tools has allowed unprecedented analysis of microbial communities of various environments [7, 16, 17]. As with any technique, pyrosequencing has its limitations. Errors associated with homopolymeric runs may lead to an overestimation of microbial diversity [18, 19]. To circumvent this problem, bioinformatics tools and analysis parameters were developed [20, 21]. The ubiquitous 16S rRNA genes are widely used to assess the bacterial diversity in the environment, to detect and quantify specific bacterial populations, and to determine phylogenetic relationships among bacterial groups [22, 23].

Several studies have described the diversity of plants [24, 25] and animals [26, 27] in Cerrado ecosystems. A few pioneering studies reported a partial characterization of bacterial and fungal microbial diversity using Sanger sequencing of 16S rRNA and 18S rRNA gene clone libraries, as well as fingerprinting technique to compare native areas with areas converted to pasture [28–31].

In the last 40 years, almost 50 % of the Cerrado's natural cover has been converted to other uses, in spite of the high biodiversity and the world's recognition of the region as one of the hotspots for biodiversity conservation [32]. A central and still open question is whether the diversity of vegetation composition and structure is related to the diversity of soil bacterial composition. The aim of the present work was to study and compare the bacterial communities from Cerrado soils associated with different types of native vegetation using different molecular techniques.

Materials and Methods

Soil Sampling and Analysis

Soil samples were collected in areas of Campo Sujo (15°56′ 54.6″ S, 47°52′11.7″ W), Cerrado *sensu stricto* (15°57′02.4″ S, 47°52′32.1″ W), Cerrado Denso (15°56′43.1″ S, 47°51′ 26.0″ W), and Mata de Galeria (15°57′06.0″ S, 47°53′18.7″ W). Samples were collected in the beginning of the rainy season of October 2006, with 302 mm of precipitation in October after five dry months with a total of 170 mm of precipitation measured at Ecological Reserve of IBGE, Federal District, Brazil. Approximately 500 g of soil from each vegetation type were collected from the upper layer (0–10 cm) and were sieved (2 mm mesh) to remove large particles. Two transects 200 m apart from each other were marked for each vegetation type. Five sampling points separated by 50 m were defined in each transect, and ten soil samples were collected per point to constitute a composite sample per sampling point, totaling five samples per transect. Five composite samples per transect were then combined making one sample per transect (two composite samples per vegetation type). The two composite samples per vegetation type were named "1 to 5" and "6 to 10." For the Mata de Galeria samples, there was a topographic gradient perpendicular to the river, with sample "1" being the closest to the water and the lowest point in the gradient. Physicochemical properties of the soil samples were determined by standard methods (Solo Quimica, Inc., Brazil; see Table S1).

Soil DNA Extraction

Total microbial community DNA present in the soil samples was extracted using the PowerSoil™ DNA Isolation Kit (MOBIO Laboratories, Inc.) according to manufacturer's instructions. DNA was separated by electrophoresis in 1 % agarose gels that were then stained with ethidium bromide and photographed.

Ribosomal RNA Intergenic Spacer Analysis

The intergenic spacer region from *Eubacteria* was amplified using 20 ng of microbial DNA from soil as a template using oligonucleotide primers 1406F (5′- TGY ACA CAC CGC CCG T - 3′) and L1R (5′- CAA GGC ATC CAC CGT - 3′), in a 20-μl PCR reaction mixture containing 1× PCR buffer (Tris–HCl 10 mM Ph 8.3, KCl 5 mM, $MgCl_2$ 1.5 mM), 0.25 mM dNTP, 5 μg bovine serum albumin, 1.5 U Taq

DNA polymerase (Phoneutria, Belo Horizonte, BR) and 10 pmol of each primer. PCR amplification was performed using an initial denaturation step for 3 min at 95 °C, followed by 29 cycles of denaturation for 60 s at 95 °C, annealing for 90 s at 53 °C, and extension for 3 min at 72 °C, followed by a final extension for 30 min at 72 °C and cooling to 10 °C. PCR products were separated by electrophoresis for 2 h at 45 W on a 6 % polyacrylamide gel. Band profiles were visualized after staining with silver nitrate and analyzed with the software Bionumerics (Applied Maths, Belgium). Dendrograms were constructed using the unweighted pair group method with arithmetic mean (UPGMA) algorithm and the Dice's similarity coefficient.

