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SOIL SCIENCE

Impact of land use on soil function and bacterial community in the Brazilian savanna

LENKA BOBUL'SKÁ, SUÉLLEN P. ESPÍNDOLA, MICHELLE A. COELHO, ADÃO S. FERREIRA

Abstract: Land use systems have a great impact on soil function and microbial diversity in tropical soils. Our study aimed to evaluate soil biochemical indicators and community composition and to assess the relationship between soil biochemical and microbial indicators and bacterial diversity of three agroecosystems (pine forest, soya and sugarcane) and native Cerrado forest in the Brazilian savanna. Soil biochemical indicators (soil organic matter and enzymes) and high-throughput sequencing of 16S rDNA were performed in two topsoil depths (0-5 cm and 5-10 cm). Soil microbial and enzyme activity showed that agricultural soil usage has a negative impact on soil function compared to native and pine forests. Results also revealed higher enzyme activities in 0-5 cm depth compared to 5-10 cm depth, but enzymatic activities depend on land use systems. Soil bacterial community was affected by land use systems and depth, revealing changes in structure and abundance of bacterial composition. Alpha-diversity indexes were higher in the agricultural systems than in the forests, however they showed a significant negative correlation with most of the studied soil microbial and biochemical indicators. Our research had brought new relevant information about the relationship between the soil biochemical indicators and the bacterial diversity in the Brazilian Cerrado.

Key words: soil quality indicators, bacterial diversity, depths, native and pine forest, soya and sugarcane agroecosystems.

INTRODUCTION

Land use systems have caused several impacts on soil function, soil chemical, physical and biological properties (Gil-Sotres et al. 2005, Thomson et al. 2015, Manoharan et al. 2017, Vinhal-Freitas et al. 2017). Soil functions are essential for the biosphere and include nutrient cycling, C storage and turnover, water maintenance, soil structure arrangement, regulation of soil biota diversity, biotic regulation, buffering, etc. (Arnold 2016). The impacts on soil functions and soil properties result mainly in the loss of soil organic matter (SOM) and soil microbial properties, such as microbial activity and microbial biomass (Vinhal-Freitas et al. 2013, 2017). Soil enzyme activities, which are associated with carbon transformations and nutrient cycling, are also affected by land use systems. Hydrolytic enzymes have an extracellular activity and are mainly produced by soil microorganisms. Such indicators are important for assessing the intensity of soil degradation among different use ecosystems. However, the effects of land use on soil function are also determined by agricultural practices, soil type and environmental conditions (Gil-Sotres et al. 2005, Wallenius et al. 2011). Soil microbial community seems to be highly responsive to all or any soil physical, chemical and biological changes, as well as

environmental conditions. Therefore, studies with different long-term agroecosystems are very important for assessing the changes of soil quality indicators, soil function and soil microbial community (Fernandez et al. 2016, Vinhal-Freitas et al. 2017). The relationship between soil quality indicators (biochemical and microbial attributes) and soil bacterial composition can be useful for better understanding the changes of microbial community and soil function under land use systems.

Bacterial community has paramount importance in several soil ecological processes and plays a key role in soil function. However, soil bacterial community structure, including abundance, richness and diversity depends on several and integrate abiotic factors such as soil pH, nutrient content, moisture and temperature (Nemergut et al. 2011, Zhalmina et al. 2015, Fernandez et al. 2016). Particularly, studies that determine diversity through pyrosequencing of the 16s rRNA gene have shown that soil pH has a strong effect on bacterial diversity, indicating that higher bacterial diversity is usually found in pH from 6.0 to 7.0 (Nemergut et al. 2011, Zhalmina et al. 2015, van der Bom et al. 2018, Liu et al. 2019). Soil microbial diversity is also affected by nitrogen addition, but such changes still depend on N source, soil type and management practices (Zhalmina et al. 2015). However, soil pH and moisture appear to be the major drivers of microbial community composition in agroecosystems (Lauber et al. 2008, Fernandez et al. 2016, Liu et al. 2019, Byers et al. 2020). Moreover, these abiotic factors in the soil surface layer are strongly altered by land use type (i.e. plant cover), altering the microbial community structure and affecting soil function.

The shallow depth of topsoil has a role of utmost importance in the productivity of agroecosystems due to higher nutrient stocks than subsoil. Soil depth are strongly influenced by the deposition litter as well as environmental conditions, which depend on daily and seasonal fluctuations. In topsoil, the structure and composition of soil microbial community might sensitively be changed within a shallow depth, and this might govern many soil ecological processes, such as decomposition and mineralization of nutrients the in topsoil (Paul 2007). However, most studies have commonly been performed on a wide layer of surface topsoils (Bobuľská et al. 2015, Engelhardt et al. 2018, Sarto et al. 2020), decreasing the microbial activity indices and microbial community composition were showed.

The Brazilian Cerrado, as one of the most humid savanna region of the world, occupies over 200 million hectares and is equivalent to 22% of the Brazilian territory. It is also the second largest biome in the country and moreover, the region is a global biodiversity hotspot (Batlle-Bayer et al. 2010, Carranza et al. 2014). The majority of soils are old, highly weathered (such as Oxisols and Ultisols), rich in iron and aluminum oxides, acidic and poor in nutrients (Vinhal-Freitas et al. 2013). In the recent years, the ongoing conversion of the native Cerrado ecosystems into agricultural lands is of high concern. Several authors have reported that changes in the use and managements of Cerrado soils have promoted significant changes in physical and biochemical indices (Lobato et al. 2018, Costa et al. 2020). In the Brazilian Cerrado, surveys of shallow depths (0-5 and 5-10 cm layers) on topsoil have been recently reported for microbial and biochemical indicators (Vinhal-Freitas et al. 2013, 2017), as well as soil bacterial community composition using of next-generation sequencing (Rampelotto et al. 2013, Catão et al. 2014). However, the relationship between soil function and bacterial community composition is still unknown. This relationship can help us better understanding the transformation

of nutrients of soil under land use systems in the Brazilian savanna. Therefore, the present study aimed (i) to compare the changes in soil microbial and biochemical properties in the native Cerrado forest and different long-term agricultural agroecosystems, (ii) to evaluate the soil bacterial community composition and diversity under different land use conditions, and (iii) to determine the relationship between soil function and bacterial community composition in three agroecosystems of the Brazilian Savanna and the native Cerrado system.

MATERIALS AND METHODS

Sites

The study was performed in soil samples collected in the native Cerrado forest, pine forest, soya field (~ 17 years old with the crop rotation using corn every 4 years) and sugarcane field (~ 18 years old with new cycles every 5 years) in the region of the Uberlândia city (Minas Gerais State), in the south-eastern of Brazil (Fig. 1). The dominant plant species of Cerrado ecosystem is composed of a wide range of species such as *Qualea grandifolia* Mart. (pau-terra), *Bowdichia virgilioides* Kunth (sucupira-preta), *Pterodon pubescens* (Benth.) (sucupira), *Caryocar brasiliense* Cambess. (pequi), *Vatairea macrocarpa* (Benth.) Ducke (angelim do cerrado), *Astronium fraxinifolium* Schott (Gonçalo-alves), *Eugenia dysenterica* DC. (cagaita), *Hymenaea stigonocarpas* Mart. (jatobá) and others with no anthropogenic alteration (Vinhal-Freitas et al. 2013). The pine forest was under a dense planted forest represented by species *Pinus caribaea* Morelet var. *hondurensis* (Sénéclauze). The pine forest was fertilized only once when the seedlings were planted in 1976 and there was a 10-cm layer of litter on the soil surface consisting of needles, cones and woodchips. The sites are in the same climatic zone, which was classified as Cwa according to the Köppen's classification. The sites are in areas with the same soil type, classified as Oxisols (Soil Taxonomy, USA, 1992). More information on management of the sites was described in the previous reports (Vinhal-Freitas et al. 2013, Leite et al. 2018). In April 2016, four soil samples per site were taken in an area of 600 cm^2 (20 cm x 30 cm) and two depths (0-5 and 5-10 cm), accounting 32 soil samples. Within each site, the soil samples were approximately spaced in 100 meters from each other. Four subsamples were collected and subsequently well mixed for making the soil samples. After the samples were transferred into the laboratory, they were sieved (3 mm) and stored in the plastic bags at 4°C until analysed.

