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The Adaptations Of The Microbial Communities Of The Savanna Soil Over A Period of Wildfire, After The First Rains, And During The Rainy Season

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Research Article

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Abstract

Context Annually the Cerrado ecosystem alternates between dry periods and long rainy seasons. During the dry season, severe forest fires occur, consuming a considerable part of the native vegetation, which impacts directly on the soil of the microbiome

Objective Evaluate the adaptations of the soil microbiome to drought, rain and wildfire.

Methods It was realized sequencing of the 16S rRNA gene was carried out for three significant conditions: drought and forest fires (“Fire”), after the first recorded rains (“First_Rain”), and during the rainy season (“Rainy”).

Results It has been shown that under the “Fire” condition, there was a predominance of Phylum Actinobacteria, followed by Proteobacteria and Firmicutes. With the advent of the rainy season, “First_Rain”, there was a change in the predominant taxonomic groups, with a higher prevalence of members of Proteobacteria and Firmicutes. During the rainy season, Proteobacteria and Firmicutes continued as the most prevalent groups. However, it was noted that in this period, there was an increase in bacterial diversity when compared with other periods analyzed.

Conclusions These results show how environmental factors influence adaptations in microbial communities. This allows for a better understanding of how to link the structure of the microbial community to the performance of ecosystems, and assist in preventing the consequences of increased frequency of wildfires, and long periods of drought.

Introduction

The savanna ecosystem is an important part of planetary land area, comprising about 22% of Earth’s surface, and 40% of the forests present in tropical latitudes (Ramankutty and Foley 1999; Miles et al. 2006). This ecosystem is widely distributed across continents, being present in Australia, Africa, and South America (Lehmann et al. 2014; Lehmann and Parr 2016). The savanna biome is characterized by exhibiting two well-defined climatic seasons, with an intense rainy season, followed by a long dry season (Powers et al. 2015). Savanna formations are among those that suffer most from the destruction of their areas. The surprisingly high rate of loss of savanna forests makes this biome the most threatened in the world, and it is predicted that about 80% of this area may be reduced, together with a fall in precipitation of at least 50mm per year until 2055 (Bejarano et al. 2014). This is due to soil overexploitation - especially deforestation for monoculture agricultural practices, pasture for livestock, and removal of wood to serve as charcoal, which is still widely used in these regions (Lambin et al. 2003; Searchinger et al. 2015). In addition, savannah ecosystems suffer annually from forest fires during the dry season, between June and September especially. Although fires in the savanna is a natural event, and an integral part of its annual cycle, many of these fires are of anthropogenic origin, and often uncontrolled (Archibald et al. 2013). Deforestation and fires combined have profound consequences for vegetation and macrofauna, especially for the microbiome present in the soil.

The soil microbiome constitutes most of the planet's biodiversity, corresponding to one-third of the Earth's biomass, participates as a key player in several biogeochemical cycles, and is closely related to soil health (Fierer et al. 2012; Procópio 2020b). Changes in microbial profiles following stress factors, such as soil contamination, removal of vegetation cover, and farming and livestock activities show their enormous adaptations, and genetic plasticity (Fierer and Jackson, 2006; Baldrian 2017). Other environmental aspects, for example, rain seasonality, temperature, and vegetation type, combined with soil properties, such as pH, organic matter content, and nutrients, also influence the structures of soil microbial communities (Strickland et al. 2009; Pajares et al. 2016). In tropical savanna forests in particular, wildfires are also a considerable component of influence over the soil microbiomes. Despite fires in savanna biomes being a natural event, in recent years there has been an increase in the frequency of fire in many areas, and the frequency and intensity of said fires are expected to increase further in the next century (Westerling et al. 2006). Wildfires influence microbial structuring and changes in underground communities, either by altering the inputs of organic matter driven by plants into the soil or by directly affecting the microbial composition by heating the superficial layers (Docherty et al. 2001; Hart et al. 2005; Pérez-Valera et al. 2019). Studies have demonstrated annual wildfires in savanna forests stimulate microbial growth by increasing ammonium (NH_4^+) levels, and concentrations of dissolved organic C by 20–52% in the soil (Poth et al. 1995; Andersson et al. 2004).

Soil microorganisms are the main agents responsible for long-term soil health and are considered one of the main drivers of the recovery of the fire ecosystem (Neary et al. 1999; Nannipieri et al. 2003). Microbes respond quickly to disturbances imposed on the soil, through changes in biomass, metabolic activity, and in the structures of microbial communities (Certini 2005). In the last years the high-throughput sequencing technologies emerged, and more recently improved it was possible to identify microorganisms from environmental samples. This allowed us to characterize microbial communities in different conditions, and, and establish correlations between diversity and abundance with biogeochemical processes (Daniel 2005). For example, assess microbes in different land uses, comparing pristine and contaminated environments, and monitoring changes in community structures over a period of time (Procópio 2020a). Nevertheless, the huge amount of information of 16S/18S rRNA gene sequences has brought another challenge to the taxonomy of microorganisms. How accurate is the identification of microbial species in environmental samples? Since we know that many of the biochemical processes differ at the level of strain, establishing an ecological relationship between taxonomic levels below gender and their role in the ecological niche may be inaccurate (Gutleben et al. 2018).

Brazilian savannas, also known as Cerrado, constitute the second largest morpho-floristic domain in Brazil, covering about 2 million km^2 (Forzza et al. 2010). The vegetation of the Cerrado is composed of a complex mosaic of different forest formations described as: "clean fields" and "dirty fields", i.e. pastures with low shrubs and scattered trees; “Cerrado stricto sensu” or typical savanna; and “Cerradão” or “Cerrado denso”, which is to say, seasonal deciduous and gallery forests (Oliveira-Filho and Ratter 2001; Ribeiro and Walter 2008). Cerrado soils are acidic, usually poor in nutrients, with high levels of aluminum,

leading this soil to be classified as an oxisol (Bustamante et al. 2012). The rainfall regime is characterized by a period of relatively high rainfall (1500 mm), followed by a prolonged dry season of 4 months, from June to September. This marked rain seasonality contributes to a high weathering of the soil, which also contributes to the low availability of nutrients, mainly carbon (C), nitrogen (N), and Phosphate (P) (Haridasan 2001). The fire regime in the Brazilian Cerrado biome occurs annually during the dry season. The fires in the Cerrado are historically described as natural events, initiated by spontaneous causes, such as lightning striking dry vegetation during the long drought period (Ramos-Neto and Pivello 2000). However, in recent decades, more intentional fires have also been common in this period, as a common practice of clearing "clean fields" to be used as pastures and mono-areas (Myers et al. 2000; Eloy et al. 2019).

The climatic conditions imposed on the Cerrado biome have a direct effect on the soil microbiome. Annual cyclical events such as the entry of organic matter during the period of leaf fall, wildfires, and intense rains influence soil microbial communities, especially in the upper layer, between 5 to 10 cm deep. With the intense and rapid degradation of the Cerrado biome, the soil microbiota will also be lost, causing devastation to the genetic resources available in this ecosystem. A better understanding of the resilience and reshaping of bacterial communities inhabiting this dynamic environment will help in future approaches to biome recovery. The objective of this study was to evaluate, through the sequencing analysis of the 16S rRNA gene, the bacterial communities present in the Cerrado soil after a wildfire in the vegetation during the long drought period, after the first rain recorded at the site of this study, and during the rainy season.

Material And Methods

Study area

The microbial communities investigated in this study were obtained from soil samples from the savanna ecosystem of the Chapada do Guimarães National Park (CGNP), which is located in the Center-South region of the Midwest of Brazil. The CGNP is a protected area of the Cerrado with 326.30km^2, with typical Brazilian savanna formation, well-defined rainy seasons, between October and March, and a long period of drought, from April to September. The locations determined for soil collections correspond to environments of native and pristine vegetation, without anthropogenic interference on flora or soil. The first collection occurred during the dry season, after a period of fire, in August 2019, and the samples for this period were described as “Fire”. The second collection was carried out after the first recorded rains in the locations chosen for soil sampling, which occurred in October 2019, and these samples were labelled “First_Rain”. A third and final soil collection was carried out in February 2020, which corresponds to the final rainy season, which extends approximately from October to March, and the samples were labelled “Rainy”. Approximately 500 gr of bulk soil were collected from each point at a depth of 10cm, and stored in a thermal box, and immediately transported to the laboratory, where they were preserved at a temperature of -20ºC, until further analysis.

