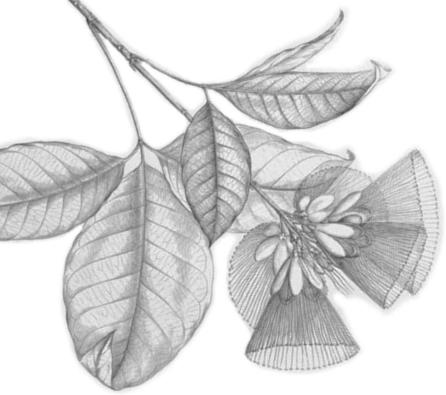

What Physical Aspects Influence Biochemical Activity in Soil in a Natural Landscape?

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ABSTRACT

The objective of this paper was to evaluate the effect of depth, seasonality and phytophysiognomies on the biochemical activity of soils in native Cerrado biome. The chemical parameters that influence the activity of the microbiota and enzymes in these environments were also listed. For eight selected areas of Cerradão (Forest) and four areas of Cerrado (shrub land) the chemical characteristics and biochemical activity were evaluated. The depth, sampling site and seasonality directly affected soil metabolic activity. Microbial biomass carbon was influenced by interaction between soil depth and sampling site and the enzyme activity tends to be higher in the superficial layer of the soil. The analysis of redundancy showed strong influence of environmental variables (phosphorus and CEC) on the biological activity of the soil. Thus, the metabolic activity of microorganisms is highly influenced by intrinsic soil characteristics of each sampling site.

Keywords: Soil Depth; Seasonality; Cerrado; Soil Biochemistry.

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Brazilian Cerrado is the second largest domain in South America, has approximately 2,036,448 km2 and is considered one of the planet's hotspots (Myers et al. 2000). This phytogeographic domain is a large mosaic composed of forests, savannas and grassland formations (Batalha 2011).

The heterogeneity of the physical-chemical and biological properties of the soil determines the diversity of phytophysiognomies (Dick and Schumacher 2015) and the functioning of the Cerrado (Vezzani 2015). The Cerrado phytophysiognomies present different types of soils that vary in depth, porosity, permeability, drainage, texture and nutrient content (Sano, Almeida, and Ribeiro 2008).

In general, topsoil has the highest content of organic matter, organic elements, macro and micronutrients. The diversity of soil microorganisms is responsible for the decomposition of such components, and allows nutrient cycling, plant growth, nitrogen fixation (Burns et al. 2013). Soil functioning depends on the interaction of biotic factors, such as diversity and activity of microorganisms, as well as abiotic factors, such as soil type, physicochemical properties, humidity and temperature (Lacerda et al. 2013; Nogueira et al. 2006).

To establish soil conservation policies, it is fundamental to monitor the physical and chemical properties and to understand the dynamics of the soil microbiota (Silva, Valentini, and Faria 2016). In addition, the evaluation of microbial processes (biochemical activity) in native Cerrado areas can result in benchmark to support research that evaluates soil quality in anthropogenic ecosystems (Mendes, Sousa, and Junior 2015).

Within this context, the objective of this paper was to evaluate the effect of depth, seasonality and phytophysiognomies on the biochemical activity of soils with Cerrado native vegetation. Additionally, we listed which chemical parameters influence the activity of the microbiota and enzymatic activity in these environments.

MATERIALS AND METHODS

SAMPLING AREAS

Sampling was performed in areas with typical Cerrado vegetation, located in the state of Goiás, Brazil. We selected twelve sampling areas of which eight were characterized, according to vegetation type, as Cerradão (Forest) and the others (4), characterized as Cerrado (shrub land) (Sano, Almeida, and Ribeiro 2008) (Table 01).

According to the classification of Köppen, the predominant climate of the region is the Aw type. The data on the temperature and precipitation of each sampling point are given in Table 01.

Table 01. Sampling locations, geographic coordinates, Cerrado classification, temperature and precipitation of each sampling point.