Soil physicochemical analyses

A portion of soil samples was air-dried for 3 days and completely crushed in a porcelain crucible. This sample was used to determine the sand, silt and clay content according to the pipette method (Gee & Bauder 1986), which were used to find the soil textural class of each site (Table I). Soil pH was determined in water (1:2.5 soil/water). Soil organic carbon (SOC) was analysed in acid solution containing potassium dichromate (Yeamans & Bremner 1988) and total nitrogen (NT) was evaluated by the Kjeldahl method (Black 1965).

P, K^+, Ca^{2+}, Mg^{2+}, and Al^{3+}, were determined according to Tedesco et al. (1995), after the samples that had been dried, sieved (< 2 mm) and crushed in a porcelain crucible. All the analyses of soil physicochemical characterizations are shown in Table II.

Soil microbial and biochemical analyses

Soil microbial respiration (SMR) was measured by CO_2 gas emissions from 100 g field moist soil in sealed bottles (500 mL) using the standard method (Stotzky 1965) for 21 days at 25°C. Microbial

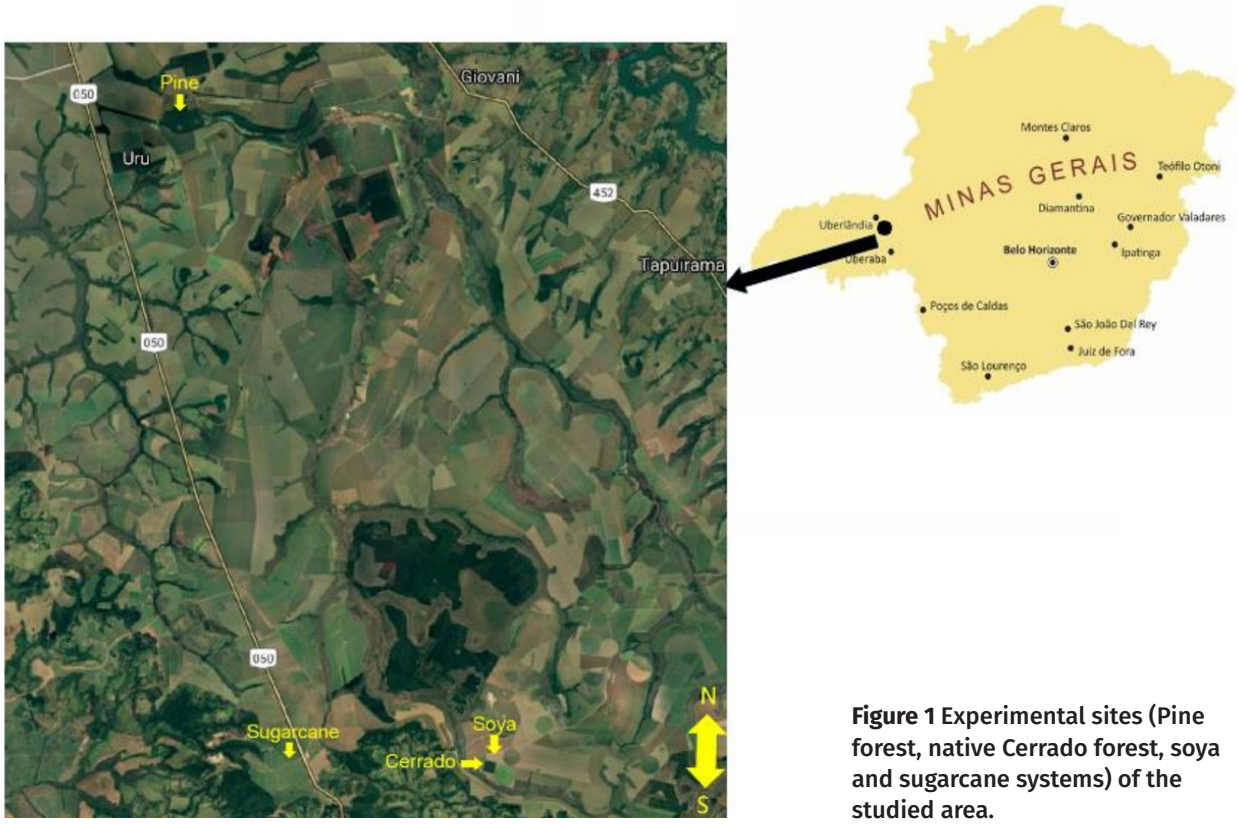

Figure 1 Experimental sites (Pine forest, native Cerrado forest, soya and sugarcane systems) of the studied area.

Table I. Sites and soil characterization of different land use types.

Land use	Geographical coordinates	Altitude	Clay	Silt	Sandy
		m	-------- g kg^{-1} ---------		
Cerrado	19°20'46" S 48°00'59"W	970	705	122	173
Pine	19°04'58" S 48°10'49"W	970	792	97	111
Soya	19°20'30" S 48°00'48"W	976	705	122	173
Sugarcane	19°20'49" S 48°06'15"W	925	732	151	117

biomass carbon (MBC) was determined by the extraction method in the solution of potassium sulphate (0.5 mol L^{-1}) as described by Vance et al. (1987). In the same extract, N concentration was quantified for assessing microbial biomass nitrogen (MBN) (Brookes et al. 1985). Metabolic quotient (qCO_2) of the soil was calculated using SMR to MBC ratio (Anderson & Domsch 1993). Enzymatic activity assays, beta-glucosidase (GLU), urease (URE), fluorescein diacetate (FDA), dehydrogenase (DHA), phosphatase (PHO) and arylsulphatase (ARY), were determined using field-moist soil samples using specific substrates of each enzyme (Sigma). All the essay conditions are shown in Table III previous published (Vinhal-Freitas et al. 2017).

Table II. Physical and chemical properties (values a) of soils investigated in two depths and different land use systems in the Brazilian Savanna.

