



Soil microbial C:N:P ratio across physiognomies of Brazilian Cerrado

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Abstract: Different physiognomies across the Cerrado could influence the microbial C:N:P ratio in the soil since these physiognomies present different abundance and diversity of plant species. Thus, the aim of this study was to evaluate the microbial C:N:P ratio in soil across three different physiognomies of Cerrado in the Northeast, Brazil, namely campo graminóide (dominance of grasses), cerrado stricto sensu (dominance of grasses, shrubs, low trees, and woody stratum), and cerradão (dominance of woody stratum). Campo graminóide was characterized by lower values of total organic C, N, microbial C:P, N:P, and soil C:N. Cerrado stricto sensu presented average values for most of the measured parameters, while cerradão presented higher values of microbial C, N, P, organic C, N and soil C:P and C:N ratios. The principal component analysis showed that the samples grouped according to the sites, with a clear gradient from campo graminóide to cerradão. Therefore, the differences of vegetation across physiognomies of Cerrado influenced the soil microbial C:N:P ratio, where cerradão showed highest microbial C:N:P ratio than soil under campo graminóide.

Key words: Microbial biomass, soil quality, organic matter, tropical soil.

INTRODUCTION

Soil organic matter (SOM) and microbial biomass (SMB) are suitable indicators of soil fertility in both native and managed ecosystems (Andrews et al. 2004, Jiménez et al. 2011). SOM presents the contents of C, N, and P that are important for biogeochemical cycles and also for plant growth (Liu et al. 2013). On the other hand, SMB is

considered the living component of SOM being constituted by soil microorganisms (Sousa et al. 2015) and can be estimated by microbial biomass C (MBC), N (MBN) and P (MBP). Specifically, soil MBC varies from 1 to 7% of total organic C (TOC), while MBN and MBP vary from 1 to 5% of total N (TN) and P (TP) (Sparling 1992).

SMB presents importance on the biological process in soil, such as immobilization and mineralization of nutrients (Marinari et al. 2006). It means that SMB can act as the sink (immobilization) or the source (mineralization) of nutrients and it

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depends on the relationship between microbial C, N and P, expressed as the C:N:P ratio (Gnankambary et al. 2008). Thus, the C:N:P ratios are important for the understanding of microbial nutrient limitations in soils, since the microbial C:N:P ratio is a good indicator of nutrient status in soil. In native ecosystem, the C:N:P ratio is influenced by the type and structure of vegetation and soil environment (Cristina et al. 2010). Therefore, the knowledge about the microbial C:N:P ratios in native ecosystem is important to assess the soil nutrient status and ecological implications for vegetation.

As a native ecosystem, the Brazilian Cerrado is a biome with high plant diversity (Amaral et al. 2006) distributed in several physiognomies, such as grassland, shrubby and arboreal vegetation (Coutinho 1978). These physiognomies present different types and structures of vegetation that influence soil environmental conditions and, consequently, can affect the soil microbial biomass. Indeed, previous studies have found the highest MBC in shrubby and arboreal vegetation than in grassland (Mendes et al. 2012, Carvalho et al. 2018). In addition, some studies have observed the influence of these different physiognomies on the structure and diversity of soil microbial community (Araujo et al. 2018a, b, 2019).

However, the knowledge about the microbial C:N:P ratio across different physiognomies of Cerrado remains unclear. Also, part of the microbial biomass is threatened, mainly due to the conversion of native areas to agricultural fields. In this sense, the expansion of knowledge about microbial biomass is necessary to protect the native areas and regulate the use for agricultural purposes. Considering the different plant physiognomies found in Cerrado, we hypothesized that these plant physiognomies could influence the microbial biomass ratio in the soil due to their different abundance and diversity of plant species. Thus, the aim of this study was to evaluate the microbial C:N:P ratio in soil across different physiognomies of Cerrado in the Northeast, Brazil.

MATERIALS AND METHODS

This research was conducted in an area under preserved Cerrado inside the Parque Nacional de Sete Cidades (PNSC), located in the Piauí State (04°02'-08'S and 41°40'-45'W). In this region, the climate is sub-humid with two distinct seasons (wet and dry) and presents an annual average temperature of 25°C and rainfall of 1,558 mm, concentrated between February and April. The soil is classified as Fluvisol (FAO) and presents 926 g kg⁻¹ of sand, 39 g kg⁻¹ of silt, and 30 g kg⁻¹ of clay.

In this study, three preserved sites (about 1.000 m² each one) were evaluated across different physiognomies, namely campo graminóide (CG), cerrado stricto sensu (CSS), and cerradão (CD) (Table I). CG is characterized by the dominance of grasses, CSS is dominated by grasses, shrubs, short trees, and woody stratum; while CD is dominated by woody stratum.