Locality	Code	Temperature (°C)	Precipitation (mm year^{-1})	Latitude S'	Longitude W'
			Cerrado (CD)		
Santa Helena	L1	24.3	1539	17°52'28.0"	50°33'07.7"
Itapirapuã	L2	25.6	1575	15°33'48.7"	50°38'13.4"
Matrinchã	L3	25.7	1525	15°28'42.3"	50°40'27.3"
Edeia	L4	24.1	1423	17°37'43.8"	50°11'59.8"
			Cerradão (C)		
Palmeiras	L5	24.0	1457	16°48'30.0"	49°52'31.1"
Turvelândia	L6	24.7	1468	17°49'13.7"	50°18'56.9"
Cristalina	L7	20.1	1422	16°09'56.1"	47°36'59.8"
Inhumas	L8	23.1	1516	16°19'35.6"	49°27'33.4"
Morrinhos	L9	23.3	1368	17°47'40.8"	49°12'01.0"
Itumbiara	L10	24.6	1119	18°30'25.3"	49°22'07.9"
Anicuns	L11	23.6	1535	16°24'34.9"	49°54'31.1"
Goiatuba	L12	23.0	1369	18°05'06.3"	49°39'30.4"

Source: Climate-data (2016).

SOIL SAMPLING

The soil sampling in the 12 areas was carried out from January to March / 2016 (rainy season) and June to July / 2016 (dry period). The soils were collected at four depths (0-0.1m, 0.1-0.2 m, 0.2-0.5 m and 0.5-1.0 m), using Dutch auger for deformed sample. We repeated this procedure three times at each collection point, so the three subsamples of each depth were pooled to form one composite sample.

Samples were homogenized and sieved (2 mm) to remove impurities (branches, roots and gravel) and then stored in polyethylene bags at 4 °C. For each sample, the moisture content was determined by drying 5 g of soil at 105 °C for 24 h (Sinsabaugh, Klug, and Collins 1999). We used dry mass as reference to calculate all biochemical soil data.

SOIL CHEMICAL ANALYSIS

We determined pH, nutrient content (calcium, magnesium, aluminum, potassium and phosphorus), aluminum saturation (m%), base saturation (V%), soil organic matter (SOM), total organic carbon (TOC) and total nitrogen (TN), using the methodology of EMBRAPA (2011).

SOIL BIOLOGICAL ACTIVITY

ENZYME ASSAYS

We evaluated the activities of extracellular enzymes (hydrolases) related to the nitrogen cycle (glycine aminopeptidase), carbon cycle (β-glucosidase) and phosphorus (acid phosphatase). The activity

of glycine aminopeptidase we determined according to the methodology of Allisson and Vitousek (2005), with modifications. The soil (0.1 g dry mass) was incubated with 900 µL p-nitroanilide, prepared in sodium acetate buffer, at 37 °C for 60 min. The activities of β-glucosidase and acid phosphatase we measured according to the methodology of Baldrian et al. (2005). All enzymatic assays were performed in triplicate. In the control assays, the substrate was replaced with the acetate buffer, used in the preparation of the substrates.

The enzyme activity we calculated based on the calibration curve and expressed in micromol of product formed per gram of soil dry mass per hour of reaction (µmol of p-nitroaniline g^{-1} h^{-1}, µmol of p-nitrophenol g^{-1} h^{-1}, respectively).

The microbial biomass carbon (MBC) we quantified by the irradiation-incubation method. The amount of CO_2 was estimated after 10 days of incubation by titration with HCl (1 mol L^{-1}). The results were expressed in micrograms of carbon per gram of soil (µg C g-1), using the conversion factor (kc) of 0.45 (Ferreira, Camargo, and Vidor 1999). All MBC determination were carried out in triplicate.

DATA ANALYSIS

We used generalized linear mixed models (GLMM) to test the effects of locality, soil depth and interaction between locality and depth in MBC and the activity of the aminopeptidase, beta glucosidase and phosphatase enzymes. We performed this analysis controlling the effect of seasonal seasons (dry and rainy seasons). We constructed the models considering the locality, depth and the interaction between locality and depth as variables of fixed effects and the seasonal period as random effect. GLMM parameters were estimated by maximum likelihood with Poisson distribution (O'Hara and Kotze 2010).

We used Variance Inflation Factor (VIF) to verify the existence of multicollinearity among the variables. We selected seven variables with lower inflation value (FV <5) to compose the set of chemical predictors: pH (FV = 4.84), Al (FV = 1.71), CEC (FV = 2.40), P (FV = 1.29), K (FV = 1.79), SOM (FV = 1.81) and V% (FV = 4.43).

In order to evaluate the influence of these variables on the MBC and enzyme activities versus localities, we used the Analysis of Redundancy (RDA). We use the mean depths, first for the seasonal periods together, and then separately. The influence of each predictor variable we tested through correlation analysis with the first two axes summarized by the RDAs, obtained with 999 randomizations.

