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RESEARCH ARTICLE

# BIOCHEMICAL INDICATORS AS PARAMETERS OF CHANGES IN THE FERTILITY OF BRAZILIAN CERRADO SOILS

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**ABSTRACT**

Biochemical indicators have been proposed as a tool to monitor soil quality, but the degree of precision of these indicators is not yet known to identify changes in fertility over time. The present study evaluated the influence of monocultures on the quality of the Brazilian Cerrado soil, using biochemical indicators. Forty-eight samples of native Cerrado soil and soils converted to sugarcane crops were submitted to chemical and enzymatic evaluation. The implantation of monoculture crops promoted a reduction of 60% in the content of organic matter. The 79% reduction in β-glucosidase activity, 84.5% in acid phosphatase activity and 32% in glycine aminopeptidase activity occurred in relation to the activity found in native Cerrado soils. The results indicate that the implantation of sugarcane crops in substitution to the Cerrado can negatively affect the chemical and biochemical properties of the soil, causing degradation and loss of fertility.



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## INTRODUCTION

Sugarcane cultivation in recent years has been highlighted in the economic scenario by the increase of planted area, production and productivity. In Brazil, the state of Goiás has a strong tradition in agribusiness with approximately 64% of its territory converted into agro ecosystems (agriculture and livestock) and is considered the fifth most anthropized Brazilian state (Sano *et al*., 2010). In Goiás the expansion of monoculture planting areas is mainly due to three factors: the first is due to the flat relief, because this territory occurs on the Central Plateau (Sano *et al*., 2010). The second factor is explained by the tropical climate, which presents a hot and humid season and a cold and dry season, which provides ideal conditions for germination, maturation and elevation of sucrose in the plant. Another factor that contributes to the expansion of sugarcane plantations in Goiás is the photoperiod, that is, the region provides adequate sun exposure time necessary for the development of this plant species (Araldi *et al*., 2010). In 2016, the increase in planted area in Goiás was about 68.6 thousand hectares, which is equivalent to 7.7% more than in the previous harvest and due to the increase in food and fuel demand. The estimative for 2025 is that 1,131 thousand hectares will be cultivated with sugarcane in this state (MAPA, 2015, CONAB, 2016). Within this context, the cultivation of sugarcane plays a fundamental role in the dynamics of substitution of native vegetation and occupation of soils. Although agricultural activities are economically and socially important for food, fiber and fuel production, it is important to consider that soil degradation processes are closely related to the intensity of exploitation of this ecosystem and deforestation of areas of natural vegetation (Cunha *et al*., 2008; MAPA, 2015). The removal of native vegetation cover for large-scale agricultural production has led to environmental problems such as soil compaction, erosion and loss of biodiversity, which in turn endanger the health of the soil and other ecosystems (Cunha *et al*., 2008; Sano *et al*., 2010; Nogueira *et al*., 2006). Improper tillage leads to loss and / or changes in organic matter composition and disturb the soil physical and chemical properties, directly impacting the soil microbial community. As a consequence of this, the biochemical processes are adversely affected, thus compromising the cycling and availability of nutrients in the soil (Grandy *et al*., 2009). Soil functioning is closely related to its physicochemical composition and the interaction between biotic and abiotic factors. This is the reason why the changes caused by the anthropic action generate an imbalance in the soil functions, compromising the sustainability of this

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ecosystem and its productive capacity, which can reduce future agricultural productivity (Lacerda *et al*., 2013). Biological attributes or bioindicators such as enzymatic activity and microbial biomass may indicate the current condition of agro ecosystem functioning, since they are subject to changes according to the type of management and type of vegetation cover (Mendes *et al*., 2013). It is important to consider that the evaluation of soil fertility should be associated to the analysis of chemical parameters, such as organic matter, which is an attribute that determines the microbial activity and production of extracellular enzymes by soil microorganisms. These enzymes determine the rate of decomposition, immobilization and mineralization of organic matter, allowing nutrient flow, species survival and soil functioning (Nannipieri *et al*., 2012). Thus, soil quality assessment is essentially important to detect fertility losses in these environments and impacts resulting from agricultural practices and decision making on management practices that amplify soil sustainability conditions (Mendes *et al*., 2013). In order to evaluate the influence of the implantation of sugarcane on bioindicators of soil quality, the hypothesis is that the microbial activity (enzymatic activity of hydrolases and microbial biomass) is negatively affected and the quality of the soil is reduced in monocultures.