Analysis of soil parameters

About 100 g of each sample referring to the three conditions studied were sent immediately for analysis of the physical-chemical parameters. The percentage of moisture, pH, total organic carbon (TOC), nitrogen (N) and phosphate (P) were evaluated. The pH was determined through the activity of hydrogen ions by potentiometric measurement using a standard hydrogen electrode and a reference electrode. After preparing the soil, the hydrogen potential is measured using an electrode immersed in liquid soil suspension. The determination of TOC was carried out dryly through the loss of mass by ignition. The total organic carbon content was quantified by the difference between the mass of the kiln-dried soil and the mass of the residue obtained after muffle incineration. Phosphate levels were determined by direct reading of the sample, without a preliminary step of acid hydrolysis or oxidative digestion, and measured in a spectrophotometer at 880nm. The total nitrogen method was carried out in three stages: a wet digestion of the sample to convert organic nitrogen to ammonium ion, distillation of the ammonia with steam entrainment for later quantification of the ammonia by the colorimetric indophenol method. Ammonia reacted with sodium hypochlorite in a slightly alkaline solution forming monochloramine, which, in the presence of phenol and excess of sodium hypochlorite, formed the indophenol blue. The reaction was catalyzed by Sodium Nitroprusside and the color intensity developed proportional to the amount of ammoniacal nitrogen in the sample was measured in a spectrophotometer at 640 nm.

Microbial community analysis

Soil samples from the three studied conditions were used to analyze the present microbiome. Each condition, “Fire”, “First Rain”, and “Rainy” was evaluated in replicate. For this, 0.25 gr of each sample was used for DNA extraction using the commercial kit DNeasy PowerSoil Kit (Qiagen), following the conditions suggested by the manufacturer. After extraction, the total DNA was analyzed for integrity and then quantified using the Qubit system. The next step consisted of in the preparation of the sequencing library. For this, the 16S rRNA V3/V4 region (Wang and Qian 2009) was amplified using primers 341F (5´-CCTACGGGRSGCAGCAG-3´) and 806R (5´-GGACTACHVGGGTWTCTAAT-3´), with Illumina adapters, necessary for the sequencing, previously prepared. Amplification was performed in 35 cycles at 50ºC of annealing temperature, which was tripled for each sample. The sequencing was performed by Illumina MiSeq using V2 kits, with 300nt single-ended runs. To increase the reliability of the reading, excluding the possible diversity generated by chimeric amplicons or erroneous nucleotides incorporated in the PCR, only groups of 100% identical readings were considered (Bolyen et al. 2019). In addition, clusters represented by less than 5 readings were discarded, and not considered in further analysis. The final sequencing products were used for downstream analysis. Initially, the primer and adapter sequences were trimmed from the reads and only sequences with 275nt or more were used in subsequent analysis. In turn, all reads with one or more indeterminate “N” bases and truncated sequences with two or more consecutive bases with quality scores below Q20 were eliminated. Reads processing in OTUs was performed following the clustering-based method described by the Brazilian Microbiome Project (Pylro et al. 2014), using the QIIME 2 software (Bolyen et al. 2019). Then, OTU Picking was performed using BLASTN 2.2.28 against the GreenGenes 13.8 database. To attribute taxonomy, only sequences with 99%

of identity hits in an alignment covering over 99% were considered. The OTUs sequences are available in the NCBI Sequence Read Archive (SRA) database under accession no. PRJNA670130.

Data analysis

The alpha-diversity measures of the sequenced samples were evaluated using the abundance and richness indices, using the phyloseq package available for the R software (R Core Team 2017). Chao and ACE measures were used to define the abundance parameters, while Shannon and Simpson indices were used to assess richness. For Betadiversity measurements, data from the OTUs table were used. The relationships between the different communities were determined using the Non Metric Multidimensional Scaling (NMDS) using Bray-Curtis distances. The interactions between the physical-chemical parameters were determined using Canonical Correspondence Analysis (CCA) using Bray-Curtis similarity. Both graphics, NMDS, and CCA, were built using Past4 software. Stacked bar, boxplot graphs and Sectors diagrams were performed in an R environment. A heatmap graph was made using the heatmap2 package available for R. Co-occurrence patterns were explored and visualized through a network analysis using the phyloseq package, available for software R. A valid co-occurrence event was considered a robust correlation if the Spearman correlation coefficient (ρ) was > 0.6 and highly statistically significant ($P < 0.001$) (Junker and Schreiber 2008).

Results

Physical-chemical parameters of the study area

The comparison of the physical-chemical parameters of the soil samples is shown in Fig. 1. The results indicate that throughout the analyzed period there were differences in the characteristics of the samples. The dry season had the highest percentage of solid mass, in contrast to the lowest value in moisture. Under these conditions, the pH value was slightly above 4.5 (± 0.96). The TOC level was above 2.2 (± 0.35) mg/kg, while the amount of N and P showed the highest values among all the conditions analyzed in this study, with 8.3 (± 0.23) mg/kg and 8.77 (± 0.45) mg/kg, respectively. After the first rainfall, the results of the soil analysis showed changes in its parameters. Despite the values of solid mass and humidity maintaining percentages similar to during the dry season, a sharp drop in the pH value was noted (3.86 ± 0.46). However, there was a small increase in the percentage of TOC, to just above of 2.5 (± 0.32) mg/kg. The values of N (5.3 ± 0.22 mg/kg) and P (11.59 ± 0.99 mg/kg) under this condition also showed a marked decrease in their measurements (Fig. 1). The analyses of samples during the rainy season indicated falls in the values of measured parameters. A decrease in the percentage of solid mass was accompanied by an increase in humidity values, which reached about 25%. The pH under this condition of intense rains showed a considerable increase when compared to the condition "First Rain", near 6.4 ± 0.39, but still remaining below the pH measured during the dry season, "Fire". The percentage of TOC (0.84 ± 0.08 mg/kg) was the lowest among all analyses. This decrease was accompanied by the parameters of N, which was measured at 4.5 ± 0.17 mg/kg, and P had an abrupt decrease in its value, presenting only 0.3 ± 0.13 mg/kg (Fig. 1).

Community structures

The bacterial communities presented different profiles of richness estimation, Chao1 and ACE, and diversity indices, Shannon and Simpson. The results obtained from samples referring to the "Fire" condition presented the highest measures of richness Chao1 and ACE, followed just below by the values obtained from soil samples of the "First Rain" condition. The samples referring to the “Rainy” condition presented considerably smaller values of richness estimation when compared with the previous samples (Fig. 2). When analyzing the diversity indices of the samples of the “Fire” condition, it was observed that this sampling also presented the highest values in Shannon in relation to the other conditions. However, when assessed by Simpson, this condition was below the communities obtained under the “First Rain” condition. Further, the communities present in the “Rainy” samples continued to show the lowest values for both, Shannon and Simpson indices (Fig. 2).

The results presented in NMDS graph describe the groups "First Rain" and "Rainy" apart from each other, resulting in three distinct groups (Fig. 3a). To check the effects of environmental factors, humidity, pH, TOC, N and P, on the bacterial communities present in the samples, a CCA plot was used. The results showed that the bacterial communities identified in the "Fire" condition were significantly correlated with the parameters N, P and soil pH, and in lesser association with TOC. On the other hand, "Rainy" showed robust affinities with the humidity parameter (Fig. 3b).