All analyzes were performed using R (R Core Team 2017) software. The data (log + 1) to meet the assumptions of normality and homogeneity.

RESULTS AND DISCUSSION

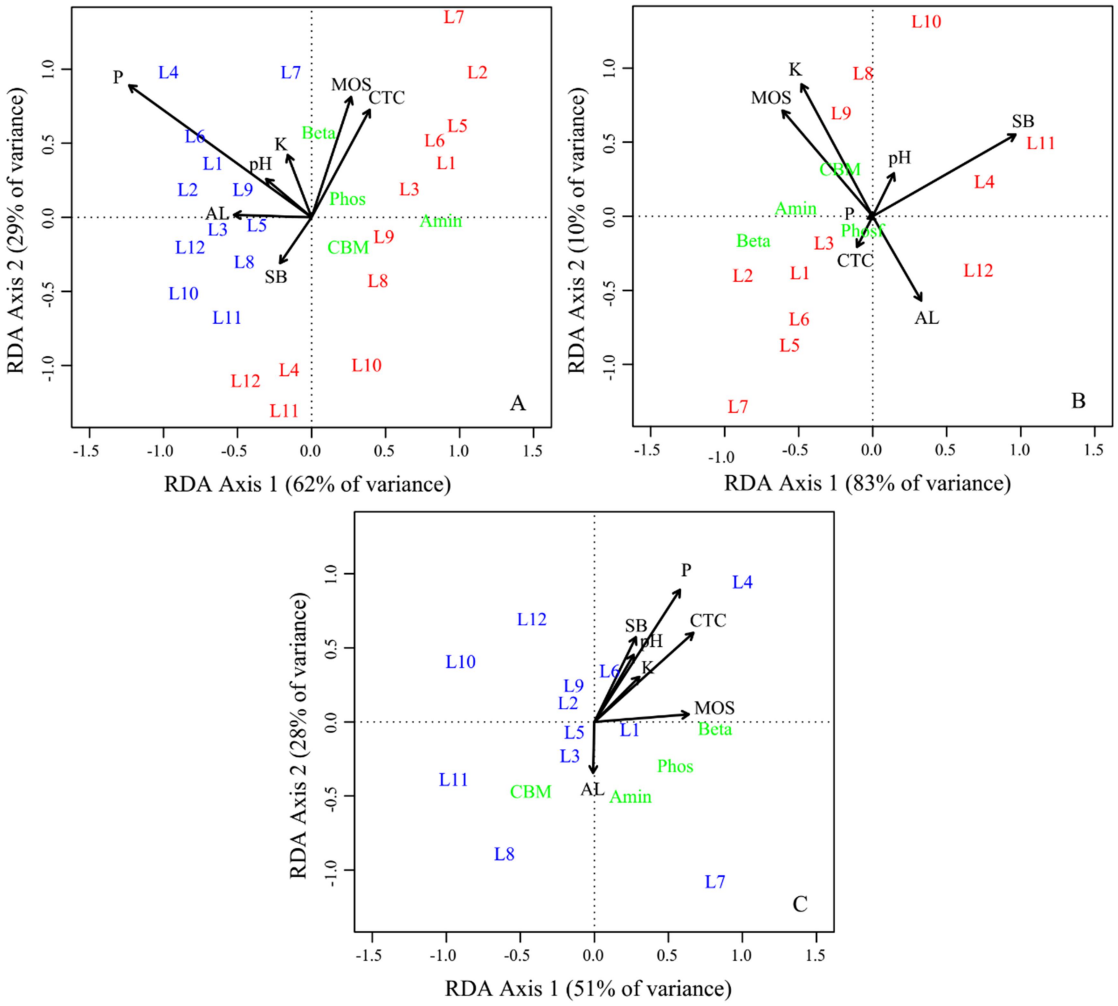
The RDA brought the relation among chemical variables, biochemical activity, sampling areas and seasonality (Table 02, Figure 01).

Table 02. Predictor variables and the two first components of RDA for enzyme activity in Cerrado soils.