## MATERIALS AND METHODS

### *Description of sampling area*

This survey was conducted in Oxisols of Sugar and Alcohol Mills and native Cerrado areas located in the state of Goiás, Central-West region of Brazil. For the evaluation of the chemical characteristics and biological activity of the soil, 12 sampling points were selected, of which six presented sugarcane cultivation, considered as a perennial crop. Sampling was carried out in fields with 24 ha (P1: Itumbiara), 21 ha (P2: Goiatuba), 12 ha (P3: Morrinhos), 29 ha (P4: Edeia), 16 ha (P5: Inhumas) (P6: Anicuns). In such areas, the soil preparation was carried out by conventional planting techniques, using planed plows and grids for soil rotation. All cultivated areas received approximately 350 kg $ha^{-1}$ of fertilizer containing nitrogen, phosphorus and potassium (NPK) in the composition before planting. In three of the six collection points (P1, P3, P5), in addition to these fertilizers were added about 40 L $ha^{-1}$ of vinasse two months before planting. Cover fertilization was performed six months after planting, using approximately 25 L $ha^{-1}$ of vinasse in these same areas. In all sites the cover fertilization with NPK (average 300 Kg $ha^{-1}$) was realized. Except in one of the sites (P6), pest elimination was performed using herbicides (Provence®, Combine®, Advance®, Ancosar 720® and/or DMA 806®) on average 1.2 L $ha^{-1}$, fungicide (Glifosate®, average 1.2 L $ha^{-1}$) and/or insecticides (Regent® and/ or *800WG®,* average 0.03 L $ha^{-1}$). The six areas of natural vegetation are located near the agricultural areas (Table 1) and present 27 ha (P1: Itumbiara), 21 ha (P2: Goiatuba), 12 ha (P3: Morrinhos), 69 ha), 20.4 ha (P5: Inhumas), 22 ha (P6: Anicuns). These areas were used as reference and consist of regions of vegetation characteristic of the Brazilian Cerrado Biome, with predominance of mesophytic forest. The climate of the region is, according to the classification of Köppen, type Aw. The average annual rainfall ranged from 1100 to 1500 mm $year^{-1}$, with a minimum average temperature of 19 ºC and a maximum of 38 ºC (LAPIG-MAPS, 2016; CLIMATE-DATA, 2016).

### Soil sampling

Sampling was carried out in the months of January and July, for two consecutive years (2015 and 2016). Composite samples (three subsamples) were collected in the six sugarcane plantations and six native Cerrado areas, at depth of 0-10 cm. The 48 samples obtained (12 samples collected for each period) were homogenized and sieved (2 mm) for the removal of roots, sticks, stones and gravel and packed in polyethylene bags at 4 $^{o}$C. The moisture content of the soil was determined after drying of 5 g of sample at 105 $^{o}$C for 48 h to evaluate the biological activity of the soil based on its dry mass.

### Soil chemical and biochemical analyzes

Soil chemical analyzes consisted by determination of the nutrient content ($Ca^2$+, $Mg^2$+, K+ and P), organic matter (OM), total organic carbon (TOC) and total nitrogen (TN), according to Embrapa (1997). The microbial biomass carbon (MBC) was determined by the irradiation-incubation method described by Ferreira *et al.* (1999). The amount of $CO_2$ released from each sample was obtained by titration with HCl (1 mol $L^{-1}$) and the results were expressed in micrograms of carbon per gram of soil (µg C $g^{-1}$). Monitoring of the extracellular enzymes involved in the cycling of carbon (β-glucosidase), nitrogen (protease and aminopeptidase) and phosphorus (acid phosphatase) was performed in triplicate. The activities of β-glucosidase (EC 3.2.1.21) and acid phosphatase (EC 3.1.3.2) were determined according to the methodology described by Baldrian *et al.* (2005). The soils were incubated with specific substrates (p-nitrophenyl-β-D-glucopyranoside and p-nitrophenyl phosphate, respectively) and the control was only differed by the addition of sodium acetate buffer (0.05 mol $L^{-1}$, pH 5.0) to replace the substrate. The activity was monitored at 400 nm and expressed in micromol of p-nitrophenol formed per gram of dry soil per hour of reaction (µmol p-nitrophenol $g^{-1}$ $h^{-1}$). For the determination of the glycine aminopeptidase activity (EC 3.4.11) the methodology of Allisson and Vitousek (2005) was used, with some modifications. Soil samples (0.1 g dry mass) were incubated at 37 $^{o}$C for 1 h using 900 µL of p-nitroanilide (pNA) (0.005 mol $L^{-1}$) prepared in acetate buffer (pH 5.0). For controls, the substrate was replaced with sodium acetate buffer (0.05 mol $L^{-1}$, pH 5.0). The formation of glycine p-nitroaniline released in the reaction was measured at 405 nm and expressed in micromol of p-nitroaniline formed per gram of dry soil per hour of reaction (µmol of p-nitroaniline $g^{-1}$ $h^{-1}$). The protease activity (EC 3.4.2.21) was evaluated by the method of Ladeira et al. (2010), using 0.05 g soil (dry mass), incubated with azocasein (0.2%) at 37 $^{o}$C. The control was differed by the addition of 5 mL of TCA before the substrate and the activity expressed in enzyme units (U) per gram per hour of reaction (U $g^{-1}$ $h^{-1}$). According to Janssen *et al.* (1994), a protease unit was defined as the amount of enzyme required to produce an increase in absorbance at 420 nm, equal to 0.1 after 60 min of assay.

### Statistical analyzes

The data were evaluated via analysis of variance in the Assistat program 7.7 and the graphs were plotted in Sigmaplot version 12.0. Statistical analyzes were performed, considering a completely randomized design with two treatments (sugarcane and Cerrado), six replicates (sampling sites) and four replications (corresponding to rainfall and dry sampling in the year of 2015 and 2016), totaling 48 sample plots. The Tukey test was applied to evaluate the differences between each chemical attribute, microbial biomass carbon and the means of activity of each enzyme, between the two ecosystems and two periods of the year, considering $p < 0.05$ as a significant result. The relationship between biological parameters (enzymatic activity and CBM) and chemical parameters ($Ca^{2+}$, $K^{+}$, $Mg^{2+}$, P, OM, TOC, TN) were identified by Pearson's correlation analysis (at a 5% significance level), using the mean values of such attributes for each land use class.