Property *	Cerrado		Pine		Soya		Sugarcane	
	0-5cm	5-10cm	0-5cm	5-10cm	0-5cm	5-10cm	0-5cm	5-10cm
pH	3.75 ± 0.05	3.85 ± 0.05	3.67 ± 0.08	3.9 ± 0.00	5.87 ± 0.08	5.37 ± 0.08	5.90 ± 0.12	5.40 ± 0.14
SOC, mg C kg^{-1}	30.4 ± 2.6	23.9 ± 2.5	19.9 ± 3.2	12.5 ± 0.3	18.7 ± 1.6	16.7 ± 0.98	18.7 ± 0.51	18.3 ± 2.2
N total, mg kg^{-1}	3.43 ± 0.15	2.34 ± 0.11	1.25 ± 0.14	0.89 ± 0.14	2.01 ± 0.16	1.50 ± 0.06	1.64 ± 0.12	1.17 ± 0.09
P available, mg kg^{-1}	1.21 ± 0.11	1.12 ± 0.01	1.07 ± 0.02	1.03 ± 0.01	3.47 ± 0.11	2.43 ± 0.81	1.47 ± 0.09	1.64 ± 0.36
K available, mg kg^{-1}	44.2 ± 7.1	32.7 ± 2.0	25.7 ± 7.4	11.5 ± 0.86	116 ± 47	94.1 ± 32	116 ± 23	66.5 ± 16.7
Ca, $cmol_c$ dm^{-3}	0.3 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	2.8 ± 0.1	2.4 ± 0.1	2.9 ± 0.2	2.5 ± 0.1
Mg, $cmol_c$ dm^{-3}	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.3 ±0.1	0.6 ± 0.1	0.5 ± 0.1
Al, $cmol_c$ dm^{-3}	0.9 ± 0.3	1.0 ± 0.3	0.4 ± 0.1	0.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
H + Al, $cmol_c$ dm^{-3}	12.3 ± 0.2	12.1 ± 0.3	12.4 ± 0.3	11.1 ± 0.4	3.2 ± 0.1	3.5 ± 0.1	3.1 ± 0.1	3.4 ± 0.1

***Values of physicochemical properties per gram of dry soil following its standard deviation; SOC, total organic carbon; N, nitrogen; P, phosphorus; K, potassium; Mg, magnesium; Ca, calcium; Al, aluminium.**

Table III. Incubation conditions of enzymes used with biochemical indicators.

Enzyme	Incubation conditions					Reference
	Substrate	Buffer (pH)	Soil (g)	Temperature (°C)	Time (h)	
FDA	Fluorescein diacetate	7.6 (PPB)	2	30	1	Green et al. 2006
DHA	Iodonitrotetrazolium chloride	7,5 (TB)	1	40	5	Von Mersi & Schinne 1991
PHO	p-Nitrophenyl phosphate	6.5 (SAB)	1	37	1	Tabatabai & Bremner 1969
GLU	4-Nitrophenyl glucopyranoside	6.0 (SAB)	1	37	3	Eivazi & Tabatabai 1988
URE	Urea	6.7 (CB)	5	37	3	Kandeler & Gerber 1988
ARY	p-Nitrophenyl sulphate	5.8 (SAB)	1	37	1	Tabatabai & Bremner 1970

FDA, fluorescein diacetate; DHA, dehydrogenase; PHO, phosphatase; GLU, B-glucosidase; URE, urease; ARY, arylsulphatase; PPB, potassium phosphate buffer; SAB, sodium acetate buffer; CB, citrate buffer; TB, tris buffer.

DNA extraction, 16S rRNA amplification and pyrosequencing

Genomic DNA was extracted from 0.250 g of soil sample using the PowerSoil DNA Isolation Kit (MoBio, Qiagen). The genomic DNA concentration was determined by using the Qubit Fluorometer Kit (Invitrogen, Carlsbad, CA) following the manufacturer's recommendations. The V4-V5 region of the 16S rRNA gene was amplified using archaeal/bacterial primers 515F and 806R (Caporaso et al. 2012) and amplicons sequenced in the PGM Ion Torrent (Life Technologies). To distinguish each sample, a unique barcode sequence was inserted into the forward primer. The forward and reverse primers were tagged with adapter, pad and linker sequences. Each used 25 ul of the PCR mixture consisted of 2.5 ul of 10 x PCR Buffer (Invitrogen), 1.5 ul of $MgCl_2$ (50 mM), 5 ul of dNTP mix (0.01 nM), 0.5 ul of each primer (10 uM 515 F and 806R), 0.5 ul of Platinun™ *Taq* DNA Polymerase (Invitrogen), 100 ng of genomic DNA and 5 – 10 ul of sterile ultrapure water. The PCR conditions were 94°C for 2 min, 30 cycles of 94°C per 45 s denaturation; 55°C per 45 s annealing and 72°C per 1 min extension; followed by 72°C per 6 min. The triplicate amplicons were pooled and purified with the Agencourt® AMPure® XP Reagent (Beckman Coulter, USA) and magnetic rack. The final concentration of the amplified DNA was estimated by using the Qubit Fluorometer Kit (Invitrogen, Carlsbad, CA). Equimolar concentrations of amplicons from all samples were mixed. This composite sample was used for library preparation with the Ion OneTouch™ 2 System with the IonPGM™ Template OT2 400 Kit Template. Sequencing 400 on Ion PGM™ System using Ion 314™ Chip v2 (Thermo Fischer Scientific, Waltham, USA).

Statistical analyses

Statistical analyses were carried out in R using the Vegan and Phyloseq packages (Oksanen et al. 2007, R Core Team 2012, McMurdie & Holmes 2013). Soil microbial and biochemical properties were compared with box-plots showing differences among land use changes. Constrained Analysis of Principal Coordinates (CAP) was used to partition the UniFrac distance matrices of variation among samples using soil physicochemical, microbial and biochemical attributes (Anderson & Willis 2003). Beta-diversity was evaluated using Weighted UniFrac distances to assess phylogenetic differences between samples (Anderson & Willis 2003). Shannon diversity index (H') was determined in each replicate, depth, and ecosystem within taxonomic groups (phylum, class, order, family, genus and species). Person's correlation (r) was performed to assess the relationship between the bacterial compositions and soil quality indicators, which was tested at 5 % significance level according to the Student's test.

RESULTS

Soil microbial and biochemical indicators

The values of soil microbial indicators varied among different land use and two soil depths (Fig. 2). The greatest values of MBC, SMR, MBN, and FDA were found in native Cerrado forest, but soil DHA was higher under pine system in 0-5 cm depth compared to other ecosystems. SMR had a lower change among ecosystems (pine, soya and sugarcane), but high values were observed under native Cerrado forest (Fig. 2b). Overall, the values of microbial indicators were higher in 0-5 cm depth than in 5-10 cm. In contrast to microbial indicators, soil metabolic quotient (qCO_2) values were higher in agricultural systems than in forest systems (Fig. 2d).

Soil biochemical indicators also presented changes with land use systems and depths in topsoil. Beta-glucosidase activity (0-5 cm depth) was higher in agroecosystems (soya and