Predictor variable	RDA1	RDA2	R^2	Pr(>r)
Both season				
pH	-0.77	0.64	0.04	0.65
Al	-1.00	0.03	0.07	0.50
CEC	0.48	0.88	0.17	0.14
P	**-0.81**	**0.59**	**0.58**	**<0.01***
K	-0.36	0.93	0.05	0.58
SOM	0.31	0.95	0.18	0.12
V%	-0.57	-0.82	0.04	0.70
Dry season				
pH	0.44	0.90	0.02	0.93
Al	0.50	-0.87	0.07	0.71
CEC	-0.46	-0.89	0.01	0.96
P	-0.91	-0.42	0.00	1.00
K	-0.48	0.88	0.17	0.44
SOM	-0.65	0.76	0.14	0.45
V%	0.87	0.50	0.20	0.37
Rainy season				
pH	0.51	0.86	0.23	0.30
Al	-0.03	-1.00	0.10	0.58
CEC	**0.74**	**0.67**	**0.67**	**0.01***
P	**0.54**	**0.84**	**0.94**	**<0.01***
K	0.71	0.71	0.15	0.50
SOM	1.00	0.08	0.34	0.17
V%	0.44	0.90	0.34	0.15

Source: The Authors.

Figure 01. Analysis of Redundancy (RDA) for enzyme activity on the dependence of soil chemical variables of 12 sampling areas of Cerrado, during drough and rainny periods.



Source: The Authors.

(A), only for dry periods (B) and only for rain (C). Red colors are samples from dry periods, blue colors correspond to rainny periods and green are respective to enzymes and MBC.

In Table 02 the content of phosphorus (P) was the strongest component over the data variation ($R^2 = 0.58$ $p < 0.01$). P is negatively associated to the first axis ($r = -.81$), but positively with the second axis ($r = 0.59$), which indicates that more values can be found during the rainy period (Table 02).

In Figure 01A the biological activity in soil between drought and rain is distinct especially in first RDA axis, which explained 62% of data variation. The negative influence of phosphorus on acid phosphatase activity, aminopeptidase and MBC is also observed. Moreover, there is a positive tendency on the increments of β-glucosidase, cation Exchange capacity and soil organic matter.

The influence of seasonality was identified when data were analyzed simultaneously (Figure 01A) and included (Figure 01B and 01C), as observed by other researchers (Zago et al. 2016; Zago,

Sousa, and Caramori 2017). According to those authors, the soil moisture and temperature alter the enzyme quantity produced and released by the microbiota. Thus, catalytic activity can be increased or reduced, according to the period of the year (Borowik and Wyszkowska 2016; Giacometti et al. 2013).

Data from the RDA using only the drought period showed that the first axis explained 83% and the second, only 10% of the data variance. No variables significantly influenced the explanation of the RDA axes during drought ($p < 0.05$) (Table 02).

However, both enzymes and MBCs were grouped according to biochemical variables. The research was carried out with the negative association of the enzymes with the base saturation. For some authors, V values are not representative, because nutrient levels are the most important factor to maintain the growth of plants and microorganisms in the soil (IPNI 1998).

For the analysis of the sampling data in the rainy period, the first data from the RDA explained 51% and 28%, respectively. CEC ($R^2 = 0.67$, $p = 0.01$) was exerted with significance in the first axis ($r = 0.74$) and P ($R^2 = 0.94$; $p < 0.01$), without the second axis ($r = 0.84$) of the RDA (Table 02).

In Figure 01C, we observed the negative influence of CEC and P, both on MBC, as well as aminopeptidase and acid phosphatase activity. These findings reaffirm the results shown in Figure 01A, that is, that acid phosphatase activity is negatively influenced by soil phosphorus content.

The synthesis and release of phosphatases in the soil depends on the concentration of soluble phosphorus (Pi) in the soil and the demand of plants and animals. If the concentration of Pi in the soil is low, the phosphatase activity will be high (Nahas 2015). In addition, the production of this enzyme can be affected by the availability of water in the soil (Allison and Vitousek 2005) or by the set of environmental variables (Neal et al. 2017).

It was also verified the relationship between glycine aminopeptidase activity and aluminum content in the soil, which can be explained by the acidic characteristics of Cerrado soils (Sano, Almeida, and Ribeiro 2008). In addition, the positive relationship between soil organic matter (SOM) and biochemical variables (β-glucosidase and MBC) was observed (Figure 01C), indicating that this chemical parameter exerts a direct influence on the action of the soil microbiota (Araújo et al. 2017; Zago, Sousa, and Caramori 2017; Kivlin and Treseder 2014).