Terminal Fragment Restriction Length Polymorphism

For terminal fragment restriction length polymorphism (T-RFLP) analysis, 16S rRNA gene fragments were amplified from the soil DNA samples using the primers Eub-8Fm (5′ AGA GTT TGA TCM TGG CTC AG 3′) labeled with 6-carboxyfluorescein at its 5′ end and Eub-926R (5′ CCG TCA ATT CCT TTR AGT TT 3′) [33]. Each PCR reaction had a total volume of 20 μl containing 1× PCR buffer (Invitrogen), 3.0 mM $MgCl_2$, 10 pmol of each primer, 0.25 mM dNTP, and 1.5 U Taq DNA polymerase (Invitrogen). PCR amplification was performed with an initial denaturation step for 3 min at 95 °C, followed by 25 cycles of denaturation for 30 s at 95 °C, annealing for 45 s at 60 °C, and extension for 2 min at 72 °C, followed by a final extension for 5 min at 72 °C and cooling to 10 °C. The quality and quantity of amplification products were examined by electrophoresis in agarose gels (1 %) followed by ethidium bromide staining. Five microliters of PCR product was digested at 37 °C for 8 h with 10 U of restriction endonuclease *Msp*I (Promega). The digested PCR amplicons were precipitated with 0.1 volume of 3 M sodium acetate and 2.5 volumes of 99 % ethanol. PCR product was digested at 37 °C for 8 h with 10 U of restriction endonuclease *Msp*I (Promega).

Terminal restriction fragments (T-RFs) were analyzed on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). GS-500 LIZ (Applied Biosystems–Life Technologies) was loaded as an internal size standard in each lane. The electrophoretic resolution of the T-RFLP fragments ranged from 50 to 500 bp. T-RFLP data quality check and formatting were performed using the T-RFLP Expedited (T-REX) online tool [34]. The data were subjected to quality control procedures: T-RF (peaks) alignment (clustering threshold=0.5) and noise filtering (peak area, standard deviation multiplier=1). Matrices containing peak area data were evaluated for the contribution of each variable in the community conformation using the Additive Main Effects and Multiplicative Interaction (AMMI) model available in the T-REX tool. The experiment was performed with two replicates.

Pyrosequencing and Statistical Analysis

For pyrosequencing analysis, 16S rRNA gene fragments were amplified from the soil DNA samples using the primers that flank the V5 to V9 hypervariable regions of bacterial 16S rRNAs [35]. For amplification of *Eubacteria* sequences, primer A-787F (5′- gcctccctcgcgccatcag ATTAGATACCCIGGTAG-3′) and B-1492Rm (5′- gccttgccagcccgctcagGITACCTTGTTACGACTT-3′) were used. The oligonucleotide design was modified from the Roesch et al. [11] sequences to include 454 Life Science's A or B sequencing adapters (shown in lowercase) at the 5′ end of each primer. The PCR reaction in a total reaction volume of 20 μl contained 1X PCR buffer (Invitrogen), 3.0 mM $MgCl_2$, 10 pmol of each primer, 0.25 mM dNTP, and 1.5 U Taq DNA polymerase (Invitrogen). PCR amplification was performed using an initial denaturation step of 3 min at 95 °C, followed by 25 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 58 °C, and extension for 1.40 min at 72 °C, followed by a final extension for 7 min at 72 °C and cooling to 10 °C. PCR products were purified with the QIAquick PCR Purification Kit (QIagen). Amplicons were sequenced by the GS FLX Titanium (454 Life Sciences). The data were deposited in GenBank under the accession number SRA029260.

Data processing was performed using the QIIME pipeline [36]. Scripts were run using default options and orchestrated by the GALAXY workflow system [37]. The starting point was flowgram denoising to reduce pyrosequencing errors [38]. OTUs were defined by clustering sequences using UCLUST [39], and taxonomic levels were assigned according to the Ribosomal Database Project (RDP) classification system [40]. OTU counts per sample were rarefied and used to compute alpha (within a sample) and beta (between samples) diversity indices [41]. For the latter, unweighted and weighted UniFrac were used as the phylogenetically based distance metric [42]. Statistical tests about taxonomic differences between the samples were computed using the software Statistical Analysis of Metagenomic Profiles (STAMP) [43].

Results

Soil Analysis

Analysis of soil physicochemical properties for Campo Sujo, Cerrado *sensu stricto*, Cerrado Denso, and Mata de Galeria (see Table S1) shows that the soil pH is acidic in all areas, ranging between 4.3 and 4.9. The principal

component analysis (PCA) of soil physicochemical parameters (see Fig. S2) grouped the Cerrado *sensu stricto* and Cerrado Denso samples in the same quadrant and separated Campo Sujo and Mata de Galeria samples in different quadrants. The soil of Cerrado *sensu stricto* and Cerrado Denso shows higher clay content in comparison to Campo Sujo and Mata de Galeria. This physicochemical parameter was the major factor grouping Cerrado Denso and Cerrado *sensu stricto* in the same PCA quadrant. The organic carbon content in samples from Mata de Galeria is almost twice the content found in the other three areas (see Table S1). The concentration of available phosphorus (P) in soil samples from Mata de Galeria is approximately five times higher than that in the other areas. Moreover, the concentration of calcium (Ca) in Mata de Galeria is approximately two times higher than in the other areas studied. Thus, organic carbon, P, and Ca were the major factors separating Mata de Galeria from the other vegetation soil types (see Fig. S2). In general, it was observed that the soil physicochemical properties of Cerrado *sensu stricto* and Cerrado Denso are similar to each other and Campo Sujo and Mata de Galeria are different from all the others.