Each site was divided into three transects (replications) where soil samples (500g) were collected at a depth of 0 - 20 cm (three points per transect) in May 2016 (rainy season). At each soil sampling, the soil temperature was measured for 5 minutes at 10 cm depth using a probe thermometer. A portion of the soil samples (300g) was stored in bags and kept at 4°C for microbial analysis, while another portion (200g) was air-dried, sieved through a 2-mm screen and homogenized for the chemical analyses.

Soil chemical properties (pH, P, K, Ca and Mg) were determined and measured using standard laboratory procedures (Embrapa 1999). Total organic C (TOC) was determined by the wet combustion method using a mixture of potassium dichromate and sulfuric acid under heating (Yeomans and Bremner 1998). Total N (TN) was determined by Kjeldahl digestion as described by Bremner (1996). Total P (TP) was determined by perchloric acid digestion according to Sommers and Nelson (1972).

TABLE I
Vegetation indexes of the evaluated sites (Oliveira et al. 2007).

	campo graminóide	cerrado stricto sensu	cerradão
Plant richness*	4.7	11	17
Plant diversity**	0.2	0.85	1.10
Plant density***	4.7	27.1	35.0
Vegetation****	a	b	c

* species/100 m²; ** H/100 m²; *** individual/100 m²; **** species of plants present.

H – Shannon-Weaver index.

^a *Andropogon fastigiatus*; *Aristida longifolia*; *Eragrostis maypurensis*;

^b *Andropogon fastigiatus*; *Aristida longifolia*; *Terminalia fagifolia*; *Magonia pubescens*; *Hymenaea courbaril*; *Plathymenia reticulata*; *Qualea grandiflora*; *Combretum mellifluum*; *Lippia organoides*; *Anacardium occidentale*; *Simarouba versicolor*; *Vatairea macrocarpa*;

^c *Aspidosperma discolor*; *Parkia platycephala*; *Terminalia fagifolia*; *Piptadenia moniliformis*; *Plathymenia reticulata*; *Qualea parviflora*; *Anacardium occidentale*; *Copaifera coriacea*; *Thiloua glaucocarpa*; *Casearia grandiflora*.

The microbial biomass C (MBC) was determined by the chloroform fumigation-extraction method according to Vance et al. (1987); the microbial biomass N (MBN) was determined by the method of Brookes et al. (1985); and the microbial biomass P (MBP) was determined by a fumigation-extraction according to the method of Brookes et al. (1982). The extraction efficiency coefficients of 0.38, 0.45 and 0.40 were used to convert the difference in C, N and P between fumigated and unfumigated soil in MBC, MBN, and MBP, respectively. The analyses were conducted in triplicate and expressed as dry weight.

The data obtained were then compared between the treatments using a one-way analysis of variance (ANOVA), with the means being compared using least significant difference values calculated at the 5% level. Principal component analysis (PCA) biplot was used to visualize the differences between the treatments. First, the matrices were analyzed using Detrended Correspondence Analysis (DCA) to

evaluate the gradient size of the species distribution, which indicated linearly distributed data (length of gradient < 3), suggesting the PCA as the best-fit mathematical model for the data. To test whether the sample clusters harbored significant differences among the treatments, we used the analysis of similarity ANOSIM. PCA plot was generated using Canoco 4.5 software (Biometrics, Wageningen, The Netherlands) and ANOSIM was calculated using Past 3 software (Hammer et al. 2001).

RESULTS

The results of soil and microbial properties are shown in Table II. Soil pH, K content and temperature did not vary between sites, while soil moisture and P content increased from campo graminóide to cerradão. The values of TOC, TN, MBC, MBN and MBP also increased from campo graminóide to cerradão. Campo graminóide presented the lowest value of TP than cerrado stricto sensu and cerradão.

The values of soil C:N and microbial biomass C:N were higher in cerrado stricto sensu than in campo graminóide and cerradão (Table III). On the other hand, the values of soil C:P and N:P were higher in cerradão than cerrado stricto sensu and campo graminóide. Similarly, microbial biomass C:P and N:P presented the highest values in cerradão and lowest in campo graminóide (Table III).

The microbial indices were different across sites (Table IV). MBC:TOC ratio were higher in campo graminóide and cerrado stricto sensu than cerradão, while MBN:TN ratio did not vary across sites. On the other hand, MBP:TP ratio was higher in cerradão than cerrado stricto sensu and campo graminóide.