We observed that the cation exchange capacity (CEC) also influenced the biochemical activity of the soil (enzyme β-glucosidase and MBC) (Figure 01C). However, the connections between the

macronutrients and the soil matrix also depends on the availability of these chemical elements, which come from the soil itself and the litter (Saidi 2012).

In this line of reasoning, the scientific evidences emphasize the direct relations between the chemical components and the soil microfauna. Soil microbiology studies confirm that the interaction between abiotic factors and the microbiota may interfere with microbial cell metabolism in the soil (Margalef et al. 2017).

The analysis of generalized linear mixed models (GLMM) showed that soil biochemical activity (glycine aminopeptidase, acid phosphatase, β-glucosidase and MBC) was significantly influenced by soil depth and sampling site. Except for MBC, the interaction between the variables (depth and location) was not important to explain the variations observed in the enzymatic activity (Table 03).

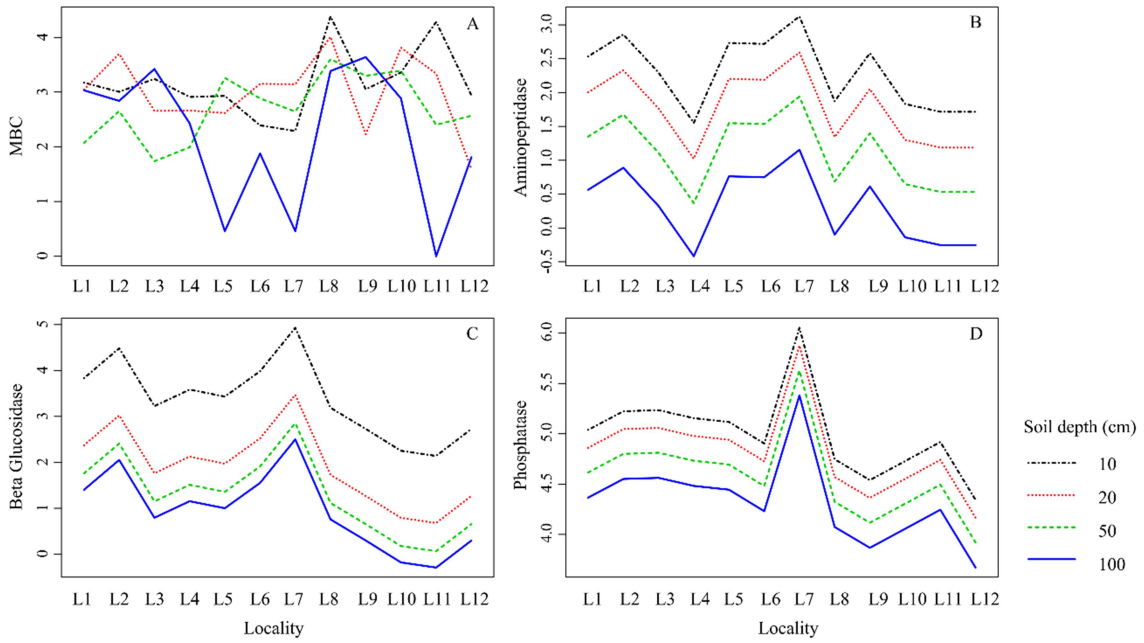
Table 03. Effects of soil depth, locality and their interactions on MBC and glycine aminopeptidase, acid phosphatase and β-glucosidase activities using GLMM with seasonal period as random effect.

Predictor variables	MBC			Aminopeptidase			Phosphatase			β-glucosidase		
	Df	F	Pr(>F)	Df	F	Pr(>F)	Df	F	Pr(>F)	Df	F	Pr(>F)
Depth(D)	3	6.1	0.00*	3	21	0.00*	3	15.2	0.00*	3	23.6	0.00*
Locality(L)	11	2.8	0.01*	11	2.7	0.01*	11	10.9	0.00*	11	4.9	0.00*
L:D	33	1.7	0.04*	33	0.5	0.98	33	0.6	0.89	33	0.6	0.92

Source: The Authors.

Overall, GLLM indicated that the variable predictor depth was important to explain the variation on enzyme activity. The activity of cycling-related enzymes of different types of organic compounds in the soil (nitrogen: glycine aminopeptidase; phosphorus: acid phosphatase, carbon: β-glucosidase) exhibited the same trend of variation along the soil profile. We observed that enzymatic activity presented values inversely proportional to soil depth, that is, as we increased the sampling depth, the activity values were reduced (Figure 02B, C and D).