Bacterial Community Profile in Cerrado Savanna Soils

DNA was successfully extracted from soil samples of all studied areas. Analysis of each ribosomal RNA intergenic spacer analysis (RISA) profile revealed differences in band distribution reflecting distinct compositions of bacterial communities associated with the vegetation types. However, there were also several bands of similar molecular weight, indicating that there may be many bacterial species in common among the areas of the Cerrado. Analysis with Bionumerics software (Fig. 1) grouped the composite replicated samples "1 to 5" and "6 to 10" (see "Materials and Methods") of each area in the same clade, showing no major differences between the two sampling transects within each area. Campo Sujo, Cerrado Denso, and Cerrado *sensu stricto* were grouped in the same cluster, but Cerrado Denso and *sensu stricto* showed a bacterial community profile more similar to each other (73 % of similarity) when compared to Campo Sujo and were grouped closer together. Mata de Galeria presented the most different bacterial community profile in comparison to the other areas. The relationship of soil bacterial communities from different vegetation types was also investigated by AMMI analysis of T-RFLP data using the software T-REX (Fig. 2). The number of T-RFs ranged between 44 and 59 in the 16 samples (i.e., eight soil samples, two for each vegetation type, with two replicates). T-RFLP also indicated no major differences between composite replicated samples "1 to 5" and "6 to 10" of each area. Distinct differences in bacterial community composition among vegetation types were observed by T-RFs analysis. Interaction principal components axes (IPCAs), as a result of AMMI analysis, located Cerrado Denso and Cerrado *sensu stricto* very close in the same quadrant, suggesting a similar bacterial community composition between these two vegetation types. Mata de Galeria and Campo Sujo were located alone in different quadrants suggesting more distinct bacterial communities.

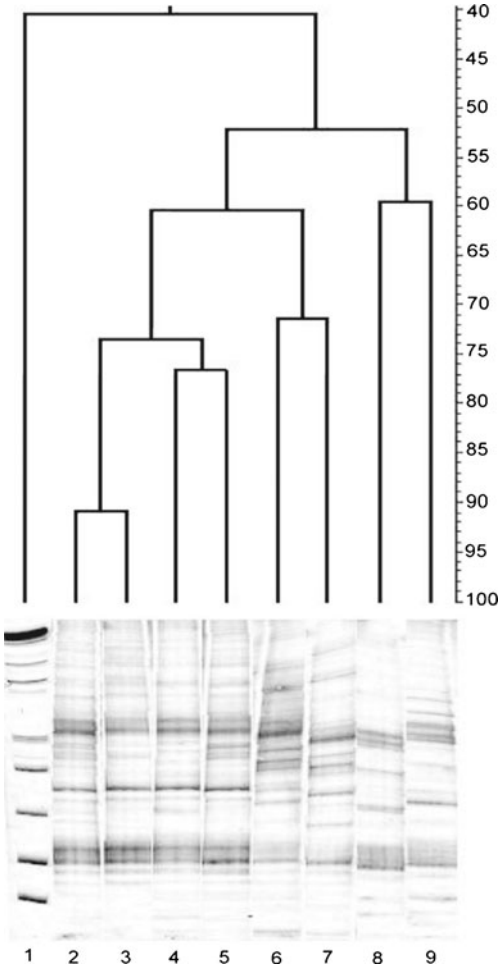

Figure 1 Dendrogram based on RISA profiles of bacterial communities associated with Cerrado vegetation physiognomies. The dendrograms were constructed using the UPGMA algorithm and DICE similarity coefficient. *1* 1 kb plus ladder; *2* Cerrado *sensu stricto* sample "1 to 5;" *3* Cerrado *sensu stricto* sample "6 to 10;" *4* Cerrado Denso sample "1 to 5;" *5* Cerrado Denso sample "6 to 10;" *6* Campo Sujo sample "1 to 5;" *7* Campo Sujo sample "6 to 10;" *8* Mata de Galeria sample "1 to 5;" *9* Mata de Galeria sample "6 to 10"

Composition of Soil Bacterial Community under Different Cerrado Vegetation Types

About 300,000 high quality 16S rRNA gene sequences were obtained in this study (27,926–44,832 sequences per sample). Using Classifier (RDP), a total of 17 phyla were identified in the four Cerrado vegetation types (Fig. 3). Seven phyla (Acidobacteria, Proteobacteria, Actinobacteria, Verrucomicrobia,