Composition of microbial communities

The structures of the microbial communities analyzed described a remodeling to different environmental conditions. However, despite changes in the composition of different taxonomic levels between different climatic situations, there was a predominance of members of the phyla Actinobacteria, Proteobacteria, and Acidobacteria in all analysis (Fig. 4). During the dry season, the first sampling occurred during the vegetation burning period. In this condition, the phylum Actinobacteria dominated, which comprised about 55% of all identified OTUs (Fig. 4a). The second most present group was Proteobacteria (27%), followed by Firmicutes (18%). After the first rains on the study site, there was a change in the predominance of bacterial groups. Proteobacteria became the most representative group, with 55% of OTUs, followed by Firmicutes (27%), and Actinobacteria became the third most abundant Phylum (13%) (Fig. 4b). For the first time in this study, members of the Phylum Acidobacteria were identified, which was represented in 4% of the OTUs (Fig. 4b). During the period of intense rains, the general profile of the distribution of OTUs among the different phyla proved to be close to the taxonomic structure described in Fig. 4b. In “Rainy” the Proteobacteria Phylum remained the most prevalent (65%), followed by Firmicutes (19%), and Actinobacteria (11%), while Acidobacteria continued to appear in this analysis with about 5% representativeness (Fig. 4c).

In Class level analyses, differences between taxonomic levels were also evident. The first condition, "Fire", showed a predominance of Actinobacteria, which comprised more than 55% of the OTUs (Fig. 5). Then, the most abundant Class was Alphaproteobacteria, which had 22.6% representation, followed by Bacilli, with 17.5%, and Betaproteobacteria with 2.8% (Fig. 5). The results after the first rain showed a considerable decrease in the abundance of Class Actinobacteria (12.5%), with a concomitant increase in the representativeness of Betaproteobacteria (34.3%), followed by Bacilli (27%), and Alphaproteobacteria

(20%) (Fig. 5). Class Acidobacteria was represented with 4.3% of OTUs, while other classes showed relative abundance below 1%. In the rainy season, there was an increase in the abundance of members of Class Alphaproteobacteria (36.8%), and a consistent presence of members of Bacilli (18%), Betaproteobacteria (16.8%), and Acidobacteria (5%) (Fig. 5). In this analysis, there was a modest increase in the abundance of Gammaproteobacteria (12%).

During the dry season, when fires were constant in the sampling area, there was a predominance of members of the orders Streptomycetales (36.2%), Bacillales (17.5%), and Rhizobiales (13.5%) (Fig. 6). Among Streptomycetales, the *Streptomyces* genus constituted almost the totality of OTUs of this Order, representing 35%. *Bacillus* was also the main genus of the Bacillales, making up 15.3% of OTUs. Among the genera belonging to Order Rhizobiales, *Bradyrhizobium* (4.9%), *Microvirga* (2.4%), *Chelatococcus* (1.3%), Ensifer (1.7%), and *Mesorhizobium* (1.1%) emerged. After the first rain, there was a clear redefinition of the taxonomic structure, both at the level of the Order, as well as its members at the level of Genus. Under this environmental condition, Order Burkholderiales was the most abundant, making up about 34.3% of OTUs (Fig. 6). The main genera of Burkholderiales were *Burkholderia* (22.2%), *Cupriavidus* (9.3%), and *Massilia* (1.7%). The second most abundant Order was Bacillales with 27% of total OTUs under this condition (Fig. 6). Once again, the *Bacillus* was dominant in the Order to which it belongs, alone constituting 24.2% of the detected OTUs. Rhizobiales (11.2%) remained among the most numerous groups in the “First Rain” sample (Fig. 6). The *Bradyrhizobium* genus was the most present among the detected OTUs, with 8.9% representativeness. For the first time, Order Caulobacterales was considerably detected in this study, with about 6.5% of OTUs, and having the *Phenylobacterium* genus as its only representative (Figs. 6 and 7). The Order Streptomycetales was also described in this analysis, although, relative to the number of OTUs, well below that described in “Fire”, with only 5.9% (Fig. 6). *Streptomyces* was the most present genus (3.1%), followed by *Streptacidiphilus* (1.9%). For the first time, members of the Acidobacteriales (4.3%) were identified in this study, which was represented by the only genus *Edaphobacter*. The last condition evaluated was during the rainy season, which continued to show changes in the profiles of the microbiological structures analyzed. The most abundant order was Rhizobiales (32.9%), which had the *Bradyrhizobium* genus (32.8%) as dominant. Bacillales (18.7%) was the most numbered Order, and *Bacillus* (13.3%) was the most present in this group, followed by *Lysinibacillus* (2%), and *Tumebacillus* (1%). A unique genus of the Burkholderiales was detected, *Burkholderia*, which was present with 16.2% of OTUs (Figure 6 and 6). Among the members of Xanthomonadales (11.2%), two genera were identified, the predominant *Dyella* (10.9%), and *Stenotrophomonas* (0.3%). The Acidobacteriales Order was again detected in the “Rainy” condition, in a slightly higher abundance than in “First Rain” (Fig. 6). In this analysis, the *Edaphobacter* genus was more prevalent (4.8%), and two more new genera *Acidicapsa* (0.1%) and *Acidipila* (0.15%) were detected for the first time, although in much smaller numbers of OTUs.

Discussion

The present study evaluated how cyclical environmental factors act on the structures of bacterial communities present in the soil of a savanna biome. Throughout the main climatic changes that occur in

this ecosystem, it was possible to describe the prevalent taxonomic groups during each period. In addition, physical-chemical analysis of the soil described the relationships among major microbial groups. In this analysis, soil pH varied considerably, with values close to 4.5 during the dry season and after the first rains, and close 6.5 during the rainy season. These results are similar to previous studies, which describe the Cerrado soil as acid, especially during the dry season, but with its values close to neutrality in the rainy season (Pinheiro et al. 2010; Rocha et al. 2019). Moisture was the physical-chemical factor that most influenced the microbial communities analyzed in our analysis. This confirms what has been described in a study on the edaphic characteristics of a hyper-seasonal cerrado that points to humidity as the main factor of influence on soil microbial activity (Amorim and Batalha 2006). This is mainly because, in water-saturated soils the oxygen supply is sufficient only for microbial activity in the first millimeters of the soil surface, while at greater depths, oxygen decreases after several days to a week following saturation by rains (Brinkman and Diepen 1990; Amorim and Batalha 2006; Eaton and Chassot 2012). Levels of TOC, N, and P also changed due to seasonality, with higher values during the dry season, followed by a sharp drop during the rainy season. The highest levels of TOC, N, and P during the dry (Fire) period occur due to the greater entry of organic material by way of plant litter (Nardoto et al. 2006; Rocha et al. 2019). Studies suggest that these differences in nutrient availability contribute to shaping the different microbial structures and diversity that inhabit the Cerrado soil (Araújo et al. 2018; Rocha et al. 2019).

The microbial changes in the soil described in this study suggest that environmental factors had a substantial influence on abundance and taxonomic diversity. In this study, the predominance of bacterial groups under different environmental conditions was described. For example, during the dry season, when fires constantly occur, there was a dominance of Actinobacteria, followed by Proteobacteria, and Firmicutes, in contrast to a high abundance of Proteobacteria under the two other conditions, "First Rain" and "Rainy". The increase in representation of members of the group of gram-positive bacteria, especially Actinobacteria and Firmicutes, has been related to environments prone to fire (Smith et al. 2008; Xiang et al. 2014). In these environments, where vegetation is adapted to events of violent fires, rhizospheric communities play an important role in the nitrogen cycle (Cobo-Díaz et al. 2015). Furthermore, members of spore-formers and actinobacteria groups are commonly characterized by the capacity to survive under extreme environments, and unfavorable conditions (Nicholson et al. 2000; Flärdh and Buttner 2009; Procópio et al. 2013; Prendergast-Miller et al. 2017). Our analyses showed the genera *Streptomyces* and *Bacillus* as the genera most present in the soil of the "Fire" sampling. Although both genera showed less representation in other conditions, with higher soil moisture, *Streptomyces* and *Bacillus* are described as common inhabitants throughout the Cerrado ecosystem (Suela-Silva et al. 2013; Araújo et al. 2017 and 2018; Sáenz de Miera et al. 2020). Another genus that was found in the analyses was *Arthrobacter*, which is also widely found in the Cerrado soil under different environmental conditions. Its metabolic diversity, with the ability to secrete different proteases, and resistance to heavy metals, enables species of this genus to inhabit different environments (Bafana et al. 2010; Suela-Silva et al. 2013). The *Paenibacillus* genus was detected in greater abundance under the "Fire" condition compared to the other two samples. In a study on immediate fire-induced changes in soil microbial community, *Paenibacillus* was also

described as more abundant when compared to the control system, without burning (Lucas-Borja et al. 2019; Sáenz de Miera et al. 2020).