The PCA analysis based on the soil and microbial C:N:P across different Cerrado physiognomies explained more than 99% of the data variation (Figure 1). The analysis showed that the samples grouped according to the treatment,

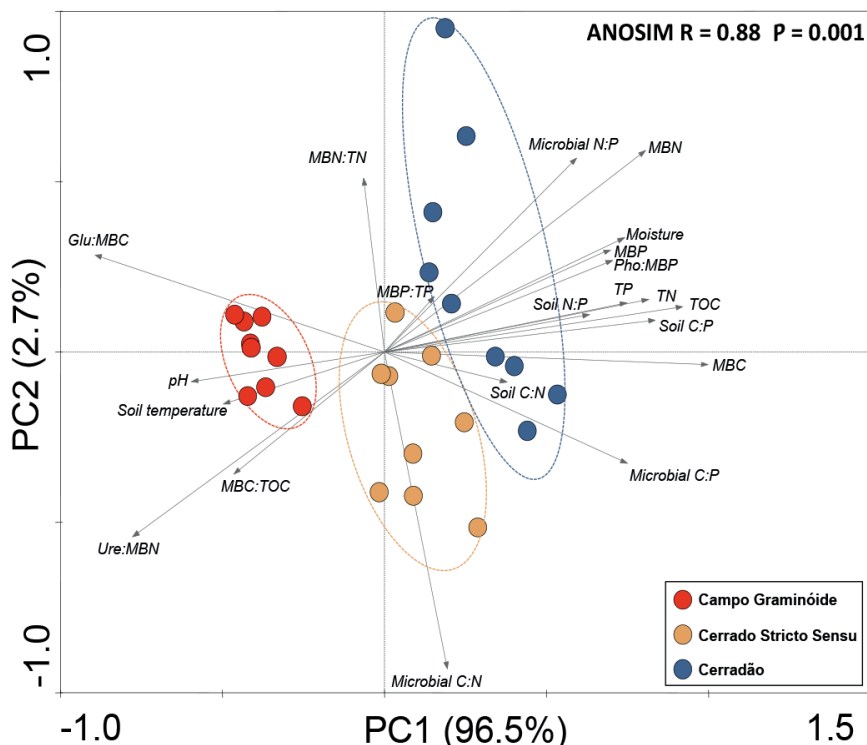


Figure 1 - Principal component analysis (PCA) based on the soil and microbial C:N:P across different Cerrado physiognomies.

TABLE II
Soil chemical parameters and microbial properties.

	campo graminóide	cerrado stricto sensu	cerradão
pH	4.97 ^a ± 0.5	4.85 ^a ± 0.7	4.79 ^a ± 0.7
P (mg kg ⁻¹)	3.57 ^b ± 0.4	4.87 ^a ± 0.7	4.81 ^a ± 0.5
K (cmol _c kg ⁻¹)	1.33 ^a ± 0.2	1.86 ^a ± 0.3	1.80 ^a ± 0.3
Moisture	8.47 ^c ± 0.8	9.90 ^b ± 1.0	12.07 ^a ± 1.4
Temperature	27 ^a ± 1.9	26 ^a ± 1.8	26 ^a ± 2.1
TOC (g kg ⁻¹)	3.31 ^c ± 0.3	6.44 ^b ± 0.5	8.47 ^a ± 0.9
TN (g kg ⁻¹)	0.35 ^c ± 0.1	0.50 ^b ± 0.1	0.78 ^a ± 0.2
TP (g kg ⁻¹)	0.036 ^b ± 0.008	0.049 ^a ± 0.01	0.048 ^a ± 0.01
MBC (mg kg ⁻¹)	113 ^c ± 9.3	198 ^b ± 13.4	228 ^a ± 15.7
MBN (mg kg ⁻¹)	28 ^c ± 2.7	41 ^b ± 5.8	60 ^a ± 6.1
MBP (mg kg ⁻¹)	10 ^c ± 0.8	12 ^b ± 1.1	15 ^a ± 1.5

TOC – Total Organic C; TN – Total N; TP – Total P; MBC – Microbial Biomass C; MBN – Microbial Biomass N; MBP – Microbial Biomass P. Different lower case letters in each line refer to significant differences between physiognomies ($P < 0.05$).

with a clear gradient from campo graminóide to cerradão (ANOSIM $R = 0.88$, $P = 0.001$). In general, samples from the cerradão presented the highest values for the most of the variables, while samples from the campo graminóide presented higher values of pH and soil temperature.