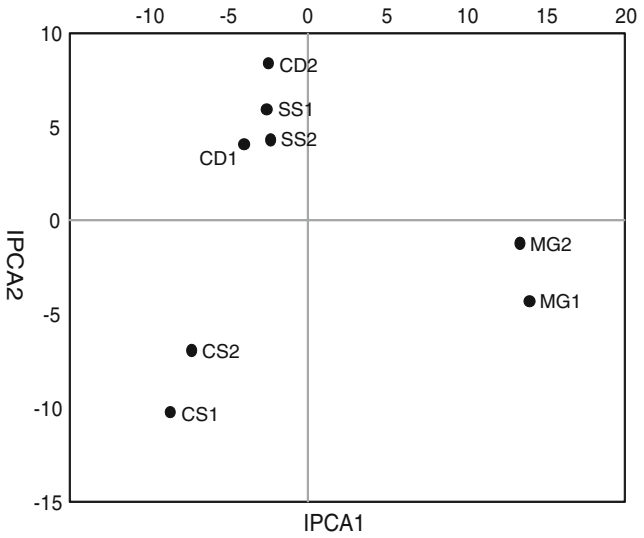

Figure 2 Interaction principal components axes (IPCA) of T-RFLP analysis of Cerrado soil bacterial communities using the T-REX Software. The bacterial community found in Campo Sujo soil sample "1 to 5" is shown as CS1, in Campo Sujo sample "6 to 10" as CS2; in Cerrado Denso sample "1 to 5" as CD1; in Cerrado Denso sample "6 to 10" as CD2; in Cerrado *sensu stricto* sample "1 to 5" as SS1; in Cerrado *sensu stricto* sample "6 to 10" as SS2; in Mata de Galeria sample "1 to 5" as MG1 and in Mata de Galeria sample "6 to 10" as MG2

Planctomycetes, Gemmatimonadetes, and Bacteriodetes) were considered abundant with sequence frequencies above 1 % (Fig. 3a), and 10 phyla (Chlamydiae, Firmicutes, OP10, TM7, Chloroflexi, Cyanobacteria, Nitrospira, Spirochaetes, Thermomicrobia, and BRC1) were considered low abundance with sequence frequencies below 1 % (Fig. 3b).

The phylum Acidobacteria was dominant in all the communities studied, corresponding to 40–47 % of the sequences analyzed. Sequences corresponding to 10 Acidobacteria subdivisions (Gp1, Gp2, Gp3, Gp4, Gp5, Gp6, Gp7, Gp8, Gp10, and Gp13) were identified (see Fig. S3). Subdivision Gp1 was the most abundant, accounting for 52 % of Acidobacteria in all four soil types (results not shown). Subdivision Gp8 was only present in Mata de Galeria (see Fig. S3) and in low abundance (0.07 %) (results not shown). Proteobacteria were also abundant in all four areas studied, corresponding to 34–40 % of all the sequences. The classes Alphaproteobacteria, Gammaproteobacteria, Betaproteobacteria, Deltaproteobacteria, and unclassified Proteobacteria were found in the four areas studied. The most abundant class was Alphaproteobacteria, corresponding to 52–57 % of all Proteobacteria sequences (results not shown). Unclassified bacteria represented the third most abundant group in all four Cerrado areas, corresponding to 8–13 % of all sequences. A slightly larger proportion of unclassified sequences was found in Campo Sujo.

Rarefaction curves (Fig. 4), calculated using a cutoff of 0.03 (defined by the threshold of 97 % sequence similarity) from the average value for each area, revealed 3,430 OTUs in Campo Sujo; 4,459 OTUs in Cerrado *sensu stricto;* 4,666 OTUs in Cerrado Denso; and 6,728 OTUs in Mata de Galeria. According to this result and to the OTU richness estimated by Chao1 (data not shown), the bacterial microbial community found in the soil of Mata de Galeria is more species rich than those found in the soils of the other vegetation types. However, the rarefaction curves indicated that the diversity at the species level did not reach an asymptote in this study despite the high number of sequences obtained. These results demonstrate the extensive bacterial diversity associated with Cerrado soils.

Simultaneous comparison of all four bacterial communities (in duplicate) was accomplished by Unifrac Principal Coordinate Analysis (PCoA). Unifrac analysis showed that soil bacterial communities from the four Cerrado vegetation types are quite distinct from each other. The analysis was carried out either unweighted (Fig. 5a), using only presence–absence information, or weighted (Fig. 5b), which takes into account the relative proportions of DNA sequences in each group. Results for both PCoA show that Mata de Galeria replicates "1 to 5" and "6 to 10" are separated from the other areas. Cerrado Denso "1 to 5" and "6 to 10" are together in unweighted analysis (Fig. 5a) and distant in the weighted analysis (Fig. 5b).