## RESULTS AND DISCUSSION

### The influence of monocultures on the chemical soil properties

The chemical analysis showed that soils collected in areas of sugarcane contain higher levels of phosphorus (20 cmolc $dm^{-3}$) and potassium (40.2 cmolc $dm^{-3}$) in relation to the Cerrado soil samples (8.9 and 31.2 cmolc $dm^{-3}$, respectively), reflecting the influence of fertilizers. The levels of magnesium (1.2 cmolc $dm^{-3}$ for sugarcane and 1.6 cmolc $dm^{-3}$ for Cerrado) and calcium (3.7 and 4.8 cmolc $dm^{-3}$ for sugarcane and Cerrado, respectively) were not significantly different among the sample types analyzed in this study. The results of the analysis of variance obtained from the evaluation of the different soil attributes indicated that the natural ecosystem modification for agro ecosystem generated changes in the chemical characteristics observed by the presence of lower organic matter (SOM) (2.4%), carbon (TOC) (1.4%), total nitrogen (TN) (0.12%) and microbial biomass carbon (MBM) (11.1 µg $g^{-1}$ C) and are in accordance with the studies conducted by Bini *et al.* (2013). It is noted that the native areas of Cerrado have 60% more of SOM, TOC and TN than was observed in the cultivation of sugarcane. The interrelation among these three attributes was confirmed in the present study, which indicated positive correlations between MOS and TOC ($r^2 = 0.99$), MOS and TN ($r^2 = 0.97$) and TOC and TN ($r^2 = 0.96$). In addition, the amount of MBC is approximately 2.5 times greater in the native area when compared to monoculture.

**Table 1. Chemical and biologycal characteristics of soil samples collected from sugarcane crops and native Cerrado soils (0-10 cm depth)**

| Soil chemical characteristics | Land use classes | |
|---|---|---|
| | Sugarcane | Cerrado |
| SOM (%) | 2.4 ± 0.72$^a$ | 3.8 ± 1.37$^b$ |
| TOC (%) | 1.4 ± 0.4$^b$ | 2.2 ± 0.7$^a$ |
| TN (%) | 0.12 ± 0.04$^b$ | 0.19 ± 0.07$^a$ |
| MBC (µg $g^{-1}$ C) | 11.1 ± 8.1$^b$ | 28.3 ± 26.2$^a$ |

Values followed by different letters in distinct columns are different (p> 0.05).

The values found for such attributes in soils planted with sugarcane (Table 1) represent a consequence of the reduction in the organic matter content in these soils, which is the result of the management associated with this crop. Monocultures consist of systems of soil exploration with a single type of plant species and do not present alternation with other agricultural crops (Araldi *et al.*, 2010). In this type of crop, the soil management is carried out in a conventional way, using plows and grids, for soil rotation, as well as high amounts of pesticides and fertilizers. The lower concentration of organic carbon and total nitrogen observed in the sugarcane crop in relation to the Cerrado reflects the conventional soil preparation system, which causes alterations in the structure of the aggregates, reduction of organic matter, which play a predominant role in the microbial activity in this system (Carneiro *et al.*, 2008). The greater accumulation of organic residues in native areas promotes greater aggregation of particles, protecting organic matter, which promotes lower C and N losses in the soil (Evangelista *et al.*, 2013). The reduction in MBC can be attributed to factors such as the amount of organic matter, as well as its quality. In natural ecosystems, the tendency is that over the years there will be more accumulation of litter, since there is no anthropic interference. The diversity of plant species, roots, macro and micro biota in natural environments generates greater availability of organic residues with varied composition and, therefore, larger quantities and different substrates are offered stimulating the microbial activity in a continuous flow. These factors provide equilibrium conditions that favor the performance of the micro biota and nutrient cycling. In addition, studies have indicated that biochemical attributes such as microbial biomass carbon are easily disturbed by changes in physical structure and chemical composition resulting from agricultural cultivation and use of agrochemicals (Souza *et al.*, 2012).

### The influence of monocultures on the soil biochemical properties

The biochemical analysis of soil samples under cultivation of native sugarcane and Cerrado showed that the enzymes β-glucosidase, acid phosphatase and glycine amino peptidase are sensitive to anthropic actions, such as the removal of native vegetation cover and soil management (Fig. 1). It was observed that β-glucosidase, acid phosphatase and amino peptidase had the lowest values of enzymatic activity in areas of monocultures (β-glucosidase: 21.6 µmol p-nitrophenol $g^{-1}$ $h^{1}$; acid phosphatase: 62.2 µmol p-nitrophenol $g^{-1}$ $h^{-1}$; aminopeptidase: 35.2 µmol *p*-nitroaniline $g^{-1}$ $h^{-1}$) and were statistically different from the enzyme activity found in areas of Cerrado native vegetation (Fig 1A, 1B and 1C). The observed differences in the activity of the enzymes related to the carbon, phosphorus and nitrogen cycle between the two ecosystems (perennial crops and native areas of Cerrado) follow the same pattern presented for chemical analysis (levels of SOM, TN, TOC) and biological (MBC). These factors confirm the hypothesis that soil management exerts influence on the metabolic activity of microorganisms and on the enzymatic activity of the soil (Bini *et al.*, 2013). Another important factor to consider is that the type of vegetation cover also exerts influence not only on biochemical processes but also on the diversity of microbial communities in the soil, promoting the proliferation of different and more complex microbial communities. The amount, type and composition of the root exudates differs depending on the plant species and influences the abundance and survival of certain groups of microorganisms, which in turn regulate the synthesis of extracellular enzymes and enzymatic activity (Gianfreda *et al.*, 2015 ). Thus, the greater microbial diversity, associated with greater abundance and types of carbon residues in the soil, will result in higher rates of enzymatic activity and higher rates of nutrient cycling, since the degradation of organic compounds is carried out from biochemical processes that depend on soil microbial respiration (Bini *et al.*, 2013, Kuwano *et al.*, 2014). Therefore, in the native Cerrado soil, the greater diversity of species allows the entrance of organic residues with varied carbon composition, increasing the metabolic rate, which was evidenced in this study by the values of MBC and β-glucosidase activity.