Another important taxonomic group under the dry and fire conditions was the phylum Proteobacteria. In fact, this phylum is widely described in microbial studies in Cerrado soil (de Castro et al. 2016; Souza et al. 2016; Araujo et al. 2017; Araujo et al. 2018; Vieira et al. 2018). A study on the influence of wildfires in soil microbial communities also reported the high abundance and diversity of this phylum in soils (Smith et al. 2008; Xiang et al. 2014; Pajares et al. 2016; Yang et al. 2020). The immediate responses of microbial communities to fires confirmed an increase of Proteobacteria and Firmicutes, with a concomitant decrease in the Acidobacteria and Bacteroidetes phyla (de Castro et al. 2013; Rodrigues et al. 2013; Lucas-Borja et al. 2019). The Alphaproteobacteria Class was prevalent in the "Fire" sample, constituting the second most abundant taxonomic group, followed by Betaproteobacteria. In contrast, most studies on the impacts of wildfires on soil microbiota describe a decrease in the abundance of Alphaproteobacteria, followed by an increase in the levels of representation of Betaproteobacteria (Smith et al. 2008; Xiang et al. 2014; Sáenz de Miera et al. 2020). A possible reason for the high level of Alphaproteobacteria during the dry season also, and in the rainy months, may be due to the fact that members of this class are classified as copiotrophic. High carbon input through plant litter allows more nutrient input during the dry season, or deciduous period (Fierer et al. 2012).

With the beginning of the rainy season, the microbial structures present in the soil showed changes in both diversity and abundance. Under "First Rain" and "Rainy", the soil became moist, which directly influenced the microbial structures analyzed. In “First Rain” there was a predominance of the Betaproteobacteria Class, followed by Bacilli and Alphaproteobacteria. Interestingly, other studies show that during wetter seasons there are higher levels of representatives of the Alphaproteobacteria and Gammaproteobacteria classes (Fierer and Jackson 2006; de Castro et al. 2016). Another striking feature described under the “Rainy” condition was a consistent representation of different taxonomic groups, which were not described in the previous settings. Members of the Gammaproteobacteria and Acidobacteria Class have been described at higher relative levels of OTUs. The low representativeness of members of Acidobacteria was an intriguing consideration in our analyses, since other studies demonstrate the high presence of these groups in Cerrado soil (Catão et al. 2014). Probably, during the rainy season, bacterial species that were dormant, due to adverse environmental factors, find more favorable conditions (Lennon and Jones, 2011). An example would be the bacteria of the Acidobacteria Phylum, which are known to tolerate environmental fluctuations, especially soil moisture (Ward et al. 2009; Placella et al. 2012; Bouskill et al. 2013).

Conclusions

This study highlighted how annual natural and anthropogenic events affect the soil microbiota of a savanna ecosystem. Our findings showed that after a long period of drought and forest fires, there was a predominance of taxonomic groups of the Actinobacteria, which possess adaptations for enduring severe stress. The beginning of the rainy season marked a change in taxonomic levels, with a predominance of

members of the Proteobacteria. With the extension of the rainy season, new representatives of different taxonomic levels were described. The results presented here describe how environmental factors contribute to changes in the diversity profiles of abundant microbial communities. These findings are essential for a better understanding of resilience and microbial adaptations, as well as for predicting the consequences of environmental factors and stress conditions.

Declarations

Funding

No funding was applied.

Conflicts of interest/Competing interests

The author declare that they have no conflict of interest.

Availability of data and materials

Metagenomic sequencing data is deposited and available on the NCBI Sequence Read Archive (SRA) database under accession no. PRJNA691503 accession numbers.

Author contributions

LCS contributed to the determination of soil collection sites, soil sampling, and helped in the characterization of soil samples. LP contributed to the conception and design of the study, extraction of total DNA from the soil, preparation of the library for sequencing. In addition, LP wrote the manuscript, performed the statistical analysis, and made all the lines. LCS and LP both read and revised the text.

Ethical Approval

Not applicable.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

References

1. Amorim PK, Batalha MA (2006) Soil characteristics of a hyperseasonal cerrado compared to a seasonal cerrado and a floodplain grassland: implications for plant community structure. Braz J Biol 66:661–670

2. Andersson M, Michelsen A, Jensen M, Kjøller A (2004) Tropical savanna woodland: effects of experimental fire on soil microorganisms and soil emissions of carbon dioxide. Soil Biol Biochem 36:849–858
3. Araujo AS, Bezerra WM, Dos Santos VM, Rocha SM, Carvalho ND, de Lyra MD, Figueiredo MD, de Almeida Lopes ÂC, Melo VM (2017) Distinct bacterial communities across a gradient of vegetation from a preserved Brazilian Cerrado. Ant Van Leeuwenhoek 110:457–469
4. Araujo ASF, Mendes LW, Bezerra WM, Nunes LAPL, Lyra MDCCP, Figueiredo MDVB, Melo VMM (2018) Archaea diversity in vegetation gradients from the Brazilian Cerrado. Braz J Microbiol 49:522–528
5. Archibald S, Lehmann CER, Gomez-Dans J, Bradstock RA (2013) Defining pyromes and global syndromes of fire. PNAS USA 10:6442–6447
6. Bafana A, Krishnamurthi K, Patil M, Chakrabarti T (2010) Heavy metal resistance in *Arthrobacter ramosus* strain G2 isolated from mercuric salt-contaminated soil. J Haz Mat 177:481–486
7. Baldrian P (2017) Forest microbiome: diversity, complexity and dynamics. FEMS Microbiol Rev 41:109–130
8. Bejarano M, Crosby MM, Parra V, Etchevers JD, Campo J (2014) Precipitation regime and nitrogen addition effects on leaf litter decomposition in tropical dry forests. Biotropica 46:415–424
9. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS 2nd, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotech 37:852–857
10. Bouskill NJ, Lim HC, Borglin S, Salve R, Wood TE, Silver WL, Brodie EL (2013) Pre-exposure to drought increases the resistance of tropical forest soil bacterial communities to extended drought. ISME J 7:384–394
11. Brinkman R, Diepen CA (1990) Mineral soils, In: Patten BC (ed). Wetlands and shallow continental water bodies, SPB Acad Publish Hague. p37–59
12. Bustamante MM, Nardoto GB, Pinto AS, Resende JC, Takahashi FS, Vieira LC (2012) Potential impacts of climate change on biogeochemical functioning of Cerrado ecosystems. Braz J Biol