Discussion

In this paper, we report the differences and similarities among soil bacterial communities associated with four Cerrado vegetation types. To obtain representative data for the soil under each vegetation type, composite samples were obtained and analyzed by different molecular methods. The application of RISA and T-RFLP to study Cerrado soil bacterial communities showed that Cerrado *sensu stricto* and Cerrado Denso have similar bacterial community profiles, since the samples from these areas clustered together in RISA analysis (Fig. 1) and were located next to each other in T-RFLP-IPCA (Fig. 2). Both Cerrado *sensu stricto* and Cerrado Denso areas showed great similarity in soil physicochemical properties and vegetation structure, followed by Campo Sujo and Mata de Galeria with more disparate soil and vegetation characteristics. The studied Mata de Galeria presents a topographic gradient that determines changes in soil properties along the slope (e.g., soil bulk density, organic matter, and humidity). The location of the sampling points along this gradient may explain some differences in the concentrations of nutrients and organic carbon in soil between the composite samples "1 to 5" and "6 to 10" (see Table S1). There was a striking similarity between the PCA of soil physicochemical proprieties (see Fig. S2) and the T-RFLP-IPCA (Fig. 2), suggesting that soil

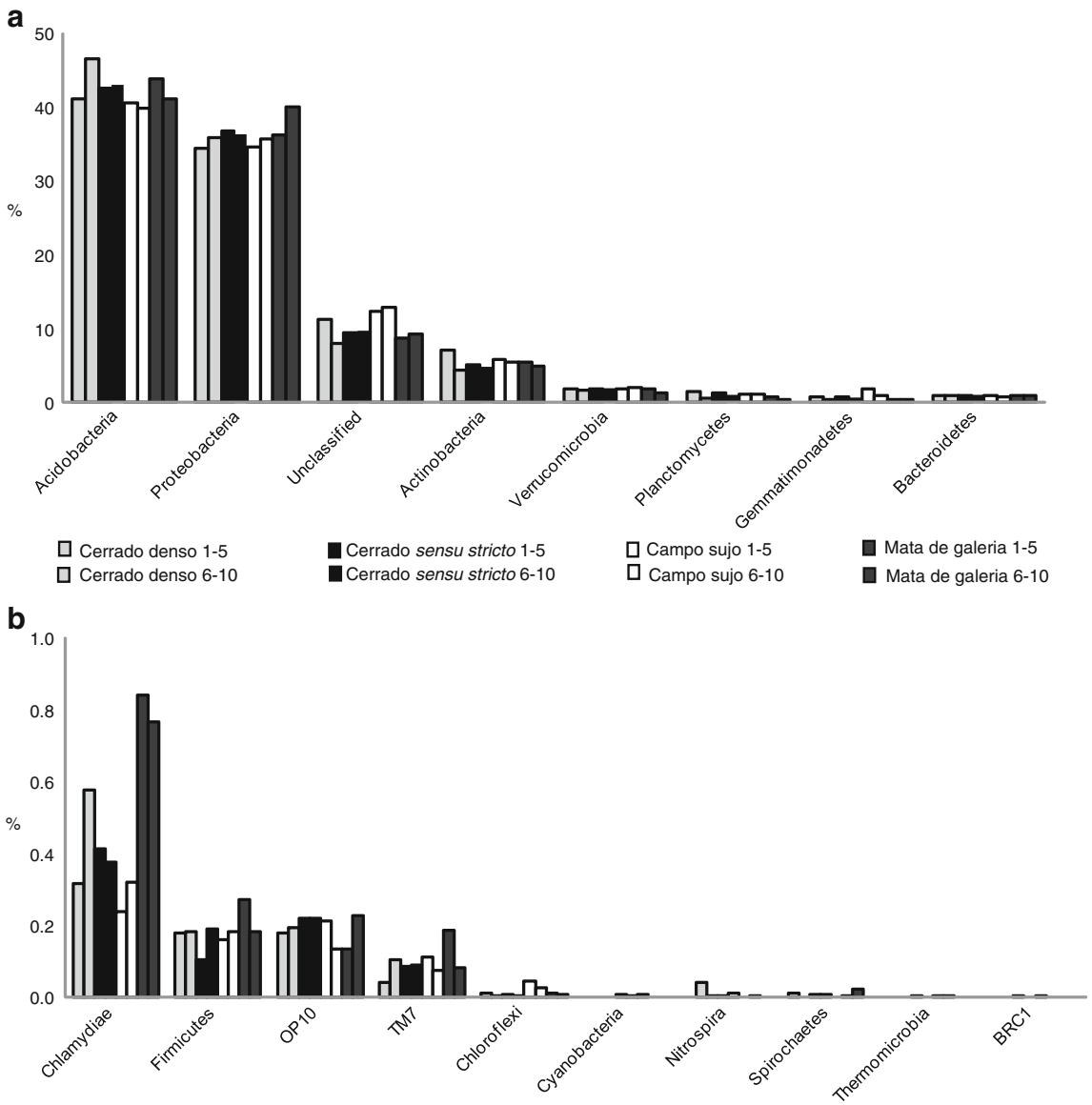

Figure 3 Frequency of bacterial phyla present in different Cerrado soil vegetation physiognomies according to pyrosequencing analysis in The classifier of Ribosomal Database Project. **a** Dominant phyla with relative frequencies >1 %. **b** Rare phyla with relative frequencies lower than 1 %

physicochemical properties may be the key determinant of Cerrado bacterial communities' profile. RISA and T-RFLP profiles were important as initial snapshot comparisons, and the results also showed no major differences between the composite samples within each area ("1 to 5" and "6 to 10").