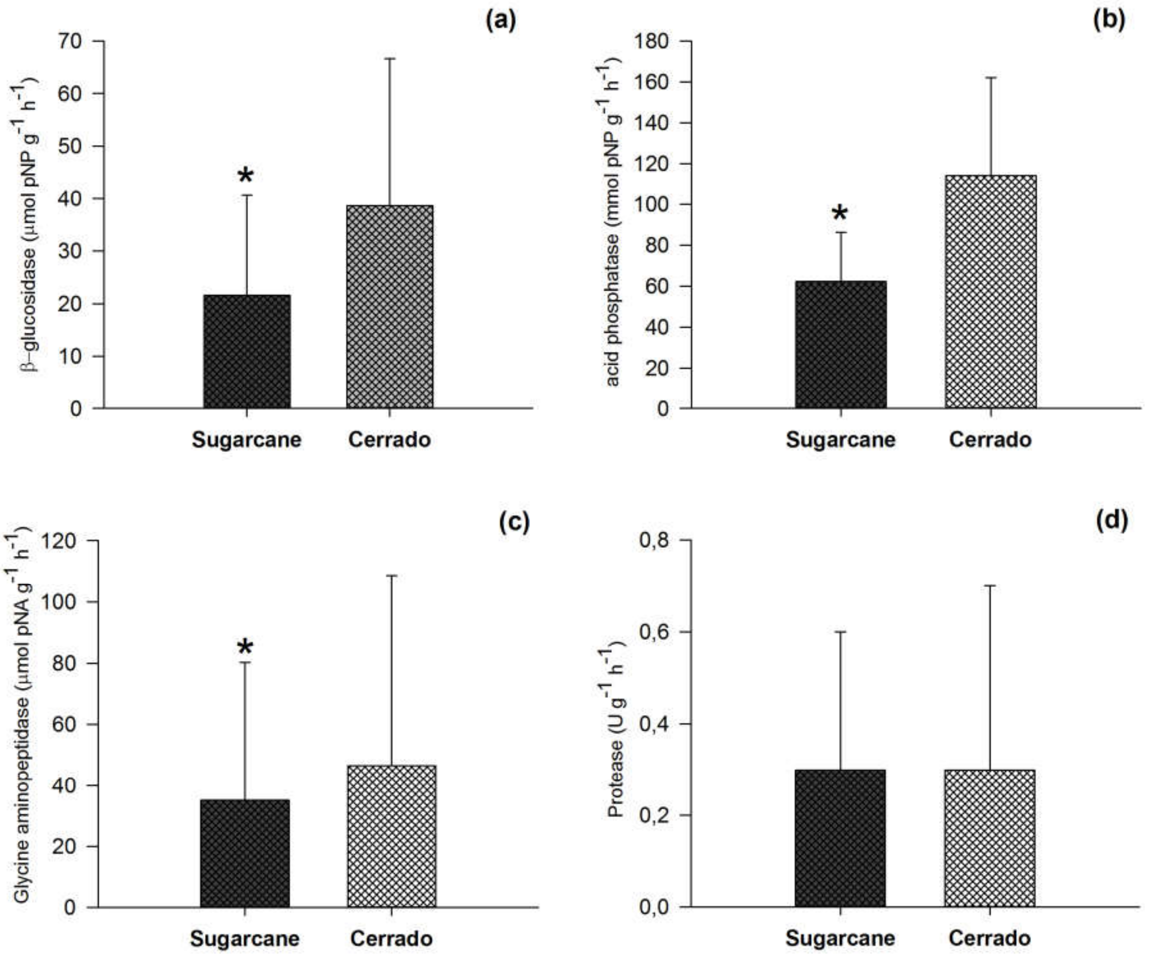


**Fig. 1. Activity of enzymes in soils of sugarcane and native Brazilian Cerrado a: β-glucosidase; b: acid phosphatase; c: glicine aminopeptidase; d: protease. * represent significant variations ($p<0.05$) between the soil land use classes (ANOVA and the Tukey test)**