72:655–671

13. Catão EC, Lopes FA, Araújo JF, de Castro AP, Barreto CC, Bustamante MM, Quirino BF, Krüger RH (2014) Soil Acidobacterial 16S rRNA Gene Sequences Reveal Subgroup Level Differences between Savanna-Like Cerrado and Atlantic Forest Brazilian Biomes. Inter J of Microbiol 2014:156341
14. Certini G (2005) Effects of fire on properties of forest soils: a review. Oecologia 143:1–10. doi:10.1007/s00442-004-1788-1788
15. Cobo-Díaz JF, Fernández-González AJ, Villadas PJ, Robles AB, Toro N, Fernández-López M (2015) Metagenomic assessment of the potential microbial nitrogen pathways in the rhizosphere of a Mediterranean forest after a wildfire. Microbial Ecol 69:895–904
16. Coleman MD, Aubrey DP (2018) Stand development and other intrinsic factors largely control fine-root dynamics with only subtle modifications from resource availability. Tree Physiol 38:1805–1819
17. Csardi G, Nepusz T (2006) The igraph software package for complex network research. Inter J Complex Sys 1695
18. Daniel R (2005) The metagenomics of soil. Nature Rev Microbiol 3:470–478
19. de Castro AP, Sartori da Silva MR, Quirino BF, da Cunha Bustamante MM, Krüger RH (2016) Microbial diversity in Cerrado biome (Neotropical Savanna) soils. PLoS One 11:e0148785
20. de Castro VH, Schroeder LF, Quirino BF, Kruger RH, Barreto CC (2013) Acidobacteria from oligotrophic soil from the Cerrado can grow in a wide range of carbon source concentrations. Can J Microbiol 59:746–453
21. Docherty KM, Balser TC, Bohannan BJM, Gutknecht JLM (2001) Soil microbial responses to fire and interacting global change factors in a California annual grassland. Biogeochemistry 109:63–83
22. Eaton W, Chassot O (2012) Characterization o soil ecosystems in Costa Rica using microbial community metrics. Trop Ecol 53:25–36
23. Eloy L, Schmidt IB, Borges SL, Ferreira MC, Dos Santos TA (2019) Seasonal fire management by traditional cattle ranchers prevents the spread of wildfire in the Brazilian Cerrado. Ambio 48:890–899
24. Fierer N, Jackson R (2006) The diversity and biogeography of soil bacterial communities. PNAS USA 103:626–631
25. Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R (2012) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME J 6:1007–1017
26. Flärdh K, Buttner MJ (2009) *Streptomyces morphogenetics*: dissecting differentiation in a filamentous bacterium. Nature Rev Microbiol 7:36–49
27. Forzza RC, Leitman PM, Costa A, Carvalho AA, Peixoto AL, Walter BMT, Bicudo C (2010) Catalogo de plantas e fungos do Brasil. Instituto de Pesquisas Jardim Botanico, Rio de Janeiro
28. Haridasan M (2001) Nutrient cycling as a function of landscape and biotic characteristics. In: (In) McClain ME, Victoria RL, Richey JR (eds) The Cerrados of Central Brazil. The Biochemistry of the Amazon Basin. Oxford University Press, New York

29. Hart SC, DeLuca TH, Newman GS, MacKenzie MD, Boyle SI (2005) Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. Forest Ecol Manag 220:166–184
30. Junker BH, Schreiber F (2008) Correlation Networks. In: Analysis of Biological Networks. Wiley-Interscience
31. Lambin EF, Geist HJ, Lepers E (2003) Dynamics of land-use and land-cover change in tropical regions. Annual Rev Environ Res 28:205–241
32. Lehmann CE, Anderson TM, Sankaran M, Higgins SI, Archibald S, Hoffmann WA, Hanan NP, Williams RJ, Fensham RJ, Felfili J, Hutley LB, Ratnam J, San Jose J, Montes R, Franklin D, Russell-Smith J, Ryan CM, Durigan G, Hiernaux P, Haidar R, Bowman DM, Bond WJ (2014) Savanna vegetation-fire-climate relationships differ among continents. Science 343:548–552
33. Lehmann CE, Parr CL (2016) Tropical grassy biomes: linking ecology, human use and conservation. Philo Transac Royal Soc B: Biol Sciences 371:20160329
34. Lennon JT, Jones SE (2011) Microbial seed banks: the ecological and evolutionary implications of dormancy. Nature Rev Microbiol 9:119–130
35. Lucas-Borja ME, Miralles I, Ortega R, Plaza-Álvarez PA, Gonzalez-Romero J, Sagra J, Soriano-Rodríguez M (2019) Immediate fire-induced changes in soil microbial community composition in an outdoor experimental controlled system. Science Total Environ 696:134033
36. Miles L, Newton AC, DeFries RS, Ravilious C, May I, Blyth S, Kapos V, Gordon JE (2006) A global overview of the conservation status of tropical dry forests. J Biogeography 33:491–505
37. Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858
38. Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. Europ J Soil Science 54:655–670
39. Nardoto GB, Bustamante MMC, Pinto AS, Klink CA (2006) Nutrient use efficiency at ecosystem and species level in savanna areas of Central Brazil and impacts of fire. J Tropl Ecol 22:191–201
40. Neary DG, Klopatek CC, DeBano LF, Ffolliott PF (1999) Fire effects on belowground sustainability: a review and synthesis. Forest Eco Manang 122:51–71
41. Nicholson WL, Munakata N, Horneck G, Melosh HJ, Setlow P (2000) Resistance of bacterial endospores to extreme terrestrial and extraterrestrial environments. Microbiol Mol Biol Rev 2000, 64:548–572
42. Oliveira-Filho A, Ratter JA (2001) Vegetation physiognomies and woody flora of the Cerrado biome. In: Oliveira PS, Marquis RJ (eds) The Cerrados of Brazil: ecology and natural history of a neotropical savanna. Columbia edn University Press, New York, pp 91–120
43. Pajares S, Escalante AE, Noguez AM, García-Oliva F, Martínez-Piedragil C, Cram SS, Eguiarte LE, Souza V (2016) Spatial heterogeneity of physicochemical properties explains differences in microbial composition in arid soils from Cuatro Cienegas. Mexico PeerJ 4:e2459

44. Pérez-Valera E, Goberna M, Verdú M (2019) Fire modulates ecosystem functioning through the phylogenetic structure of soil bacterial communities. Soil Biol Bioch 129:80–89
45. Pinheiro MH, Azevedo TS, Monteiro R (2010) Spatial-temporal distribution of fire-protected savanna physiognomies in Southeastern Brazil. An Acad Bras Ciênc 82:379–395
46. Placella SA, Brodie EL, Firestone MK (2012) Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. PNAS USA 109:10931–10936
47. Poth M, Anderson IC, Miranda HS, Miranda AC, Riggan PJ (1995) The magnitude and persistence of soil NO, N_2O, CH_4, and CO_2 fluxes from burned tropical savanna in Brazil. Glob Biogeoch Cycles 9:503–513
48. Powers JS, Becklund KK, Gei MG, Iyengar SB, Meyer R, O'Connell CS, Schilling EM, Smith CM, Waring BG, Werden LK (2015) Nutrient addition effects on tropical dry forests: a mini-review from microbial to ecosystem scales. Front Earth Sciences 3:1–8
49. Prendergast-Miller MT, de Menezes AB, Macdonald LM, Toscas P, Bissett A, Baker G, Farrell M, Richardson AE, Wark T, Thrall PH (2017) Wildfire impact: Natural experiment reveals differential short-term changes in soil microbial communities. Soil Biol Bioch 109:1–13
50. Procópio L, Macrae A, van Elsas JD, Seldin L (2013) The putative α/β-hydrolases of Dietzia cinnamea P4 strain as potential enzymes for biocatalytic applications. Ant Van Leeuwenhoek 103:635–646
51. Procópio L (2020a) The era of 'omics' technologies in the study of microbiologically influenced corrosion. Biotech Lett 42:341–356
52. Procópio L (2020b) Changes in microbial community in the presence of oil and chemical dispersant and their effects on the corrosion of API 5L steel coupons in a marine-simulated microcosm. App Microbiol Biotech 104:6397–6411
53. Pylro VS, Roesch LF, Morais DK, Clark IM, Hirsch PR, Tótola MR (2014) Data analysis for 16S microbial profiling from different benchtop sequencing platforms. J Microbiol Meth 107:30–37
54. R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
55. Ramankutty N, Foley JA (1999) Estimating historical changes in global land cover: croplands from 1700 to 1992. Glob Biogeoch Cycles 13:997–1027
56. Ramos-Neto MB, Pivello VR (2000) Lightning fires in a Brazilian savanna National Park: rethinking management strategies. Environ Manag 26:675–684
57. Resende J (2001) A ciclagem de nutrientes em áreas de Cerrado e a influência de queimadas controladas. PhD thesis, University of Brasilia, Brasília
58. Ribeiro J, Walter BMT (2008) As Principais Fitofisionomias do Bioma Cerrado. In: Sano SM, Almeida SP, Riberio JF (eds) Cerrado: ecologia e flora Brasília. edn. EMBRAPA-Cerrados, Brasília