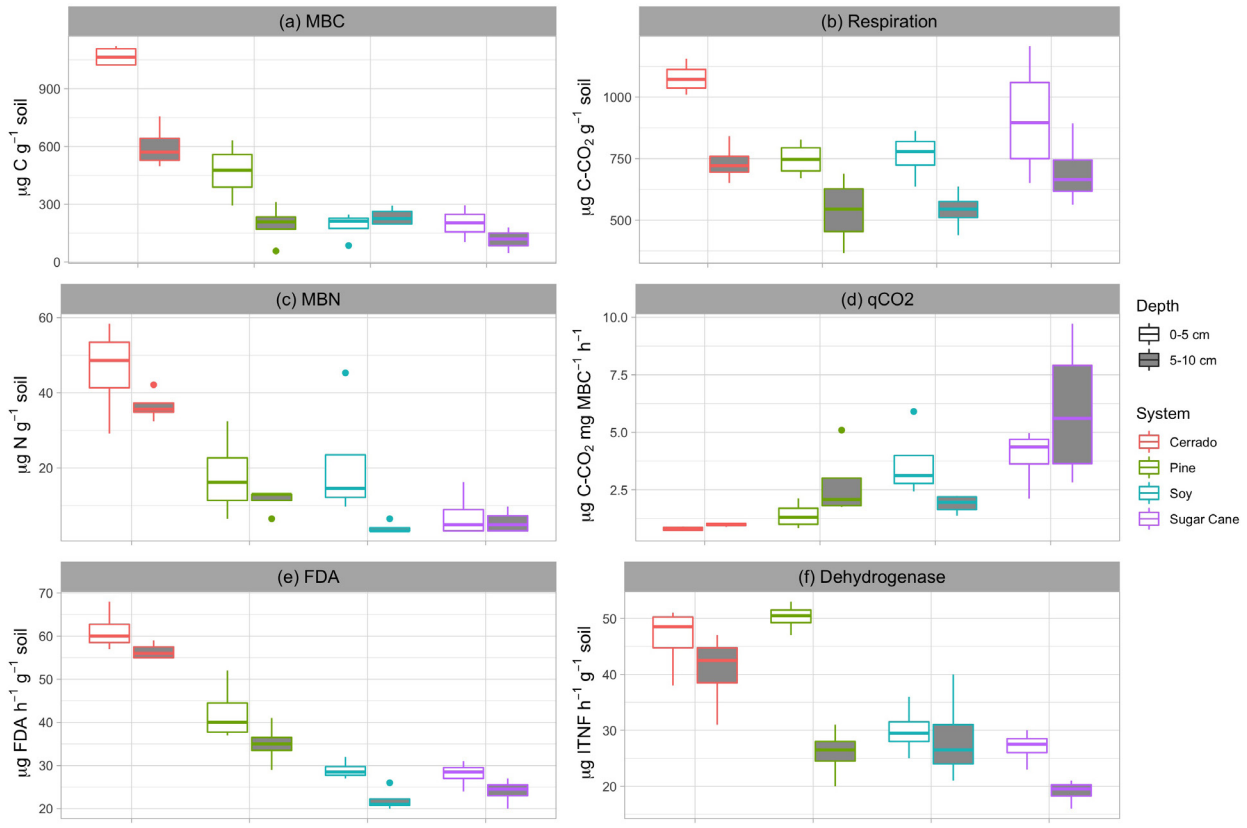

Figure 2 Soil microbial indicators in different land-uses and soil depths in the Brazilian Cerrado. (a), microbial biomass carbon (ug C g^{-1} soil dry); (b), soil microbial respiration (ug CO_2-C g^{-1} soil dry day^{-1}); (c), microbial biomass nitrogen (ug N g^{-1} soil dry); (d), metabolic coefficient (ug CO_2-C mg^{-1} MBC h^{-1}); (e), fluorescein diacetate activity (ug FDA g^{-1} soil h^{-1}); (f), dehydrogenase activity (ug INTF g^{-1} soil h^{-1}).

sugarcane) than in the native Cerrado and pine forest (Fig. 3a), and the differences in activity between both depths were accentuated with lower values in 5-10 cm depth than in the 0-5 cm depth. Urease and phosphatase activities had the same response pattern in relation to land-use systems and depths with the highest values in native Cerrado forest and lowest values in the sugarcane field (Fig. 3b and 3c). ARYL activity was higher in native Cerrado forest than in other ecosystems (Fig. 3d).

The results showed that differences in soil microbial and biochemical indicators depend on the ecosystem type and soil depth. Results also showed a low variability of indicators in each land use and depth, which can be very important to study the correlation between soil biochemical indicators and microbial community composition in topsoil.

Microbial community composition

A high number of 1.19 million high-quality 16S rRNA gene reads were obtained through high-throughput sequencing, which was classified in 463 592 OTUs at 97% sequence similarity. A total of OTUs reads, only 1.4% were identified as unassigned OTUs. The bacterial community composition was mainly dominated by *Proteobacteria* (28%), *Acidobacteria* (27%), *Actinobacteria* (14%), *Verrucomicrobia* (6%), *Bacteroidetes* (4.9%), *Chloroflexi* (4.5%), and AD3 (3.2%). However, the composition of the

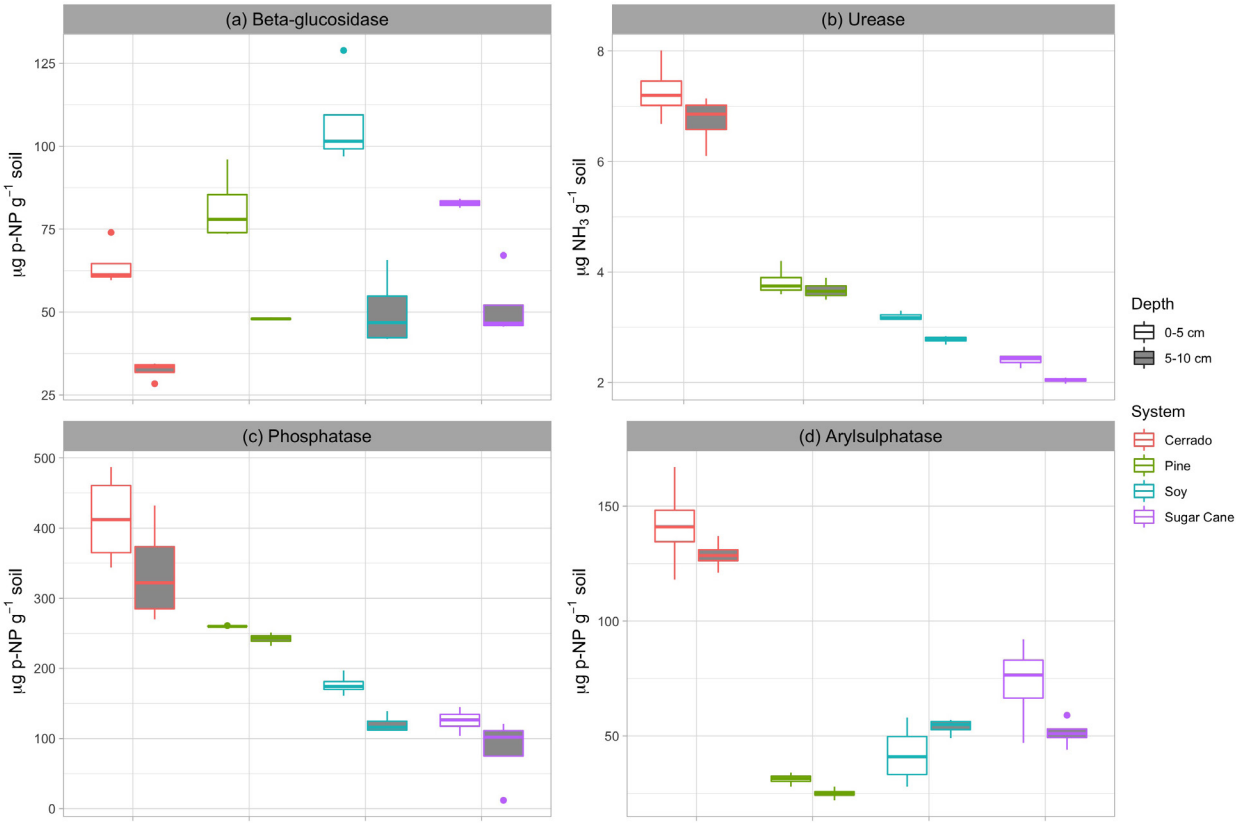

Figure 3 Soil biochemical indicators in different land- uses and soil depths in the Brazilian Cerrado. (a), β-glucosidase activity (ug p-NP g^{-1} soil h^{-1}); (b), urease activity (ug NH_3 g^{-1} soil h^{-1}); (c), phosphatase activity (ug p-NP g^{-1} soil h^{-1}); (d), arylsulphatase activity (ug p-NP g^{-1} soil h^{-1}). Result of each indicator within land use and depth is indicated by box-plots analysis.

bacterial community was strongly affected by land use systems (Fig. 4a). *Acidobacteria* phylum was negatively impacted on the number of OTUs when the land use was altered from the native Cerrado forest to pine forest (39% reduction), soya and sugarcane (both with 68% reduction). An increase of *Actinobacteria* was observed in soya and sugarcane agroecosystems. Decrease of WPS-2 phylum was also observed in soya and sugarcane agroecosystems. In general, the results showed that the top at 5-10 cm depth had a higher abundance of OTUs within phyla compared to the top at 0-5 cm depth, except for *Actinobacteria* and *Bacteroidetes* (Fig. 4a). In pine system, there was also a greater relative abundance of *Acidobacteria* at 0-5 cm depth. Fig. 5b shows that phyla under 2% relative abundance are also affected by land use agroecosystems and those specific phyla (*Armatimonadetes, Crenarchaeota, Firmicutes*, and *Nitrospirae*) also depend on the specified depth.