Based on the initial results obtained by fingerprinting techniques, a detailed analysis was pursued by amplification followed by pyrosequencing of 16S rRNA genes. The Acidobacteria phylum was the most abundant in all four areas studied. In this respect, Cerrado soil microbial communities are similar to other soil communities since Acidobacteria and Proteobacteria phyla are the most abundantly distributed bacterial groups in the soil environment [44]. Furthermore, a recent study of the Brazilian Atlantic Forest soil showed that the Acidobacteria phylum was also dominant under this vegetation type [45]. More than 30 % of the sequenced segments from 16S rRNA clone libraries from soils belonged to Acidobacteria [46]. In Cerrado soils, subdivisions Gp1, Gp2, and Gp3 were the most abundant in soil for all four studied areas, representing more than 96 % of the Acidobacteria sequences found. This corroborates the results of a recent study of soils collected throughout North and South America that showed Acidobacterial subgroups Gp1, Gp2, Gp3, Gp4, and Gp6 as the most abundant [47]. Although Acidobacteria are abundant, little is known about the physiological and ecological characteristics of the different subdivisions since most are uncultured and only known by 16S rRNA gene sequences [46]. The abundance

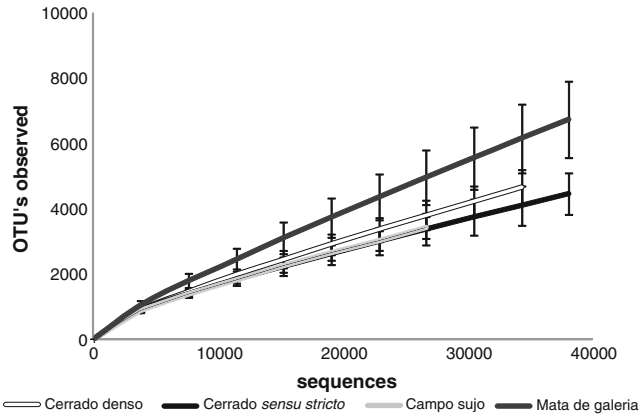

Figure 4 Rarefaction curves for soil bacterial communities associated with four Cerrado vegetation physiognomies using 16S rRNA gene sequences using a 3 % distance cutoff calculated from the number of sequences for each area. The *bars* represent the standard deviation of the two samples obtained for each area

of this phylum indicates that these organisms may be important components of soil microbial communities, although further experiments to show that they are active need to be performed. A recent study compared the complete genomes of three strains of Acidobacteria and postulated that cells of these isolates are versatile heterotrophs, exhibit slow metabolic rates under low-nutrient conditions, and are well equipped to tolerate fluctuations in soil hydration [48]. This tolerance to dystrophic soils and changes in soil moisture suggest that the percentage of Acidobacteria in Cerrado soils may be an important factor for adaptation to seasonal variations typical of savanna environments. Acidobacteria subdivisions Gp1, Gp2, and Gp3 decreased in relative abundance as soil pH increased [7], so this fact is another possible reason for the abundance of these groups in the areas of Cerrado where soil pH was between 4.3 and 4.9. Members of Acidobacteria subdivision Gp1 grow optimally in pH 4–5.5 [49]. Likewise, we can probably correlate the large percentage of Acidobacteria in Cerrado soils with the low levels of nutrients and low soil pH in the different studied areas (see Table S1).

The classes of Proteobacteria observed in this study are the most common in soils [44]. The class Alphaproteobacteria, to which the majority of Proteobacteria sequences found in Cerrado soil samples belonged, is a diverse bacterial group with many nitrogen-fixing organisms [50]. In fact, sequences from the genus *Bradyrhizobium* were commonly found in Cerrado *censu stricto* and Mata de Galeria soil samples, and sequences from other families of the Rhizobiales were found in all of the soil samples studied (results not shown). Cerrado ecosystems have conserved N cycling and are often N limited [51, 52], and biological nitrogen fixation is an important component of N budget in these ecosystems. Another interesting feature of Cerrado soils was that unclassified bacteria represented a sizeable amount of the total number of sequences analyzed (8–13 % of the sequences). This result indicates that there are a number of bacterial phyla still unknown, which highlights the need for further investigations to uncover new taxonomic groups.

The phylum Actinobacteria was the fourth in terms of relative frequency (4–7 %) in Cerrado soils. Its members have high DNA G+C content and are widely distributed in ecosystems especially in soils [53]. This group of microorganisms has been isolated from arid and acidic soils [54]. A particular group of Actinobacteria, the Rubrobacteria, has been found as the major group (70 %) present in heavy metal contaminated soils (Cd, Cu, and Zn) in Switzerland [55]. Although there is no data to support the presence of high concentrations of metals like Cd, Cu, and Zn in the studied soils, Cerrado soils have high levels of aluminum, manganese, and iron (see Table S1).