The highest acid phosphatase activity observed in natural ecosystems in relation to agricultural ecosystems (Fig. 1B) is explained by the results of the analysis of inorganic phosphate (P) in the soil. In the sugarcane plantations, the highest levels of Pi (20 cmolc $dm^{-3}$) were found in relation to the native Cerrado (8.9 cmolc $dm^{-3}$), which in turn reflected higher enzymatic activity, since the acid phosphatase activity in the soil depends, among other factors, on the amount of Pi available in the soil. Low concentration is essential for the activation of transcription factors and the expression of genes associated with phosphatases (Leal *et al.*, 2007). Thus, phosphatase activity tends to be lower when there is greater abundance of inorganic phosphate in the soil (Allison *et al.*, 2007), which probably explains the values of this enzymatic activity found in this study. The tendency is that in agricultural areas that receive Pi via fertilization the expression of the genes is smaller, since the assimilable form is available in the soil and for that reason the enzymatic activity is reduced (Esposito and Azevedo, 2004). Probably, the fertilization carried out by introduction of inorganic phosphorus in the soil increased the supply of inorganic phosphate, reducing the enzymatic activity. Other studies have confirmed this hypothesis and have also observed the same tendency to increase phosphatase activity in preserved soils, evidencing that fertilization is a factor that influences the activity of this enzyme (Carneiro *et al.*, 2008; Silva *et al.*, 2012). The activity of the glycine aminopeptidase enzyme was lower in the sugarcane areas compared to what was observed in the native Cerrado (Fig. 1C), which may result from soil management practices. The substitution of natural Cerrado vegetation by crops can provide compaction and changes in soil temperature, reduction of organic resources that are used by microbial biomass, which in turn affect the stability of the biochemical processes carried out by the microbiota and generate environmental imbalance of this ecosystem (Bini *et al.*, 2014). The action of peptidases depends on the availability and chemical composition of the organic substrates (Enowash *et al.*, 2009). In perennial crops, the diversity of resources, organic compounds, carbon and nitrogen stocks tend to be reduced in relation to natural ecosystems, which confers less quantity of substrate available for enzyme action and, consequently, lower nutrient cycling capacity (Souza *et al.*, 2012; Bini *et al.*, 2013).

Unlike the other enzymes evaluated, the protease activity did not differ among the ecosystems and presented the same value in both evaluated areas (0.3 U $g^{-1}$ $h^{-1}$) (Fig 1D). The activity of the proteolytic enzymes is stimulated by the type of organic matter, that is, availability of protein substrates, besides temperature and pH (Geisseler and Horwath, 2008; Rejsek *et al.*, 2008). In the present study, it was observed that the protease activity presented values much lower than those found for the other enzymes analyzed and even though there were variations in the organic matter content, there were no statistically significant differences in the enzymatic activity between the soil use classes. A probable explanation for this behavior is that many proteases rely on calcium to exert their