Microbial diversity

Beta- and alpha-diversity of the bacterial community were characterized using weighted UniFrac distances and Shannon's Diversity index (H'), respectively. Principal coordinate analysis (PCoA) of weighted UniFrac distances showed the similarity and differences of the bacterial community composition among ecosystems (Fig. 5a). The pine forest has a greater similarity with

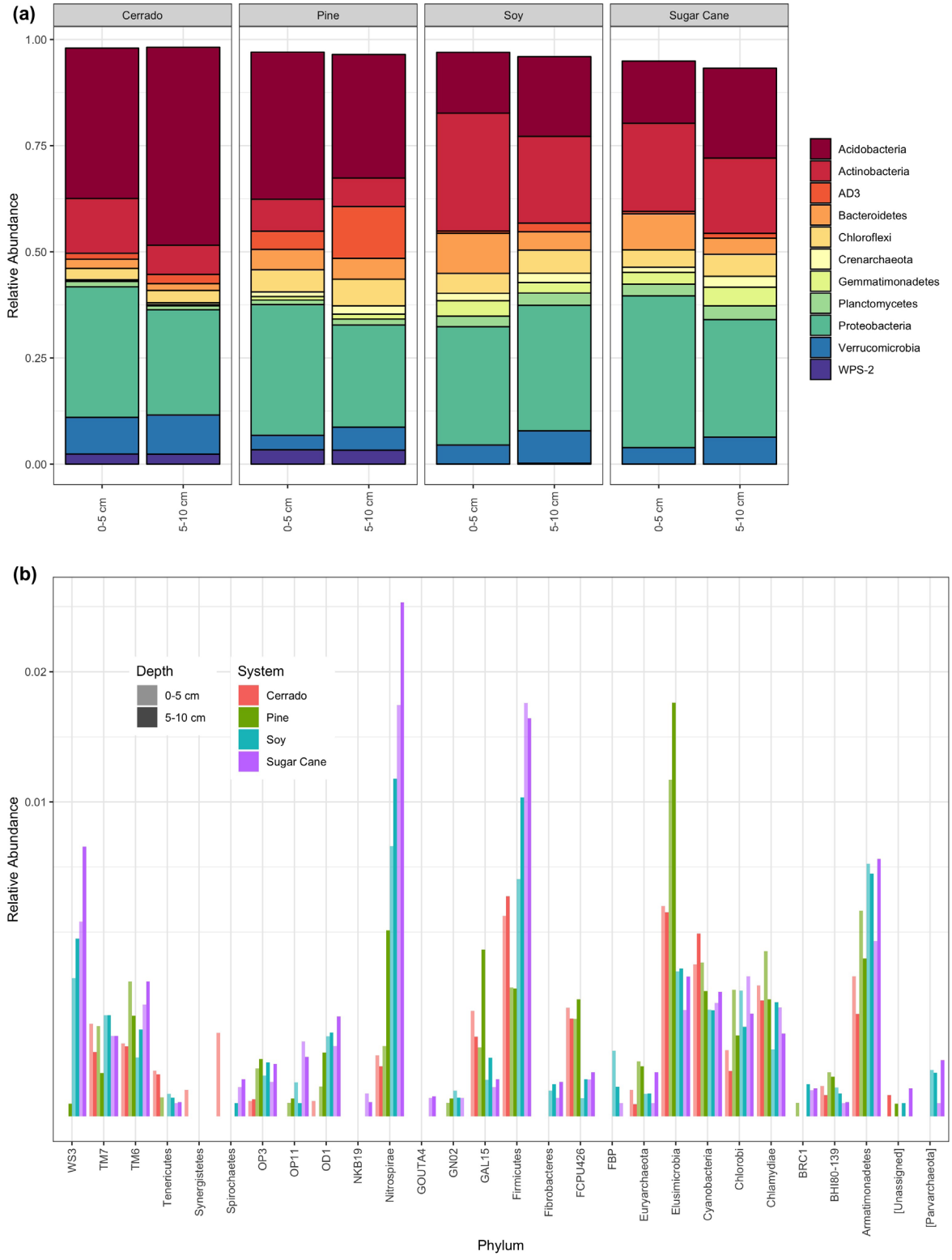

Figure 4 Taxonomic composition of soil bacterial communities in different land use ecosystems and two depths. **(a)** relative abundance of phylum greater than 2 %. **(b)** relative abundance of rare functional groups smaller than 2 %. Phylogenetic analysis was performed of high-throughput sequencing of 16S rDNA gene at 97% similarly level.