DISCUSSION

The results showed that all sites presented low soil pH and confirm that soil under cerrado is usually acid (Reatto et al. 2008). The pH has been considered the main driver of microbial

TABLE III
C:N:P ratios in soil and microbial biomass across the Cerrado gradient.

	C:N	C:P	N:P
<i>Soil</i>			
campo graminóide	9.5 ^c ±0.6	93.4 ^c ±8.1	9.8 ^b ±0.7
cerrado stricto sensu	13.2 ^a ±0.9	133.3 ^b ±9.6	10.3 ^b ±1.3
cerradão	11.0 ^b ±0.7	177.1 ^a ±12.5	16.5 ^a ±1.5
<i>Microbial biomass</i>			
campo graminóide	4.0 ^b ±0.3	10.8 ^b ±0.8	2.6 ^b ±0.3
cerrado stricto sensu	4.9 ^a ±0.5	15.6 ^a ±1.1	3.2 ^a ±0.5
cerradão	3.8 ^b ±0.2	14.6 ^a ±1.0	3.8 ^a ±0.6

Different lower case letters in each line refer to significant differences between physiognomies ($P < 0.05$).

TABLE IV
Microbial indices.

	campo graminóide	cerrado stricto sensu	cerradão
MBC:TOC	3.21 ^a ±0.7	3.08 ^a ±0.5	2.74 ^b ±0.8
MBN:TN	8.16 ^a ±0.9	8.34 ^a ±0.8	7.98 ^a ±0.6
MBP:TP	2.97 ^b ±0.3	2.66 ^b ±0.4	3.32 ^a ±0.3

TOC – Total Organic C; TN – Total N; TP – Total P; MBC – Microbial Biomass C; MBN – Microbial Biomass N; MBP – Microbial Biomass P. Different lower case letters in each line refer to significant differences between physiognomies ($P < 0.05$).

communities (Fierer and Jackson 2006). However, the soil pH did not present significant correlation with microbial biomass in our study because there was no variation in soil pH across the gradient of Cerrado. It corroborates the study of Xu et al. (2013), which did not find the influence of pH on microbial biomass across different biomes in the world. However, soil moisture varied between sites and it may have contributed for differences in the content of soil microbial biomass. Indeed, higher soil moisture found in cerradão influenced the soil microbial biomass probably due to the increase of the available organic matter (Eaton and Chassot 2012). Other factors may have influenced the higher microbial biomass observed in cerradão, such as higher plant diversity (Oliveira et al. 2007), a factor that influences this parameter (Lamb et al. 2011). Previous studies in these sites have also shown that both plant diversity and litter influenced the microbial diversity, i.e. archaeal, bacterial and

fungi diversities increased from campo graminóide to cerradão (Araujo et al. 2017a, b, 2018).

The higher amount of plant litter found in cerradão may also have contributed to the increase of soil organic C, N and P, influencing the amount of nutrient source to the microorganisms. These different characteristics found between sites, mainly between cerrado and cerradão, also contributed to shape the structure and diversity of soil microbial community (Araujo et al. 2017a, b, 2018). Nardoto et al. (2006) estimated the input of plant litter in these areas and found that campo graminóide presented about 1 ton ha⁻¹ y⁻¹, cerrado stricto sensu 2.1 ton ha⁻¹ y⁻¹ and cerradão 7 ton ha⁻¹ y⁻¹. Thus, this contribution of plant litter influenced in the highest content of soil organic C, N and P found in cerradão and cerrado stricto sensu as compared with campo graminóide. The values of microbial biomass C, N, and P observed in this study are in agreement with previous studies in Brazilian soil which ranged

from 72 to 797 mg kg⁻¹ for microbial C (Matsuoka et al. 2003, Sampaio et al. 2008), from 11 to 104 mg kg⁻¹ for microbial N (Hungria et al. 2009, Santos et al. 2004) and from 4 to 60 mg kg⁻¹ for microbial P (Rheinheimer et al. 2000).

Cerradão, as forest formation, presented higher C:P and N:P ratios and it may be the result of more plant litter content, as discussed before. Other studies have also shown higher C:P and N:P in the forest than the grassland environment (Chen et al. 2000, Ouyang et al. 2017). It corroborates a previous study that found a decrease of 33% in microbial C:P ratio from forest to grassland environment (Chen et al. 2000). It also confirms that changes in vegetation status influence the microbial C:N:P ratios. For microbial C:N ratio, the result shows that higher value found in cerrado stricto sensu may also indicate a higher presence of fungi than bacteria in this site as compared with the others sites (Araujo et al. 2017a, b).