We designated the bacterial phyla Acidobacteria, Proteobacteria, Verrucomicrobia, Planctomycetes, Gemmatimonadetes, and Bacteroidetes "dominant" in Cerrado soils because the relative frequencies of sequences belonging to these were more than 1 % (Fig. 3a). The rare phyla, with relative frequency lower than 1 % (Fig. 3b), may also contribute to important functions in the soil ecosystem. For example, members of the phylum Chlamydiae, rare in Cerrado soils, are known pathogens (obligate intracellular

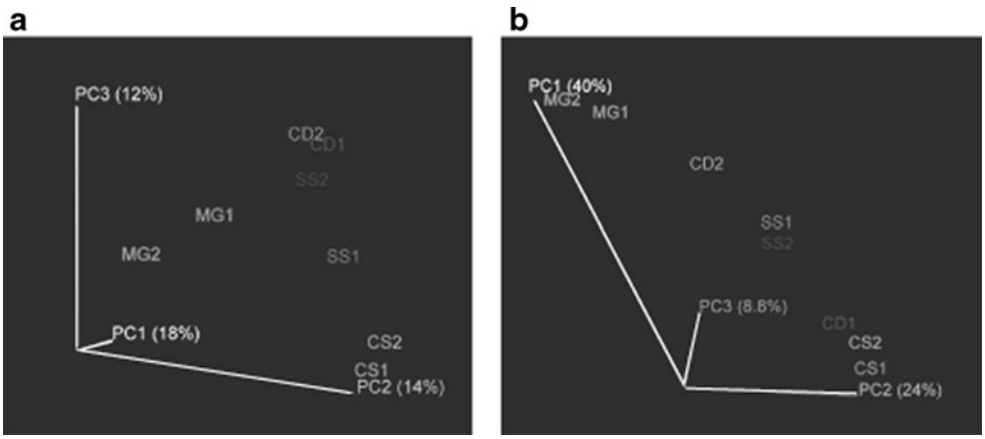

Figure 5 Unifrac principal coordinates analysis (*CS1* Campo Sujo "1 to 5;" *CS2* Campo Sujo "6 to 10;" *CD1* Cerrado Denso "1 to 5;" *CD2* Cerrado Denso "6 to 10;" *SS1* Cerrado *sensu stricto* "1 to 5;" *SS2* Cerrado *sensu stricto* "6 to 10;" *MG1* Mata de Galeria "1 to 5" and *MG2* Mata de Galeria "6 to 10"). **a** Unweighted and **b** weighted pairwise Unifrac community distances between vegetation physiognomies

bacteria characterized by a unique developmental cycle [56]) of animals and humans. Chlamydiae are present in different soil types, but their function in the soil environment is not known.

Representatives of the phylum Firmicutes are well known as soil bacteria (e.g., *Bacillus* and *Clostridium*) and only accounted for approximately 0.2 % of observed sequences. Bacteria of this phylum have cells or spores that may be difficult to lyse and are not easily detected through PCR-based analysis that rely on DNA extraction from soil. Although the bead-beating DNA extraction method used in this work is able to lyse many types of spores, it cannot be completely ruled out that members of this phylum are underrepresented in the 16S rRNA gene microbial community analysis [44].

Rarefaction curves allow comparison of communities with different sample sizes with the community whose curve lies on top being considered to be more diverse [57]. Rarefaction curves show at species level (Fig. 4) that the Mata de Galeria bacterial community is more diverse when compared to those from the other study areas. Similarly, a previous study also showed higher fungal diversity in Mata de Galeria soils in comparison to Cerrado *sensu stricto* soil samples [28]. The higher bacterial diversity in Mata de Galeria may be associated with some important characteristics of this forest formation in comparison with savanna formations, such as a higher density of different tree species, a greater amount of fine litter, the presence of superficial root mats inside the forest floor, a higher content of soil organic matter, and thus a greater input of nutrients [58, 59]. Furthermore, Mata de Galeria in the Cerrado biome are evergreen formations providing shading of the soil surface throughout the year [58], which, in association with a shallower water table, contributes to less intense water stress during the dry season compared to the savanna vegetation types studied. Another important difference is the higher concentration of available phosphorus in the soil samples from Mata de Galeria in comparison to savanna formations (see Table S1). Cerrado ecosystems are generally phosphorus limited, and a considerable proportion of available P is conserved in soil microbial biomass [60], indicating that this element is probably less limiting for soil microorganisms associated with this vegetation type.

Regarding taxonomic results, the distribution of phyla and classes is similar for the bacterial communities from the four studied areas (Fig. 3). Nevertheless, RISA, T-RFLP, and the more refined analysis of 16S rRNA gene sequences obtained by Unifrac showed that the bacterial communities in the four areas differed at the species level. The significant differences between the relative frequencies of bacterial orders of each vegetation type were further analyzed by the STAMP tool [43] (see Fig. S4). We first compared Mata de Galeria (forest type) with the other three areas (savanna type) due to its unique features such as increased bacterial diversity, distinct vegetation, and soil characteristics. It was observed that some bacterial orders are always more frequent in the Mata de Galeria soil samples, such as the Xanthomonales and Legionellales (class Gammaproteobacteria) and the Burkholderiales (class Betaproteobacteria) (see Fig. S4). Bacteria from such classes are known to be mainly decomposers and are possibly related to the higher amount of litter in Mata de Galeria [61].