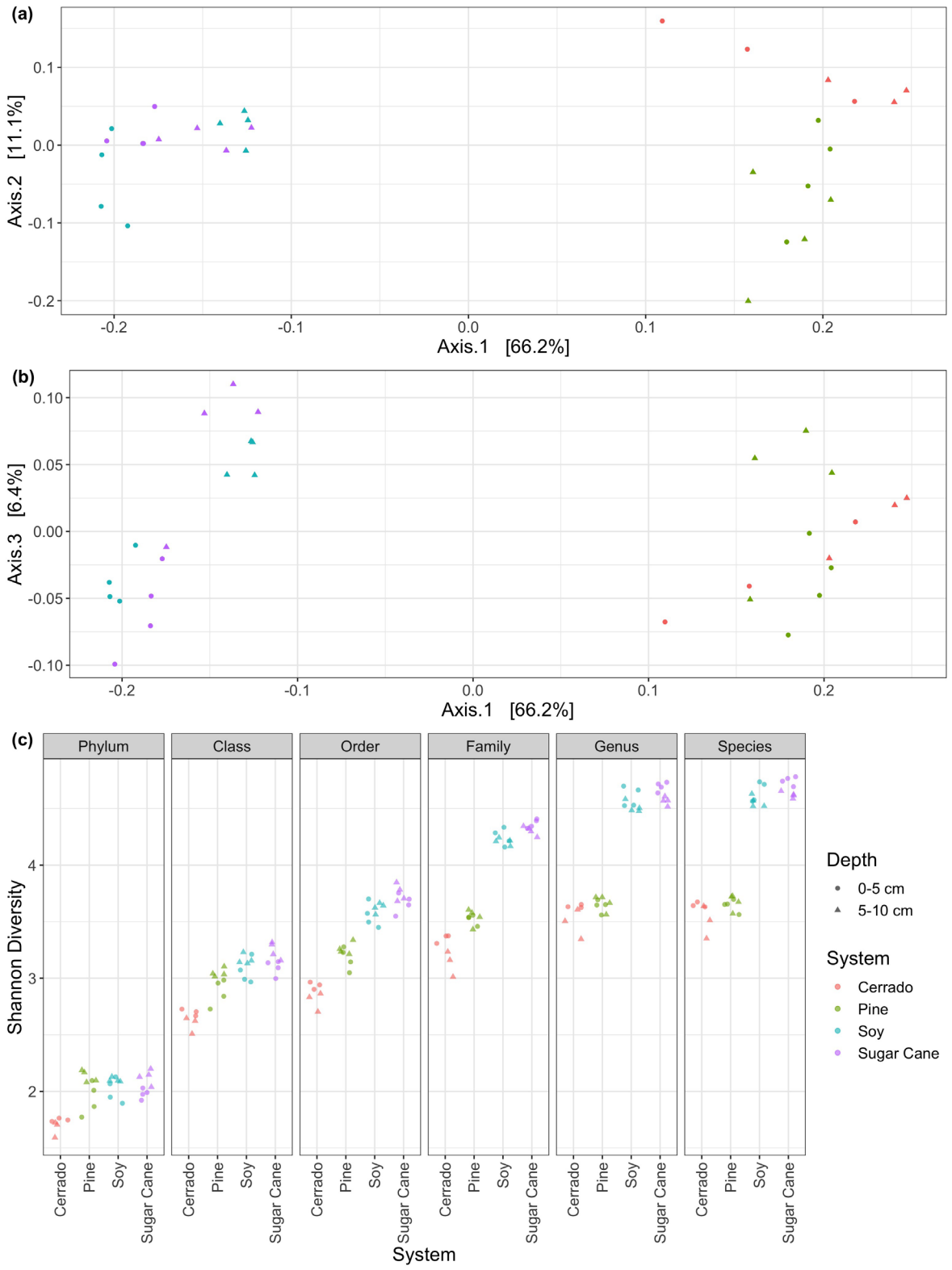

Figure 5 Weighted UniFrac distances (a and b) and Shannon diversity indexes (c) in land use ecosystems and two depths. The analysis of diversity was performed of high-throughput sequencing of 16S rDNA gene at 97% similarly level.

the native Cerrado forest, but there is a difference between two ecosystems. Principal coordinate 3 shows agroecosystems clustering by soil depth, while the native Cerrado and pine forest do not (Fig. 5b). Such results are reliable due to the high values obtained in weighted UniFrac analysis accounting higher than 72% of the variance observed. Shannon diversity index indicated a greater alpha diversity in agroecosystems than in the native Cerrado and pine forest (Fig. 5c). The H' indices increased from higher to lower taxonomic levels and were similar between two depths within the same land use. In Fig. 6, it is shown comparative alpha-diversity indices determined by four indexes (Simpson, Cha1, ACE and Shannon). All of the indexes confirmed a higher alpha-diversity in the agroecosystems than in the native and pine forests.

Correlation between soil bacterial community and quality indicators

A total of 15 bacterial taxonomic groups (including Shannon index) and 14 soil quality indicators were correlated using Person's correlation. Soil quality indicators, such as pH, MBC, MBN, qCO2, FDA, DHA, URE, and PHOP, showed significant correlations with bacterial taxonomic groups and Shannon Index (Table IV), which were associated with more than 6 bacterial taxonomic groups (including Shannon index). TOC, SMR, BGL, and ARYL showed significant correlations with less than 5 bacterial taxonomic groups. C:N rate indicator did not show any correlation with bacterial taxonomic groups.

The bacterial taxonomic groups, such as *Acidobacteria* (11/14), *Archaebacteria* (12/14), *Bacteroidetes* (8/14), *Gemmatimodadetes* (10/14), *Planctomycetes* (7/14), *Gamma-proteobacteria* (8/14), *Firmicutes* (7/14) and Shannon index (11/14) were significantly correlated with several soil quality indicators (Table IV). *Proteobacteria* (alpha-, beta- and gamma-) did not show any correlation with soil quality indicators studied in this survey. *Actinobacteria* and AD3 showed correlations with a few soil quality indicators, e.g., *Actinobacteria* and pH (r=0.55), AD3 and pH (r=-0.46) and AD3 and TN (r=-0.46).

DISCUSSION

Agricultural soils are constantly changing due to the use of different agricultural practices, such as tillage, fertilization, pesticide application, as well as transit of heavy machines in the crops management. Such practices modify the physical, chemical and biological properties and consequently alter the soil function and quality of ecosystems (Tilman et al. 2006, Tiemann et al. 2015). Microbial indicators, such as MBC, MBN, FDA, and DHA decreased in soya and sugarcane agroecosystems. These indicators are very important for ecosystem functions, because they measure a general response of soil microorganisms to environmental disturbances, indicating the efficiency of microorganisms in exploring soil resources. Generally, values of these indicators decreased in subsurface layers compared to surface layers, suggesting that microbial metabolism in the surface is more intense than in the subsurface. The major soil metabolic activity in the surface is positively correlated with a greater SOM concentration and nutrient availability in long-term agroecosystems (Coonan et al. 2020). Soil microbial respiration (CO_2 released from the soil) is an indicator used to assess the transformation and mineralization of SOM, and it is positively associated with MBC. When there is some disequilibrium between MBC and SMR, it shows that soil microorganisms have difficulty assessing SOM and SMR increases in relation to MBC (Anderson & Domsch 1993, Vinhal-Freitas et al. 2017). Thus, the metabolic quotient (qCO_2), the

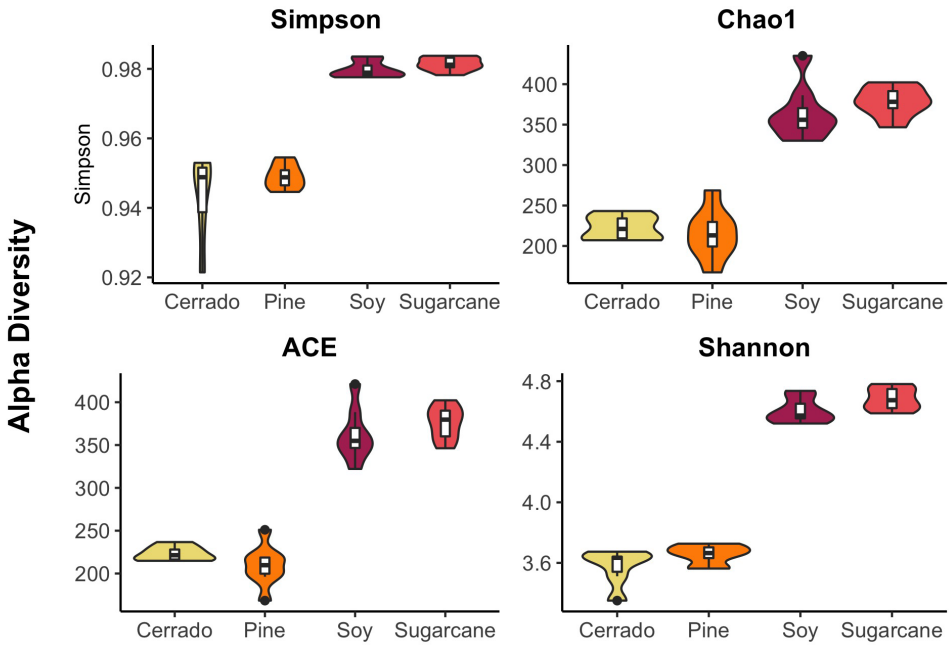

Figure 6 Comparative soil diversity indexes among land use ecosystems. The analysis of violin plot shows alpha-diversity determined by Simpson, Chao1, ACE and Shannon indices of soil bacterial species.

ratio between SMR and MBC, is an index used to measure soil disturbances and inefficiency of carbon use by soil microorganisms (Anderson & Domsch 1993). Our results show that the native Cerrado forest has a high soil microbial respiration, but metabolic coefficient (qCO_2) is lower than in pine forest and much lower than in sugarcane and soya agroecosystems.