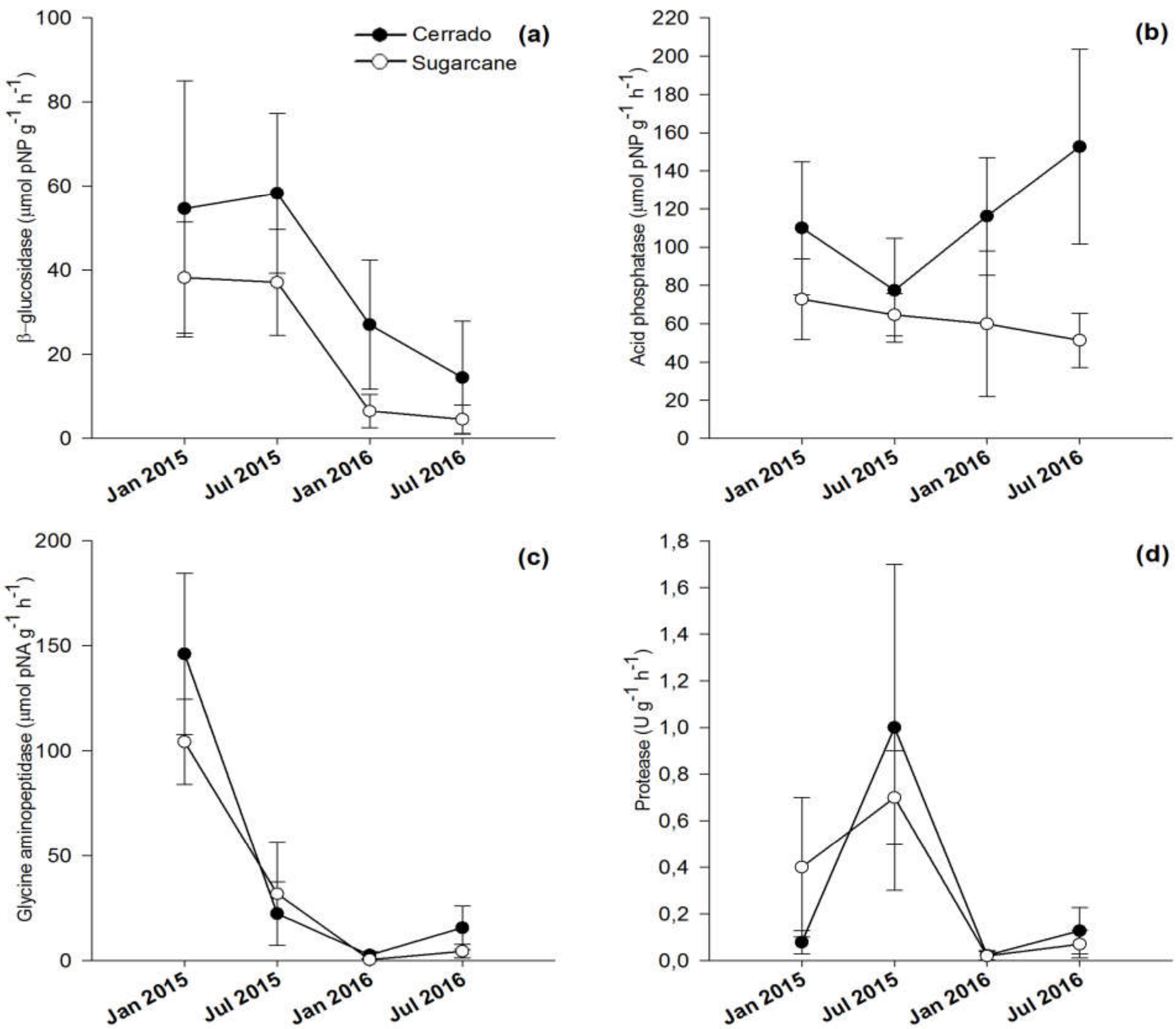


**Fig. 2. Effect of seasonality on the activity of hydrolases in sugarcane crops and native Cerrado soils; a: β-glucosidase; b: acid phosphatase; c: glicine aminopeptidase; d: protease**

activity (Balachandran *et al.*, 2012), and the levels of this element observed for the sugarcane and native Cerrado soil samples were similar to the 5% level of significance.

### Effects of seasonality on the soil enzyme activity

Data analysis in the two consecutive years indicated significant variations ($p > 0.05$) in enzymatic activity in the different ecosystems evaluated (sugarcane and Cerrado), in the different periods of the year (January and July) (Fig 2). The enzymes β-glucosidase, glycine aminopeptidase and protease showed greater activity in both the natural ecosystem and the agroecossystem in January and July 2015 than in the following year (Fig. 2a, 2c, 2d). Temperature and precipitation in 2015 were higher than in 2016 (INMET, 2017). The combination of higher temperatures and more rain provides favorable conditions for the proliferation of microorganisms, which also stimulates enzymatic activity through the quantitative increase of enzymes (Burns *et al.*, 2013). Thus, enzymes related to carbon and nitrogen metabolism should respond positively to increases in temperature and water in the soil, according to Michaelis-Menten kinetics (Davidson *et al.*, 2012). Acid phosphatase showed a distinct pattern of behavior between the two evaluated areas (Fig. 2b), especially for sugarcane crops. It is likely that inorganic phosphate fertilization practices maintain soil mineral levels and exert a greater effect on the catalytic activity of these enzymes (Esposito and Azevedo, 2004, Allison *et al.*, 2007, Leal *et al*., 2007). However, in the preserved areas (native Cerrado) the changes during the sampling periods were statistically significant. The rain shortage period in 2015 may have allowed the entry of a larger amount of litter, rich in organic phosphate, a substrate for enzymatic action. The higher rainfall and moisture content in the year 2016 provided the diffusion of the substrates and greater catalytic action capacity of this enzyme (Evangelista *et al.*, 2013).

As acid phosphatase, protease showed significant changes over time only in Cerrado soil samples, exhibiting the highest values of activity in the rainy season in 2015 (Fig. 2d). The lower values of protease activity in periods of drought in sugarcane plantations can be explained by the fact that rainwater contributed to the leaching of compounds present in chemical fertilizers and organic matter (Zago *et al.*, 2016).The results indicated that hydrolases (β-glucosidase, acid phosphatase, glycine aminopeptidase and protease) are sensitive to variations in tillage and seasonality and corroborate with other studies, indicating that biological attributes can be used to point out environmental variations caused by the conversion of ecosystems (Kaschuk *et al.*, 2010, Zago *et al.*, 2016).

In general, it was possible to notice that of the four enzymes analyzed in the present study, three (β-glucosidase, acid phosphatase and glycine aminopeptidase) showed reduced activities in sugarcane fields. Thus, monocultures have led to changes in chemical composition that may negatively impact microbial activity in the soil and the activity of some hydrolases and this may interfere on soil nutrient cycling. On the other hand, the enzyme protease behaved differently and was similar in the different ecosystems analyzed. The behavior of the hydrolases in the face of changes in ecosystem functioning conditions indicates that these enzymes are sensitive to environmental transformations and, thus, can indicate the state of conservation / quality of the soil in agroecosystems. Within this context, it is necessary to adopt new management techniques for planting sugarcane that are more sustainable and that can improve chemical and biological conditions in agricultural ecosystems (Bini *et al.*, 2014). The adoption of no-tillage techniques can be an effective alternative to improve conditions of perennial crops, since this type of management provides better biochemical conditions and increases the capacity of mineralization, enzymatic activity, nutrient cycling and immobilization of nutrients by the soil microbiota, contributing to improve the sustainability of agricultural ecosystems (Roldan *et al.*, 2005).

**Conclusion**

The soil microbiota in areas converted to sugarcane plantations has lower C, P and N mineralization capacity compared to natural ecosystems, as a result of the management practices adopted for the planting of this crop. Over time, the perennial cultivation of sugarcane promotes a reduction in the biological attributes of the soil, promoting the loss of fertility. In addition, the enzymes β-glucosidase, acid phosphatase and aminopeptidase can be used as parameters for the evaluation of soil quality, since they respond to environmental variations resulting from anthropic use and in this way may reflect the state of quality of agroecosystems.

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## REFERENCES

Allison, S.D., Vitousek, P.M. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biol Biochem.,* 37: 937–944.

Allison, V.J., Condron, L.M., Peltzer, D.A., Richardson, S.J., Turner., B.L. 2007. Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Josef chronosequence. *Soil Biol Biochem.,* 39(7): 1770-1781.