59. Rocha SMB, Antunes JEL, Araujo FF, Mendes LW, Sousa RS, Araujo ASF (2019) Soil microbial C:N:P ratio across physiognomies of Brazilian Cerrado Soil microbial biomass across a gradient of preserved native Cerrado. An Acad Bras Ciências 91:e20190049
60. Rodrigues JL, Pellizari VH, Mueller R, Baek K, Jesus Eda C, Paula FS, Mirza B, Hamaoui GS Jr, Tsai SM, Feigl B, Tiedje JM, Bohannan BJ, Nüsslein K (2013) Conversion of the Amazon rainforest to agriculture results in biotic homogenization of soil bacterial communities. PNAS USA 110:988–993
61. Sáenz de Miera LE, Pinto R, Gutierrez-Gonzalez JJ, Calvo L, Ansola G (2020) Wildfire effects on diversity and composition in soil bacterial communities. Science Total Environ 726:138636
62. Searchinger TD, Estes L, Thornton PK, Beringer T, Notenbaert A, Rubenstein D, Heimlich R, Licker R, Herrero M (2015) High carbon and biodiversity costs from converting Africa's wet savannas to cropland. Nature Clime Change 5:481–486
63. Smith NR, Kishchuk BE, Mohn WW (2008) Effects of wildfire and harvest disturbances on forest soil bacterial communities. App Environ Microbiol 74:216–224
64. Souza RC, Mendes IC, Reis-Junior FB, Carvalho FM, Nogueira MA, Vasconcelos AT, Vicente VA, Hungria M (2016) Shifts in taxonomic and functional microbial diversity with agriculture: How fragile is the Brazilian Cerrado? BMC Microbiol 16:42
65. Strickland MS, Lauber C, Fierer N, Bradford MA (2009) Testing the functional significance of microbial community composition. Ecology 90:441–451
66. Suela-Silva M, Naves Sales A, Teixeira Magalhães-Guedes K, Ribeiro Dias D, Schwan RF (2013) Brazilian Cerrado soil Actinobacteria ecology. Biome Res Int 2013:503805
67. Vieira CK, Borges LGA, Marconatto L, Giongo A, Stürmera SL (2018) Microbiome of a revegetated iron-mining site and pristine ecosystems from the Brazilian Cerrado. Appl Soil Ecol 131:55–65
68. Wang Y, Qian PY (2009) Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. PLoS One 4:e7401
69. Ward NL, Challacombe JF, Janssen PH, Henrissat B, Coutinho PM, Wu M, Xie G, Haft DH, Sait M, Badger J, Barabote RD, Bradley B, Brettin TS, Brinkac LM, Bruce D, Creasy T, Daugherty SC, Davidsen TM, DeBoy RT, Detter JC, Dodson RJ, Durkin AS, Ganapathy A, Gwinn-Giglio M, Han CS, Khouri H, Kiss H, Kothari SP, Madupu R, Nelson KE, Nelson WC, Paulsen I, Penn K, Ren Q, Rosovitz MJ, Selengut JD, Shrivastava S, Sullivan SA, Tapia R, Thompson LS, Watkins KL, Yang Q, Yu C, Zafar N, Zhou L, Kuske CR (2009) Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. App Environ Microbiol 75:2046–2056
70. Westerling AL, Hidalgo HG, Cayan DR, Swetnam TW (2006) Warming and earlier spring increase western US forest wildfire activity. Science 313:940–943
71. Xiang X, Shi Y, Yang J, Kong J, Lin X, Zhang H, Zeng J, Chu H (2014) Rapid recovery of soil bacterial communities after wildfire in a Chinese boreal forest. Sc Rep 4:3829
72. Yang S, Zheng Q, Yang Y, Yuan M, Ma X, Chiariello NR, Docherty KM, Field CB, Gutknecht JLM, Hungate BA, Niboyet A, Le Roux X, Zhou J (2020) Fire affects the taxonomic and functional

composition of soil microbial communities, with cascading effects on grassland ecosystem functioning. Glob Change Biol 26:431–442

Figures

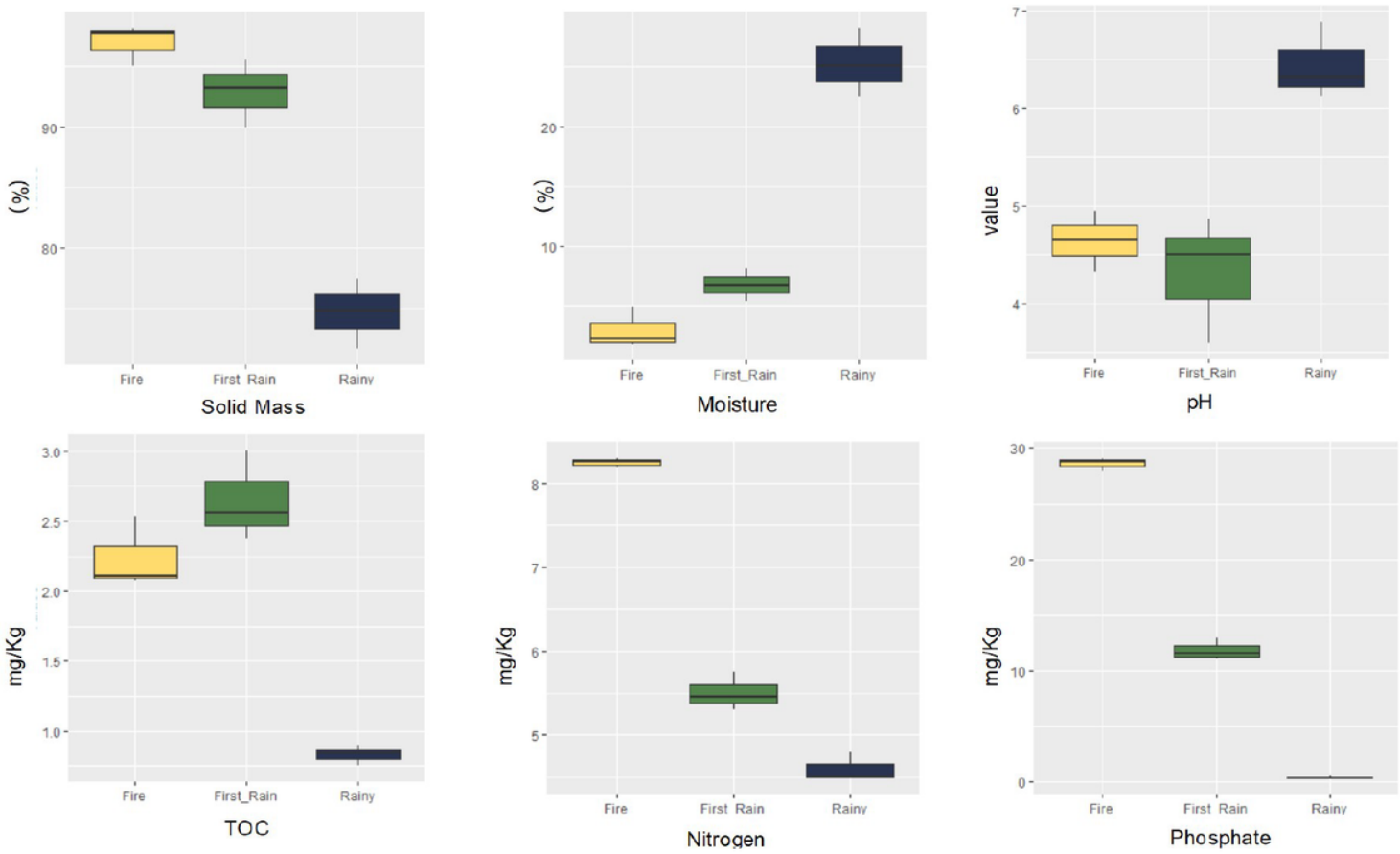

Figure 1

Boxplot showing the main physical-chemical parameters of the soil measured in the three conditions studied. Orange boxplots refer to "Fire" condition samples, green boxplots are "First_Rain", and blues are "Rainy".