Our results showed greater beta-glucosidase activity in agroecosystems compared to the native Cerrado forest. The increase in beta-glucosidase activity is usually linked with soil microbial respiration, because soil microorganisms depend on glucose production from the cellobiose-substrate reaction by beta-glucosidase (Bailey et al. 2013, Moreno et al. 2013). Nevertheless, the synthesis of beta-glucosidase may be influenced by bacterial community composition (Bailey et al. 2013, Moreno et al. 2013). In addition, our results reveal that beta-glucosidase activity can also be strongly linked to the relative abundance of *Actinobacteria* phylum. The study of Zang et al. (2017) showed that the major reservoirs of beta-glucosidase genes were the bacterial phyla *Actinobacteria*, as well as *Firmicutes*, *Proteobacteria* and *Deinococcus-Thermus*. On the other hand, other hydrolytic enzymes related to soil nitrogen (urease), phosphorus (phosphatase) and sulphur (arylsulphatase) cycling were negatively impacted by land use changes, showing the loss of soil quality and ecological soil function in agricultural systems. Except for arylsulphatase in the soya agroecosystem, a higher biochemical activity always occurs in surface layers rather than in subsurface layers. Particularly, our study does not only show comparative changes among soil microbial and biochemical properties, but also reveals the soil functional degradation by land use agroecosystems in the Brazilian Cerrado.

Assessing microbial community composition is of fundamental importance to understanding ecological processes and the functional role of microbiota in terrestrial ecosystems. Our results showed that bacterial community composition significantly altered with the land uses and soil depths. *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* were the phyla with the greatest relative abundance OTUs in sites. The dominance

Table IV. Person's correlation (r) between abundance of taxonomic groups and soil quality indicators in two depths of topsoil in Cerrado agroecosystems (n=32). Minimal significance value of ¨r¨ was estimated in 0.35 or – 0.35 (p< 0.05) according to Student's test.

Taxonomic group	pH	TOC	TN	C:N	MBC	SMR	MBN	qCO2	FDA	DHA	BGL	URE	PHOP	ARYL
Acidobacteria	**-0.51**	**0.36**	0.31	0.05	**0.47**	0.05	**0.40**	**-0.38**	**0.53**	**0.35**	**-0.37**	**0.54**	**0.48**	**0.36**
Actinobacteria	**0.55**	-0.12	0.11	-0.33	-0.30	-0.01	-0.26	0.28	-0.34	-0.24	0.31	-0.27	-0.33	-0.10
AD3	**-0.46**	-0.32	**-0.46**	0.29	0.00	-0.32	-0.09	-0.22	0.09	0.01	-0.29	0.04	0.16	-0.33
Archeabateria	**0.38**	**-0.58**	**-0.52**	0.03	**-0.55**	**-0.49**	**-0.54**	**0.39**	**-0.58**	**-0.49**	0.01	**-0.60**	**-0.55**	**-0.57**
Bacteroidetes	**0.56**	**-0.44**	-0.15	-0.27	**-0.45**	-0.01	-0.28	0.33	**-0.42**	-0.27	**0.60**	**-0.48**	**-0.44**	**-0.44**
Chloroflexi	-0.02	-0.28	-0.30	0.09	-0.15	-0.30	-0.27	0.01	-0.15	-0.08	-0.07	-0.19	-0.15	-0.32
Gemmatimonadetes	**0.70**	**-0.41**	-0.24	-0.17	**-0.54**	-0.19	**-0.49**	**0.51**	**-0.61**	**-0.51**	0.28	**-0.62**	**-0.64**	**-0.39**
Planctomycetes	**0.56**	-0.30	-0.10	-0.25	**-0.39**	-0.10	-0.35	**0.40**	**-0.45**	**-0.39**	0.10	**-0.43**	**-0.49**	-0.22
Proteobacteria	-0.03	0.11	0.17	-0.12	0.13	0.12	0.04	-0.11	0.16	0.18	-0.02	0.15	0.10	0.12
Alfa-proteobacteria	0.16	0.06	0.16	-0.19	0.00	0.09	-0.08	-0.06	-0.02	0.11	0.06	0.01	-0.05	0.05
Beta-proteobacteria	0.34	-0.10	0.13	-0.31	-0.14	0.12	-0.10	0.26	-0.09	-0.13	0,10	-0.12	-0.21	0.05
Delta-proteobacteria	0.20	-0.04	0.15	-0.26	-0.02	0.11	-0.05	0.08	-0.02	-0.10	-0.05	-0.01	-0.09	0.10
Gama-proteobacteria	**-0.69**	0.34	0.14	0.25	**0.51**	0.12	**0.36**	**-0.49**	**0.64**	**0.54**	-0.19	**0.53**	**0.59**	0.12
Firmicutes	**0.60**	-0.24	-0.02	-0.28	-0.30	0.04	**-0.39**	**0.39**	**-0.40**	**0.40**	-0.06	**-0.39**	**-0.53**	0.04
Shannon*	**0.93**	**-0.50**	-0.28	-0.22	**-0.75**	-0.14	**-0.62**	**0.57**	**-0.84**	**-0.62**	**0.47**	**-0.84**	**-0.81**	**-0.50**

TOC, total organic carbon; TN, total nitrogen; C:N, carbon and nitrogen rate; MBC, microbial biomass carbon; SMR, soil microbial respiration; MBN, microbial biomass nitrogen; qCO_2, metabolic quotient; FDA, fluorescein diacetate; DHA, dehydrogenase activity; BGL, beta-glucosidase; URE, urease; PHOP, phosphatase; ARYL, arylsulphatase.*, Shannon index of soil bacterial diversity.

of these phyla in the Brazilian Cerrado has been shown in other surveys (Rampelotto et al. 2013, Catão et al. 2014). Kielak et al. (2016) reported that *Acidobacteria* represents a highly diverse phylum resident to a wide range of environments around the Earth, but there is still relatively little information about the ecological role of this phylum. *Acidobacteria* phylum has been considered as an oligotrophic group of microorganisms in soil due to a slower growth rate and ability to metabolize nutrient-poor and recalcitrant C substrates (Davis et al. 2011, Fierer et al. 2012). In particular, our studies show that native Cerrado forest has a high microbial activity and SOM content. In addition, the native Cerrado forest presents a high plant diversity that may release different metabolic compounds such as amino acids, sugars, and organic acids. These soluble compounds are readily available to soil microorganisms. In contrast, the native Cerrado forest has a lower availability of nutrients, like nitrogen, phosphorus, calcium, and magnesium, because the majority of nutrients are bonded to SOM. Studies have also revealed that the acidobacterial community has different metabolic profiles that may metabolize many carbon sources, reduce nitrate and nitrite and resist watering stress (Catão et al. 2014, Kielak et al. 2016). In this study, the results show that the acidobacterial community is sensitive to land use changes. Although the land use causes many alterations in soil physiochemical properties, the increase of soil pH might be a primary factor in reducing of the acidobacterial community. Increasing soil pH can directly affect the metabolic function of the acidobacterial community and indirectly favour the growth of other microbial groups. In general, these effects may decrease the competitive capacity of the acidobacterial community in soil environments.

Proteobacteria and *Actinobacteria* are ecological groups of soil microorganisms with the fundamental roles in ecosystem processes due to their diversity, abundance and metabolic profiles. Our results showed that the proteobacterial community hardly changed in the community composition with the highest relative abundance detected in the sugarcane ecosystem. In general, there was a lower abundance of this phylum in subsurface layers than in surface layers, except for the soya system, which showed a similar pattern. These results reveal that the proteobacterial community may have a lower dependence on pH due to a similar pattern observed among land use systems. The distribution of this phylum in each studied ecosystem can be linked to the C source available in soil (Fierer et al. 2012). On the other hand, the abundance of *Actinobacteria* increased in agricultural soils (soya and sugarcane agroecosystems) in relation to those observed in the native Cerrado and pine forest. These results can be associated with pH-dependent values, as *Actinobacteria* have a better growth in neutral pH conditions. Fierer et al. (2012) also reported that N fertilization increased the abundance of the actinobacterial community, suggesting a positive effect of this nutrient on ecological distribution of *Actinobacteria* in soil. Concerning soil function, *Proteobacteria* and *Actinobacteria* have been putatively identified as being copiotrophic taxa, which have high growth rates in conditions of elevated C availability (Eilers et al. 2012, Fierer et al. 2012).