Figure 02. Values (log10 + 1) of the biochemical parameters of native Cerrado soils collected in 12 locations and four different depths.



Source: The Authors.

02A: MBC values; 02B: Activity of β-glucosidase, 02C: Activity of acid phosphatase and 02D: Activity of glycine aminopeptidase.

These results corroborate to the initial hypothesis that the enzyme activity decreased with the depth of the soil profile, due to the available amount of organic matter and elements to the microbiota.

With the increase of the depth, there is a gradual reduction of the organic carbon, water, oxygenation, porous space and greater compaction of the soil. This condition consequently decreases the rate of metabolic activity of the microfauna. Additionally, the reduction on diversity of bacterial and fungal communities in the deeper layers of the soil can explain such observations (Babujia et al. 2010).

The enzymes analyzed in the present study showed the same pattern of variation at the sampling depths and localities. However, for each enzyme, different values of activity were observed among the depths (Figure 02).

Those observations indicate that enzymes involved in different biogeochemical cycles behave differently within a sampling area (Zago et al. 2016). In fact, each enzyme exhibits a pattern of activity, which may vary according to the type of substrate involved in the catalysis, its soil availability, diffusion rate and nutrient concentration (Borowik and Wyszkowska 2016; Kivlin and Treseder 2014).

We found higher values of the activity of the three hydrolases in two soils that presented the same physiognomic type (L2: Cristalina and L7: Itapirapuã). The biotic and abiotic factors, such as climate and their interactions may justify the highest level of biological activity at these sites. Climatic

factors, such as temperature and humidity, soil chemical composition and plant exudates are capable of altering the soil biological activity (Giacometti et al. 2013; Nogueira et al. 2006).

The type of land cover in the soil can determine the species and processes of soil microorganisms and vice-versa. Thus, it is possible to associate these results to two factors: the soil and the type of plant cover (Araujo et al. 2017).

In the Cerrado, the phytophysiognomies differ in terms of amount of carbon, nitrogen and aluminum, macronutrients, organic matter e texture, determining different types of soil. This in turn influences the microbial activity because the difference in the physical-chemical composition directly affects the adsorption of enzymes in soil colloids (Skorupa et al. 2012).

The type and density of the vegetative cover determines the content and the variety of chemical elements in the litter, which in turn can stimulate the microbiota and determine changes in the rate of decomposition and cycling of nutrients (Nair, Kumar, and Nair 2009). Therefore, the soil-plant interaction would explain the oscillations of MBC in the natural environment of Cerrado.

CONCLUSIONS

Biochemical (enzymatic) and biological parameters (MBC) of the soil are directly influenced by soil depth and sampling site. The activity of the glycine aminopeptidase, acid phosphatase and beta glucosidase tends to be lower in the deeper layers of the Cerrado soils.

MBC was affected by the interaction between soil depth and sampling site. The metabolic activity of microorganisms is highly influenced by soil intrinsic characteristics of each sampling site.

The RDA demonstrated the influence of environmental variables on biological activity (MBC) and enzymatic activities between localities and confirmed the effect of seasonality on the biochemical activity of the soil.

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Quais Aspectos Físicos Influenciam a Atividade Bioquímica no Solo em uma Paisagem Natural?

RESUMO

O objetivo deste trabalho foi avaliar o efeito da profundidade, sazonalidade e fitofisionomias na atividade bioquímica de solos no bioma Cerrado nativo. Os parâmetros químicos que influenciam a atividade da microbiota e a atividade enzimática nesses ambientes foram também listados. Foram selecionadas oito áreas de amostragem de Cerradão (Floresta) e quatro áreas de Cerrado e avaliadas as características químicas e atividade bioquímica. A profundidade, o local de amostragem e a sazonalidade afetaram diretamente a atividade metabólica do solo. O carbono da biomassa microbiana foi influenciado pela interação entre a profundidade do solo e o local de coleta e a atividade enzimática tende a ser maior na camada superficial do solo. A análise de redundância mostrou forte influência das variáveis ambientais (fósforo e CTC) na atividade biológica do solo. Assim, a atividade metabólica dos microrganismos é altamente influenciada pelas características intrínsecas do solo de cada local de amostragem.

Palavras-Chave: Profundidade do Solo; Sazonalidade; Cerrado; Bioquímica do Solo.

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