In this study, the values of microbial C:N, C:P and N:P ratios are in agreement with the range reported for soil that are microbial C:N of 2.2 to 14.1 (Smith and Paul 1990), microbial C:P of 5.0 to 276 (Joergensen et al. 1995) and microbial N:P of 1.0 to 4.2 (Balota et al. 2003). This difference in microbial biomass ratios between the sites may be related with the different composition of the microbial community (Araujo et al. 2017a, b, 2018) and microbial C, N, and P that were influenced by the characteristic of each site. Thus, as campo graminóide presented lower plant diversity, organic matter inputs, and P content, these characteristics contributed to the lowest microbial C, N and P contents. Since the microbial biomass ratios are indicators of C, N and P availability in the soil (Balota and Auler 2011), the lowest microbial C:N and C:P found in campo graminóide may suggest a high potential of mineralization, while highest microbial C:N and C:P found in cerradão suggest a process of immobilization of N and P (Paul 2006). Therefore, soils from cerrado stricto sensu and

cerradão present more ability for storage C, N, and P with slow releasing to plants. Ecologically, these results confirm that soils from cerrado stricto sensu and cerradão present more abundance of fungi than soil from campo graminóide (Araujo et al. 2017b).

The ratios of microbial C:N:P found in all evaluated sites were lower than the ratios found by Heuck et al. (2015) and Tischer et al. (2014), that were 39:4:1 and 32:3:1, respectively. The values are also below the mean found in forest soils that was 74:9:1 (Cleveland and Liptzin 2007). On the other hand, the values of microbial C:N:P ratios are closer to the values found by Balota et al. (2003) in soils from Brazilian Cerrado, which was 18:2:1. The lower values of microbial C:N:P ratio found in this study may be related to the high microbial P found in all sites as compared with microbial C and N.

The values of soil C:N ratio did not differ between cerrado stricto sensu and cerradão, while it was lowest in campo graminóide. Soil C:P and C:N ratios were higher in cerradão than other sites. The ratios between C, N, and P are indicators of nutrient dynamic in soil (Fazhu et al. 2015). According to Paul (2006), C:N ratio higher than 25 indicates the accumulation of organic matter. In this study, the values of C:N ratios were below 25, indicating mineralization of the organic matter. However, cerradão presented higher C:N ratio than campo graminóide, indicating that this site accumulates more organic matter than campo graminóide. Similarly, the values of C:P were lower than 200, also indicating net mineralization (Paul 2006). The values of soil C:N, C:P and N:P found in this study are similar to a previous study which reported ranges from 9.9 to 25.5; 54.5 to 459.8; and 1.91 to 30.9 for C:N, C:P and N:P, respectively (Fazhu et al. 2015).

The ratio of MBC:TOC is an indicator of the potential mineralization of organic matter (Hedo et al. 2014). As reported before, the highest MBC:TOC found in campo graminóide confirms that this site presents more mineralization, leading to less accumulation of organic matter than cerrado

stricto sensu and cerrado. On the other hand, the highest of MBP:TP ratio in cerrado may represent conservation of P in soil, thus increasing the soil fertility in this site.

Finally, the result of PCA showed a clear separation of physiognomies according to the variables and confirms that the type and structure of vegetation influence the soil properties. Therefore, soil pH, temperature, and MBC:TOC ratio are more correlated with campo graminóide. On the other hand, MBC, MBN, TOC, TN, TP, microbial N:P and C:P ratios, and soil C:N, C:P, and N:P ratios are correlated with cerrado. Our analysis also revealed that the cerrado stricto sensu presents an intermediate position between campo graminóide and cerrado, revealing a gradient of physiognomies. Together, our data revealed that each physiognomy influences differently the pattern of soil microbial biomass.

CONCLUSIONS

The differences in the physiognomies of cerrado, i.e. across a gradient from grassland to arboreal vegetation, influenced the soil microbial biomass ratio. Therefore, campo graminóide presented lower soil microbial biomass compared to cerrado. This result indicates that the preserved Brazilian cerrado, such as cerrado with rich and diverse vegetation, presents higher soil microbial C:N and C:P ratios than soil under campo graminóide. Our results indicate that cerrado presents a higher capacity of accumulating soil organic matter and maintaining soil fertility.

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AUTHOR CONTRIBUTIONS

SMBR carried out the research as part of her master's degree and contributed to write the paper, prepared the tables and figures and interpreted the results. JELA, FFA and RSS performed data analysis, interpreted results and give suggestions for improving the paper. LWM carried out the statistical analysis, discussed the results and revised all paper. ASFA was the supervisor of the project, and contributed to the experimental design, data analysis, writing and revision of the manuscript, as well as the correspondence with the editors and reviewers.

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