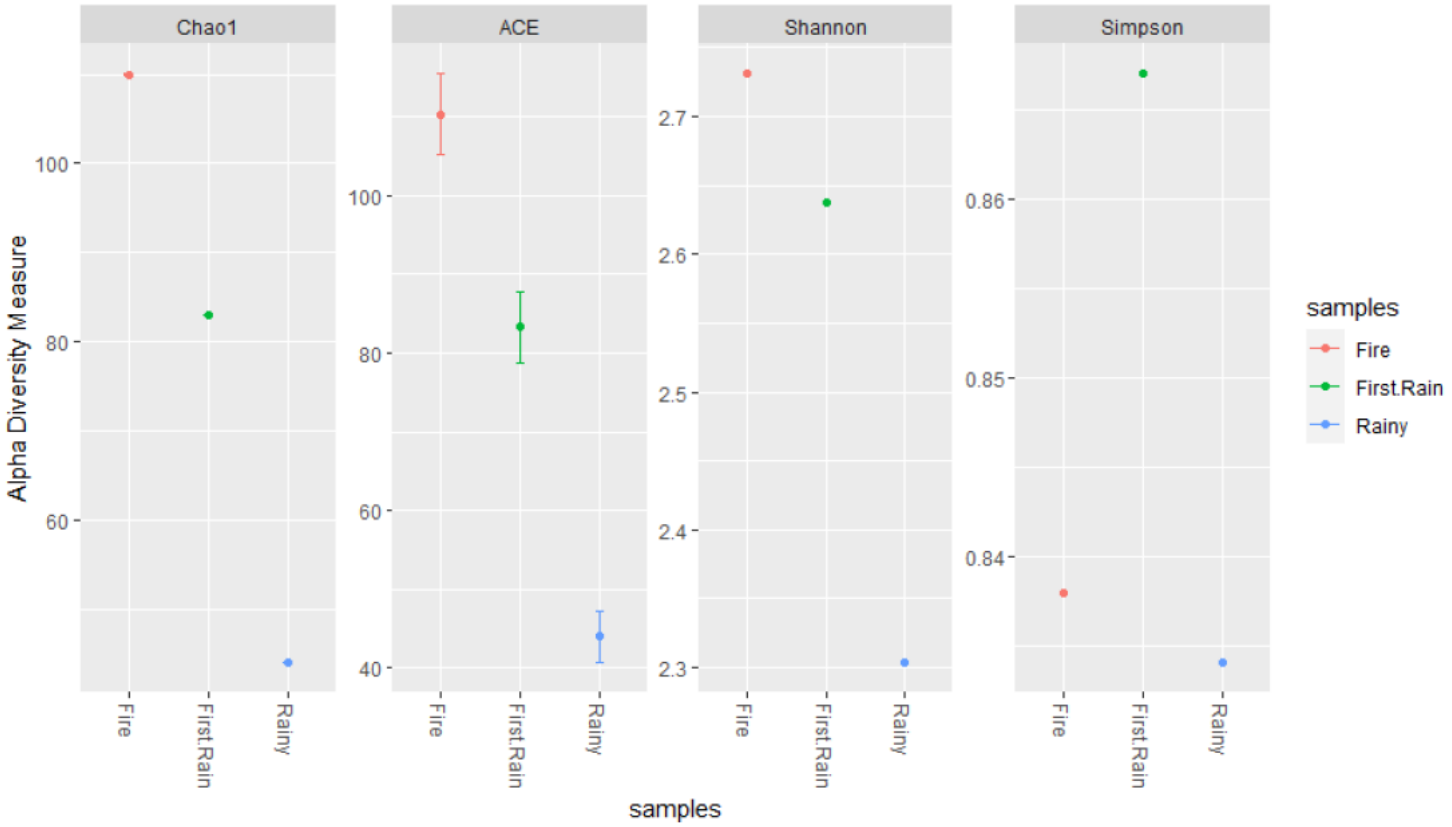

Figure 2

Alpha diversity of the soil bacterial community according to the Chao1, ACE, Shannon, and Simpson indices as affected by the environmental conditions analyzed.

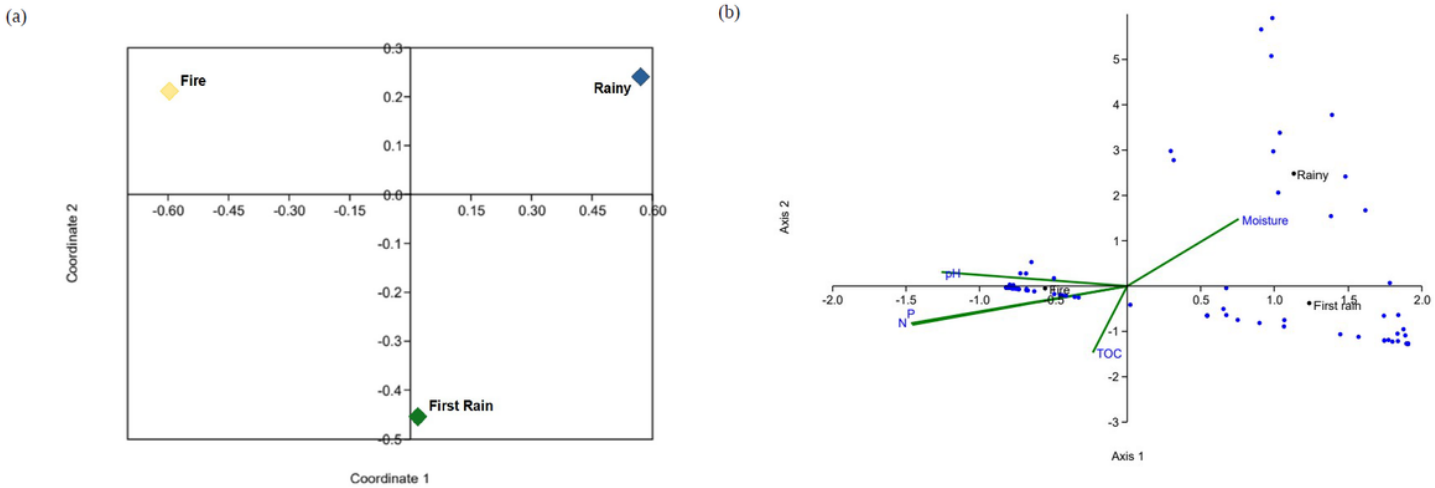

Figure 3

(a) Non Metric Multidimensional Scaling (NMDS) of bacterial communities in relation to the three environmental conditions evaluated in this study. Environmental variables, Fire, First Rain, and Rainy are just projected in the graph. (b) Canonical correspondence analysis (CCA) biplot of microbial communities

and environmental variables significantly related to microbial community variations: moisture, pH, N, P and TOC.

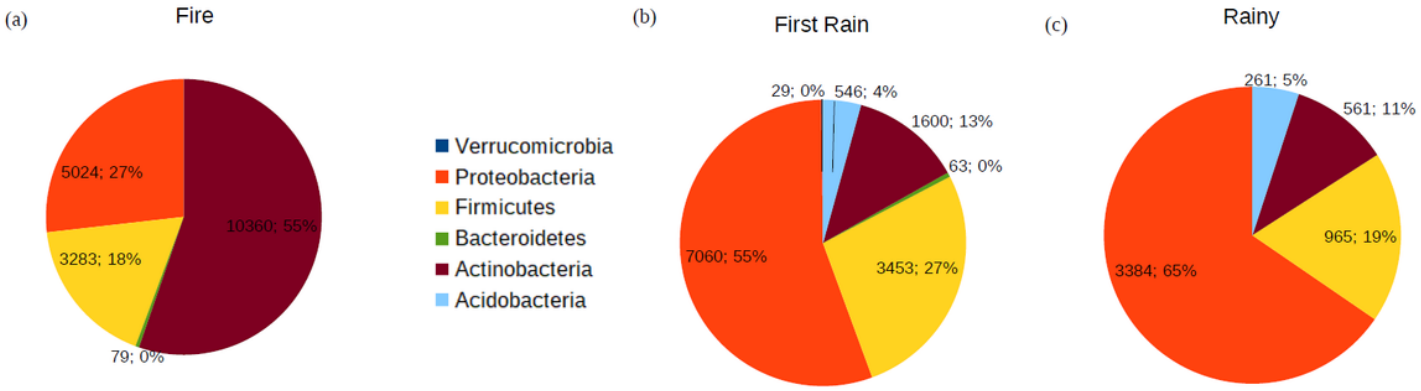

Figure 4

Sector graphs showing the number of OTUs and relative abundance of the phyla described under different environmental conditions: (a) "Fire", (b) "First Rain", and (c) "Rainy".