Araldi, R., Silva, FML., Ono, E.O., Rodrigues, J.D. 2010. Florescimento em cana-de-açúcar. *Cienc Rural.,* 40(3): 694-702.

Balachandran, C., Duraipandiyan, V., Ignacimuthu, S. 2012. Purification and characterization of protease enzyme from actinomycetes and its cytotoxic effect on cancer cell line (A549). *Asian Pac J of Trop Biomed S.*,1138-S1146.

Baldrian, P., Valáskova, V., Merhautová, V., Gabriel, J. 2005. Degradation of lignocellulose by *Pleurotus ostreatus* in the presence of copper, manganese lead and zinc. *Res Microbiol.,* 156(6): 670-676.

Bini, D., Santos, C.A., Bernal, LPT., Andrade, G., Nogueira, MA. 2014. Identifying indicators of C and N cycling in a clayey Ultisol under different tillage and uses in winter. *Appl Soil Ecol.,* 76: 95-101.

Bini, D., Santos, CA., Carmo, KB., Kishino, N., Andrade, G., Zangaro, W., Nogueira, MA. 2013. Effects of land use on soil organic carbon and microbial processes associated with soil health in southern Brazil. *Eur J Soil Biol.,* 55:117-123.

Burns, RG., Deforest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A. 2013. Soil enzymes in changing environment: current knowledge and future directions. *Soil Biol Biochem.,* 58*:*216-234.

Carneiro, MA., Siqueira, J.O., Moreira, F.M.S., Soares, A.L.L. 2008. Carbono orgânico, nitrogênio total, biomassa e atividade microbiana do solo em duas cronossequências de reabilitação após a mineração de bauxita. *Rev Bras Cien Solo.,* 32(2), 621-632.

Climate-data, 2016. Dados climáticos para cidades mundiais. http://pt.climate-data.org/. Acessado 15 janeiro 2017.

CONAB, Companhia Nacional de Abastecimento 2014. Acompanhamento da safra brasileira cana-de-açúcar: Safra 2016/2017. Terceiro Levantamento, pp.1-20. http://www.conab.gov.br. Acessado 13 janeiro 2017.

Cunha, NRS., Lima, J.E., Gomes, M.F.M., Braga, M.J. 2008. A Intensidade da Exploração Agropecuária como Indicador da Degradação Ambiental na Região dos Cerrados, Brasil. *Rev Econ Sociol Rural.,* 46(2): 291-323.

Dalchiavon, F.C., Carvalho, M.P., Montanari, R., Andreotti, M. 2013. Sugarcane productivity correlated with physical-chemical attributes to create soil management zone. *Rev Ceres.,* 60(5):706-714.

Davidson, E.A., Sudeep, S., Caramori, S.S., Savage, K. 2012. The dual Arrhenius and Michaelis-Menten Kinectics model for decomposition of soil organic matter at hourly to seasonal time scales. *Global Change Biol.,* 18(1): 371-384.

EMBRAPA, Empresa Brasileira de Pesquisa Agropecuária, 1997. Manual de análises de solo. Rio de Janeiro, Embrapa, 212 pp.

Enowashu, E., Poll, C., Lamersdorf, N., Kandeler, E. 2009. Microbial biomass and enzyme activities under reduced nitrogen deposition in a spruce forest soil. *Appl Soil Ecol.,* 43:11-21.

Esposito, E. and Azevedo, JL. 2004. Fungos: uma introdução à biologia, bioquímica e biotecnologia. Caxias do Sul: EDUCS, p. 638.

Evangelista, C.R., Partelli, F.L., Ferreira, E.P.B., Pires, F.R. 2013. Atributos microbiológicos do solo na cultura de cana-de-açúcar sob manejo orgânico e convencional. Semina: *Cien Agrar.,* 34(4):1549-1562.

Ferreira, A.S., Camargo, F.A.O., Vidor, C. 1999. Use of microwave radiation to evaluate soil microbial biomass. Rev Bras Cien Solo 23:991-996.

Figueiredo, E.B., Panosso, A.R., Romão, R.L.A., Scala, N. 2010. Greenhouse gas emission associated with sugar production in southern Brazil. Carbon Balance Manage 5(3):1-7.

Frazão, L.A., Piccolo, M.C., Feigl, BJ., Cerri, C.C., Cerri, C.E.P. 2010. Inorganic nitrogen, microbial biomass and microbial activity of a sandy Brazilian Cerrado soil under different land uses. *Agr Ecosyst Environ.,* 135*:*161-167.

Geisseler, D. and Horwath, RW. 2008. Regulation of extracellular protease activity in soil in response to diferente sources and concentrations of nitrogen and carbono. *Soil Biol Biochem.,* 40:3040-3048.

Gianfreda, L. 2015. Enzymes of importance to rhizosphere processes. *J Soil Plant Nutr.,* 15(2):283-306.

Gibbs, HK., Johnston, M., Foley, JA., Holloway, T., Monfreda, C., Ramankutty, N., Zaks, D. 2008. Carbon payback times for crop-based biofuel expansion in the tropics: the effects of changing yield and technology. *Environ Res Lett.,* 3(3):1-11.

Grandy, AS., Strickland, MS., Lauber, CL., Bradford, MA., Fierer, N. 2009. The influence of microbial communities, management, and soil texture on soil organic matter chemistry. *Geoderma.,* 150:278-286.