The frequency of the subclass Actinobacteridae was higher in Mata de Galeria than in the other vegetation types. In savanna vegetation types (Campo Sujo, Cerrado *sensu strict*, and Cerrado Denso), where the incidence of radiation is much higher than in the Mata de Galeria, the frequency of subclass Rubrobacteridae of the phylum Actinobacteria was higher than in Mata de Galeria (see Fig. S4). This may be explained by the fact that this subclass is characterized by its ability to tolerate high temperatures and high radiation [62]. The same trend of high abundance of Actinobacteria in areas with more variable conditions was observed in a Cerrado area converted to pasture. Pasture areas are more prone to temperature fluctuations and direct sunlight incidence [29]. Although the soil bacterial composition of the savanna formations clustered together in the 16S rRNA gene analysis by Unifrac-PCoA (Fig. 5), it could be observed that Campo Sujo samples tended to form a separate group. Thus, the bacterial composition in this vegetation type was further analyzed. We found differences between the savanna area sequences that were confirmed to be significant by STAMP analyses (see Fig. S4). When comparing Campo Sujo with the other savanna areas, the order Rhizobiales (Alphaproteobacteria) appears to be more abundant. This could be explained by the fact that this order is able to fix nitrogen and usually is associated with grass roots [63]. Grass cover is higher in Campo Sujo area than in the other vegetation-type areas studied here. Furthermore, Chloroflexales order sequences were only observed in Campo Sujo samples. The sequences found belong to the genus *Roseiflexus* (data not shown). Members of this genus are filamentous, thermophilic, and photosynthetic bacteria [64], which may explain their affinity for more open areas with higher light intensity. It is clear by the STAMP analysis and by Table S2, that the Gp1, Gp2, and Gp3 Acidobacteria genera are the most prolific bacteria on these native soils followed by the Burkholderia and Gemmanatimonas genera. Table S2 also shows that, although also dominated by Acidobacteria genera, the riverbank soil (Mata de Galeria samples) are indeed more diverse with the appearance of Gp5 Acibobacteria, Solirubrobacter, Bradyrhizobium, and Byssovorax genera. This result is in contrast to the dominance of a single phylum in Cerrado soils where the native vegetation was converted to pasture for more than 10 years, where the main bacterial groups belong to Actinobacterium [29]. When we

compared our data with the data by Roesh et al. [11] we can see differences that are supported by several published papers from our group and others. Two main contrasting differences frequently emerge when anthropogenic modified soils are compared with prestine soils. First, anthropogenic modified soils have a drop in bacterial and fungal richness [28, 29]. Second, dominant groups, i.e., Acidobacteria [29], are replaced by groups such as Actinobacteria and Bacteroidetes. These bacterial community shifts are possibly due to the later groups being more resistant to UV radiation, heat, and desiccation. As speculated in the work of Quirino *et al.* [29], in addition to these bacterial structural and physiological characteristics, these groups are prolific secondary metabolites producers (i.e., anti-microbial compounds), which could result in further suppression of other bacterial species.

Compositions of bacterial communities from Cerrado Denso and Cerrado *sensu stricto* were similar probably due to their similarities in soil physicochemical properties and vegetation cover. However, there are some subtle differences in the frequencies of bacteria groups. Burkholderiales sequences (from decomposing bacteria, as previously stated) appear in higher frequency in Cerrado Denso samples. This result could be related to the higher tree density and lower grass cover in Cerrado Denso, producing a thicker litter layer.

Finally, the order Acidobacteriales showed significant differences in terms of number of sequences when the different vegetation types are compared. The biological significance of these differences, however, is not well understood since the phylum Acidobacteria has few isolates identified, their functional and physiological roles are little known, and only three genomes are sequenced.

Other studies indicate that the soil characteristics (pH, organic matter content, nutrients, and particle size) and plant species (with different root exudates) are the main determinants in the structure of soil microbial communities [65]. Furthermore, it has been suggested that the main determinant of the microbial community associated with the rhizosphere is the soil type with a smaller influence from plant species [66]. The results of this work indicate that both aspects are relevant for soil microbial communities. However, the high diversity found and the lack of a deeper knowledge of ecophysiological traits of bacterial groups make it difficult to discriminate between the influences of soil and vegetation.

This survey of soil bacterial communities from different vegetation types in the Cerrado using molecular techniques represents the most comprehensive study of this microbiome to date, and it is a significant contribution to the understanding of the interactions between above and below-ground biodiversity. Despite the large size of the Cerrado biome and the intense process of land use conversion to agricultural purposes in the last 40 years, little attention has been given to the study of its microbial communities. Evidence of highly diverse soil microbial communities in Cerrado is of central importance to supporting conservation efforts as well to identifying this unexplored biotechnological potential.

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