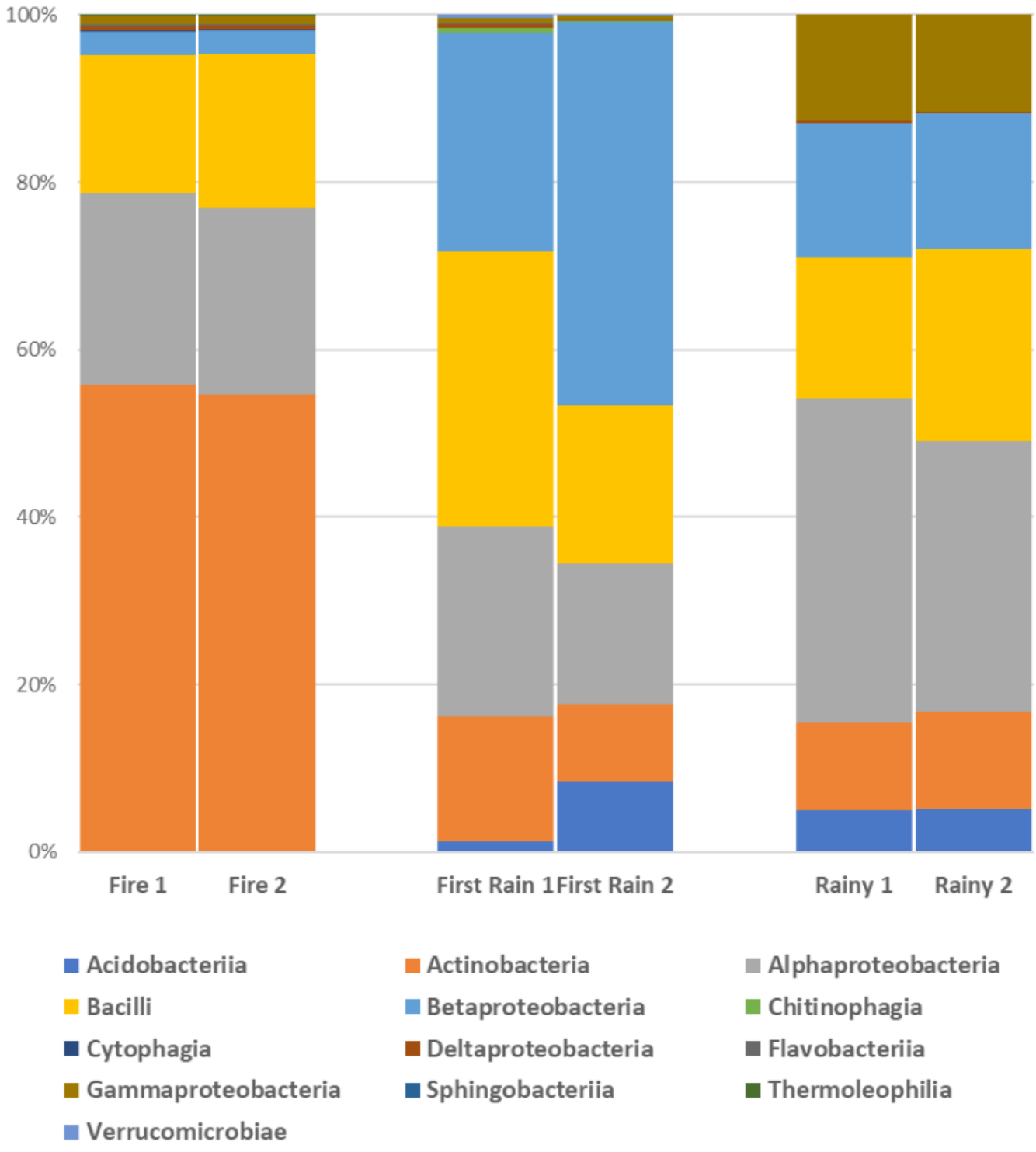

Figure 5

Distribution of microbial classes among the six samples with reference to the three conditions evaluated.

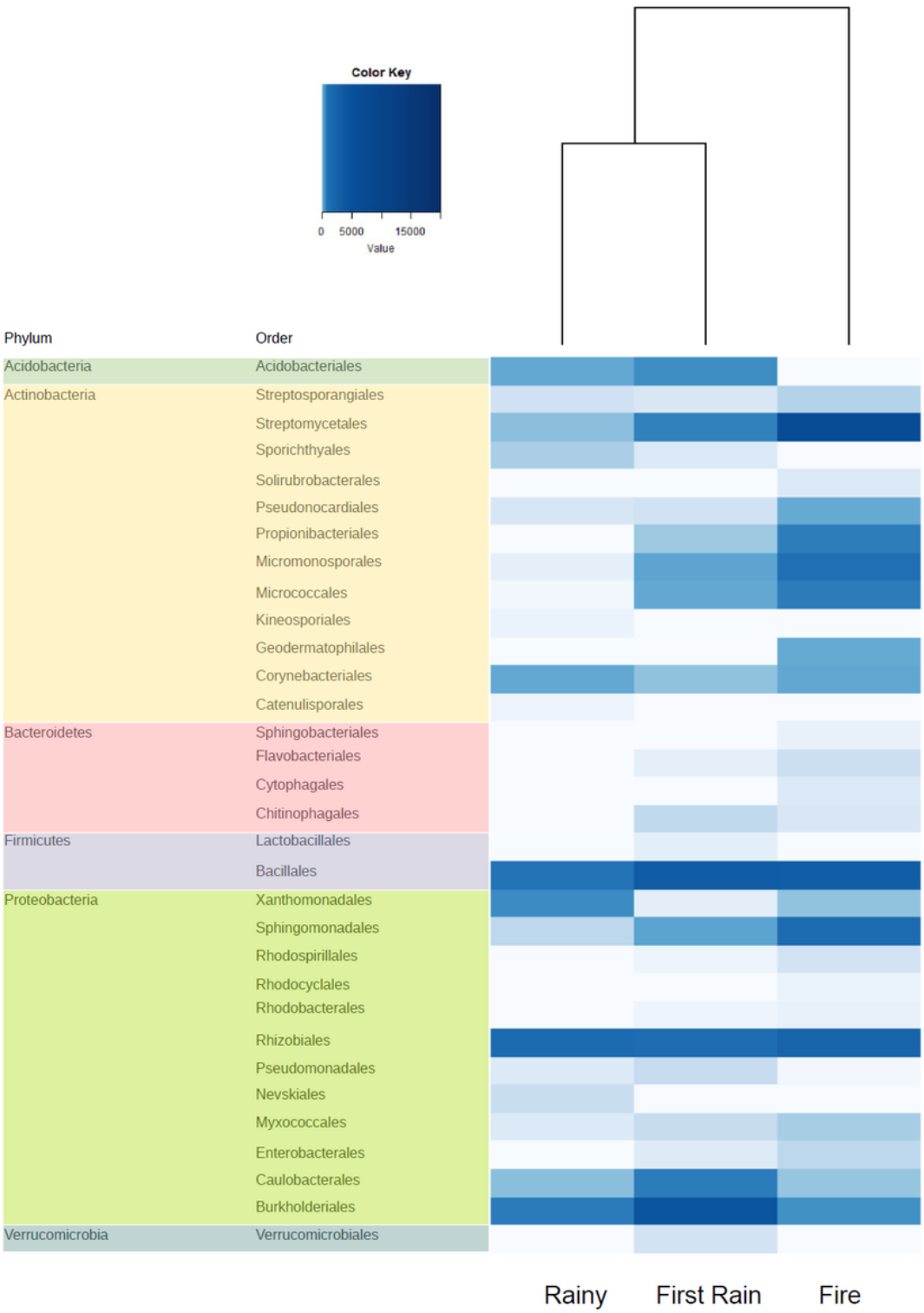

Figure 6

Heatmap of soil microbial community abundance profiles at the Order level, with related phyla shown on the right.