Huang, C., Chang, Y., Lin, J., Yu, C., Lin, H., Liu, W., Yeh, S., Tu, S., Wu, S., Ku, MSB., Li, W. 2016. Insights into the regulation of C4 leaf development from comparative transcriptomic analysis. *Curr Opin Plant Biol.,* 30:1-10.

INMET, Instituto Nacional de Meteorologia. http://www.inmet.gov.br/portal/index.php?r=tempo/grafico. Accessed 28 April 2017.

Janssen, PH., Peek, K., Morgan, HW. 1994. Effect of culture conditions on the production of an extracellular protease by *Thermus* sp. Rt41A. *Appl. Microbiol Biotechnol.,* 41:400-406.

Kaschuk, G., Alberton, O., Hungria, M. 2010. Three decades of soil microbial biomass studies in Brazilian ecosystems: lessons learned about soil quality and indications for improving sustainability. *Soil Biol Biochem.,* 42:1-13.

Kuwano, BH., Knob, A., Fagotti, DSL., Melém Júnior, NJ., Godoy, L., Diehl, RC., Krawulski, CC., Andrade Filho, G., Zangaro Filho, W., Tavares-Filho, J., Nogueira, MA. 2014. Soil quality indicators in a Rhodic Kandiudult under different uses in Northern Paraná, Brazil. *Rev Bras Cien Solo*., 38*:*50-59.

Lacerda, KAP., Cordeiro, MAS., Verginassi, A., Salgado, FHM., Paulino, HB., Carneiro, MAC. 2013. Organic carbon, biomass and microbial activity in an Oxisol under different management systems. *Braz J Agric Sci.,* 56:249-254.

Ladeira, SA., Andrade, VVM., Delatorre, AB., Perez, VH., Martins, MLL. 2010. Utilização de resíduos agroindustriais para a produção de proteases pelo termofílico Bacillus sp em fermentação submersa: otimização do meio de cultura usando a técnica de planejamento experimental. Quím Nova 33(2):324-328.

Lapig-maps, Laboratório de processamento de imagens e geoprocessamento, 2016. http://maps.lapig.iesa.ufg.br/lapig.html. Acessado 15 janeiro 2017.

Leal, J., Squina, FM., Martinez-Rossi, NM., Rossi, A. 2007. The transcription of the gene for iso-orotate decarboxylase (IDCase), an enzyme of the thymidine salvage pathway, is downregulated in the *preg*c mutant strain of *Neurospora crassa* grown under phosphate starvation. *Can J Microbiol.,* 53:1011-1015.

MAPA, Ministério da Agricultura, Pecuária e Abastecimento, 2015. Projeções do Agronegócio*:* Brasil 2014/15 a 2024/25. Brasília, MAPA.

Mendes, IC., Sousa, DMG., Reis Junior, FB. 2013. Bioindicadores de qualidade de solo: dos laboratórios de pesquisa para o campo. *Cader Cien Tecnol.,* 32*:185*-203.

Nannipieri, P., Giagnoni, L., Renell, G., Puglisi, E., Ceccanti, B., Masciandaro, G., Fornaiser, F., Moscatelli, MC., Marinari, S. 2012. Soil enzymology: classical and molecular approaches. *Biol Fert Soils.,* 48:473-762.

Rachid, C.T.C.C., Piccolo, M.C., Leite, D.C.A., Balieiro, F.C., Coutinho, H.L.C., Elsas, J.D.V., Peixoto, R.S., Rosado, A.S. 2012. Physical-chemical and microbiological changes in Cerrado Soil under differing sugarcane harvest management systems. *BMC Microbiol.,* 12:170-181.

Rejsek, K.P., Formanek, P., Pavelka, M. 2008. Estimation of protease activity in soils at low temperatures by casein amendment and with substitution of buffer by demineralized water. *Amino Acids.,* 35:411-417, 2008.

Roldan, A., Salinas-García, J.R., Alguacil, M.M., Caravaca, F. 2005. Changes in soil enzyme activity, fertility, aggregation and C sequestration mediated by conservation tillage practices and water regime in a maize field. *Appl Soil Ecol.,* 30:11-20.

Sano, E.E., Rosa, R., Brito, J.L.S., Ferreira, L.G. 2010. Land cover mapping of the tropical savanna region in Brazil. *Environ Monit Assess.,* 166:113-124.

Silva, C.F., Pereira, M.G., Miguel, D.L., Feitora, J.C.F., Loss, A., Menezes, E.G., Silva, E.M.R. 2012. Total organic carbon, microbial biomass and soil enzyme activity areas of agriculture, forestry and grassland in the middle Valley of Paraíba do Sul river (RJ). *Rev Bras Cien Solo.,* 36:1680-1689.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B,, Allison, S.D., Crenshaw, C. 2008. Stoichiometry of soil enzyme activity at global scale. *Ecol Lett.,* 11:1252–64.

Souza, R.A., Telles, T.S., Machado, W., Hungria, M., Tavares-Filho, J., Guimarães, MF. 2012. Effects of sugarcane havesting with burning on the chemical and microbiological properties of the soil. *Agr Ecosyst Environ.,* 155*:*1-6.

Zago, L.M.Z., Oliveira, R.N., Bombonato, A.K.G., Moreira, LMO., Melo, E.N.P., Caramori SS 2016. Soil extracellular enzymes from Brazilian Cerrado as quality bioindicator agricultural areas in Goiás, Brazil. Front *J Soc Tech Environ Sci.,* 5(1):104-127.

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