Soil microbial diversity is considered to be critical to the integrity, function, and long-term sustainability of soil ecosystems. Many studies have shown that microbial diversity in soil ecosystems decreases with the land use intensification, such as nutrient availability (van der Heijden et al. 2008, Tiemann et al. 2015, Zhalmina et al. 2015), nitrogen deposition and chemical contamination (Gans et al. 2005, Li et

al. 2016). In the present work, weighted UniFrac distances were used to measure beta-diversity of soil microbial communities. Beta-diversity is defined as the variation in the community composition and measurement of pair-wise dissimilarity between plots (Prober et al. 2015). Studies have shown that the β-diversity of soil microbes has a positive correlation with plant β-diversity in many environments (Prober et al. 2015). It is also shown that pine forest, as a monoculture system, is quite similar to the native Cerrado forest in relation to soil bacterial composition. Pine forest is a stable environment with the few anthropogenic disturbances and nutrient poor, containing a thick layer of litter aboveground, as well as eco-mycorrhizal fungi associations with roots are observed in this ecosystem. Such characteristics of pine forest can be determinant on bacterial communities and soil quality, but more studies are needed in order to better understand the bacterial diversity in the pine forests of tropical soils.

The values of Shannon diversity index (H') were revealed to be higher in agricultural soils compared to native Cerrado forest and pine forest soil. The Shannon index (H') has been used to assess the α-diversity and is considered a sensitive indicator to evaluate the anthropogenic perturbations such as nitrogen fertilizers, pH effects and heavy metal stresses (Jangid et al. 2008, Zhalmina et al. 2015, Liu et al. 2019). Nevertheless, Prober et al. (2015) reported that microbial α-diversity had a weak correlation with plant diversity aboveground in biogeographic scales. In our study, the high values of α-diversity of the bacterial community in soya and sugarcane agroecosystems can be a result of soil chemical properties (soil reaction, nutrients content, SOM) elapsed by land use changes. Changes in soil pH in these agroecosystems can be the main abiotic factor that drives the alpha-diversity of bacterial communities. These findings correspond with the previous research showing a strong positive relationship when assessed between bacterial α-diversity and soil pH (Lauber et al. 2008, Zhalmina et al. 2015, Li et al. 2016, van der Bom et al. 2018). Although there were differences in soil pH between two soil depths and within the same system, the present results did not indicate differences of bacterial alpha-diversity between the depths. Changes in the Shannon diversity indexes might have been influenced by the great abundance of rare bacterial communities (i.e., *Armatimonadetes*, *Firmicutes*, *Nitrospirae*, and *WS3*) in agroecosystems, as the Shannon index is affected by both the number of species and their equitability or evenness. It is also believed that a rise in copiotrophic microorganisms can increase bacterial α-diversity values in soils. The present work shows that a high α-diversity of bacterial communities might indicate a lower use efficiency of soil resources as shown above by soil quality indicators.

Some studies have separately shown the effects of land use on soil microbial diversity (Rampelotto et al. 2013, Manoharan et al. 2017) and soil quality indicators (Vinhal-Freitas et al. 2013, Vinhal-Freitas et al. 2017). To the best of our knowledge, there is no information about the relationship between soil quality indicators and soil bacterial community composition in tropical ecosystems. In this study, our results showed that soil pH had a negative correlation with *Acidobacteria*, AD3, and *Gamma-proteobacteria*, but it had a positive correlation with *Actinobacteria*, *Archaebacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Planctomycetes* and *Firmicutes* groups, as well as Shannon Index. Soil TOC was positively correlated with *Acidobacteria* and negatively correlated with three other groups (*Archaebacteria*, *Bacteriadetes*, and *Gemmatimonadestes*), including Shannon index. Soil TN had a negative correlation with

AD3 and *Archaebacteria*, and C:N rate did not interference in soil bacterial composition. These results show that soil pH and TOC are important chemical properties with the impact on soil bacterial compositions under land use systems, indicating that both properties have an opposite effect on soil bacterial composition. Some studies have reported that soil pH has a key role in governing soil microbial community structure (Ling et al. 2016, Zhalmina et al. 2015), but it appears that soil pH is more important when there were comparisons in the same site with different fertilization levels.

Our results show that the increasing Shannon diversity index is negatively correlated with most of soil quality indicators and soil function properties. A positive correlation of Shannon index is only observed with qCO_2 and beta-glucosidase activity. These results show that soil bacterial community used to determinate Shannon indices may have a low contribution on soil quality indicator and soil function. Thus, assessing Shannon indices in land use agroecosystems could not be representative for comparing the soil function, but it could be important to evaluate differences in management practices within the same land use system (Nemergut et al. 2011, Zhalmina et al. 2015).

CONCLUSIONS

This work showed ecological effects of land use change on soil function and microbial composition in the Brazilian Cerrado. Soil microbial and biochemical indicators showed that agricultural soil usage has a negative impact on soil function, indicating a lower use-efficiency of soil resources by microorganisms in agroecosystems. The effects of land use systems on soil quality indicators were higher in 0-5 cm depth than in 5-10 cm depth. Agricultural soil usage tends to increase the relative abundance of copiotrophic bacteria (*Proteobacteria* and *Actinobacteria*) and rare groups of some taxa (*Nitrospirae*, *Firmicutes* and *Armatimonadetes*) and decrease the relative abundance of oligotrophic bacteria (*Acidobacteria*). Soil pH was the soil variable that most affected bacterial community composition, but its relationship depends on the taxonomic group. Shannon diversity (H') index had a negative correlation with most of soil microbial and biochemical indicators assessed but it had a positive correlation with soil pH, qCO_2 and beta-glucosidase activity. Our survey over land use systems in the Brazilian savanna (native forest, pine forest, soya and sugarcane agroecosystems) showed that soil function might be more dependent on the microbial abundance than on the microbial diversity.

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LENKA BOBUL'SKÁ[1]
https://orcid.org/0000-0002-1803-639X

SUÉLLEN P. ESPÍNDOLA[2]
https://orcid.org/0000-0002-3135-3760

MICHELLE A. COELHO[2]
https://orcid.org/0000-0002-4930-3016

ADÃO S. FERREIRA[2]
https://orcid.org/0000-0002-1867-4308

[1]University of Prešov in Prešov, Department of Ecology, Faculty of Humanities and Natural Sciences, 17, November 1, Prešov 080 01, Slovakia

[2]Universidade Federal de Uberlândia, Instituto de Ciências Agrárias, Campus Glória, Bloco CCG, 38410-337 Uberlândia, MG, Brazil

Correspondence to: **Adão de Siqueira Ferreira**
E-mail: adaosferreira@yahoo.com.br

Author contributions

Lenka Bobul'ská: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review and editing. Suéllen P. Espíndula: Methodology, Formal analysis, Investigation, Writing - original draft. Michelle A. Coelho: Methodology, Writing - original draft. Adão S. Ferreira: Conceptualization, Methodology, Resources, Writing - review and editing, Supervision, Project administration, Funding acquisition and editing, Supervision.