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Biochemical activity in Brazilian Cerrado soils is differentially affected by perennial and annual crops

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Abstract

Organic matter mineralization depends on how microbiota access nutrients, substrates and soil fertilization. This study shows influence of perennial (sugarcane) and annual (soybean and corn) crops on the activity of enzymes participating in the carbon, nitrogen and phosphorus cycles and proposes using hydrolases as bioindicators of soil quality. Samples were collected, during dry season and rainfall, in six different plots where sugarcane, soybean, corn and native Cerrado soils could be found at the same location. We evaluated chemical properties and activity of α- and β-glucosidase, acid phosphatase, protease and glycine aminopeptidase from 48 samples collected at 0-10 cm depth. Sample of monoculture soils showed low organic matter content, total organic carbon, total nitrogen and microbial biomass carbon. Native Cerrado soils had major activities of α- and β-glucosidase, acid phosphatase and glycine aminopeptidase while sugarcane areas showed minor values. Despite native vegetation replacement decreasing biodiversity and reducing soil biochemical activity, annual crops stimulate microbial activity in this environment and maintain nutrient cycling. Soil hydrolase activities can be used as bioindicators of these ecosystems.

Keywords: agricultural crops; soil bioindicators; hydrolases; microbial biomass; soil quality.

Introduction

Last decades have been marked by expansion and intensification of agriculture in areas of Cerrado (biome consisting of savanna, forest and grassland). According to Sano et al. (2010), native vegetation areas in Cerrado were reduced by 39.5% due to agribusiness. In the coming years, new areas will be deforested for crops. Despite being important for food, fiber and fuel production, land use for agricultural purposes may cause damage to the ecosystem (Nolte et al., 2017). Changes in soil carbon storage due to anthropogenic use alter quality and quantity of soil organic matter, affecting physical and chemical properties of soils, and directly impacting microorganisms and microbial processes. As a result, cycling and availability of nutrients are affected and ecosystem sustainability is disturbed (García-Orenes et al., 2013). Removal of native vegetation exposes soil, increases soil temperature and reduces input and variability of organic matter. Management techniques modify soil physical structure, affect aggregation of particles and change their chemical composition (Babujia et al., 2010). Agricultural practices significantly decrease organic matter, main source of nutrients for microbial growth. Community and microbial processes are also negatively affected, causing changes in cycling of carbon, nitrogen and phosphorus (Frazão et al., 2010). Soil dynamics depends on the interaction between biotic and abiotic factors and, such changes imbalance ecosystem sustainability (Biswas et al., 2017), posing risks to future agricultural production. However, problems can be minimized through soil quality monitoring and more sustainable management practices (Carvalho et al., 2007). Soil quality consists of evaluating its physical, chemical, biological and biochemical properties, which are key parts of nutrient cycling processes. Such properties indicate changes in soil quality under different management systems (Babujia et al., 2010). Changes in soil organic matter from intense use and management affect physical, chemical and biological properties of soils and influence on its productive capacity (Bini et al., 2013). Evaluations of microbial biomass and soil enzyme activity are important for organic properties and knowledge of changes resulting from different agricultural practices (Biswas et al., 2017). Additionally, microbial populations and enzymes can change with land use, thus being considered important tools for assessing soil quality (García-Orenes et al., 2013). Organic matter inputs in soil differ in composition and complex breakdown depends on the synthesis and release of extracellular enzymes by various microorganisms (Biswas et al., 2017). Extracellular enzymes determine rates of decomposition, immobilization and mineralization of organic matter, allowing for flow of nutrients, species survival and soil functioning (Zhang et al., 2015).

Hydrolases participate in cycles of carbon, phosphorus and nitrogen, being most abundant enzymes in soils (Nannipieri

et al., 2012). Enzymes α- and β-glucosidase (EC 3.2.1.20, EC 3.2.1.21, respectively) participate in carbon cycle by hydrolyzing α-1,4-linked saccharide bonds present in carbohydrates and β-1,4 bonds present in molecules of cellobiose. On the other hand, phosphatase (EC 3.1.3.2) catalyzes the hydrolysis of esters and phosphoric acid anhydrides with subsequent release of inorganic phosphate (Nannipieri et al., 2010), being main phosphate mineralization source for plants. Proteases (3.4.2.21-24) and aminopeptidases (EC 3.4.11) act on protein molecules by hydrolyzing N- and C-terminus and, releasing smaller polypeptides or amino acids source of nutrients for plants and other organisms in the soil (Allison and Vitousek, 2005).

Natural remnant vegetation of Cerrado was 47% in 2010 (Beuchle et al., 2015), with threat of new areas to be deforested and converted to agriculture. Removal of native vegetation and planting crops has impacts on the chemical and biochemical properties of soil that need to be understood. Thus, we assumed implementation of sugarcane, soybean and corn inducing to negative changes in soil organic matter, microbial biomass, and biochemical processes related to cycles of C, N and P. Also, we hypothesize hydrolases present higher activity in areas with annual crops of soybean and corn compared to areas of perennial crops of sugarcane.

Results and Discussion

Soil chemical attributes indicate changes from replacement of native vegetation

Replacement of native vegetation by planting sugarcane, soybean or corn has changed chemical properties of soils such as organic matter, organic carbon and total nitrogen (Table 1). Sugarcane areas showed lower content of organic matter (OM) (2.2%), total organic carbon (TOC) (1.3%) and total nitrogen (TN) (0.09%) compared to the other land uses. The abundance of these elements in soil is associated with waste provided by vegetation and microbial ability to nutrient cycling processes (Allison et al., 2007).

Reduced content of those elements in areas converted to monoculture denotes negative effects of land use. Sugarcane crop areas had 50% less OM, 46% less TOC and 89% less TN than Cerrado areas. Low levels of TOC and TN in sugarcane soils are result of low organic matter in these soils, consequence of tillage system associated with this agriculture. No intercrops in sugarcane crop areas tend to decrease diversity of soil microbial community (Rachid et al., 2012).

Sugarcane crops are a major attraction for farmers in the Cerrado due to the Biome edaphic aspects and high productivity of this crop because of plant metabolism involving CO_2 assimilation. Sugarcane is a C4-type plant, i.e., it first absorbs CO_2 from atmosphere to form four-carbon compound then releasing this molecule in the environment, where fixation by ribulose 1,5-bisphosphate carboxylase/oxygenase occurs (Gibbs et al., 2008). This type of plant is more successful in CO_2 fixation, providing greater biomass gain with consequences for increased productivity in tropical climate (Huang et al., 2016).

Changes in chemical properties have shown the focus only on the crop and not on the soil-plant-atmosphere system (Zhang et al., 2015). This behavior can impair mineralization processes of soil organic compounds; modifying components, composition and entry of organic matter.

There is interrelationship between soil chemical properties and development of microbial community (Batola et al., 2014). In this paper, correlation analysis pointed out this relationship also for all sampled soils, being significant considering C_{mic} and total nitrogen ($p = 0.0203$), C_{mic} and total organic carbon ($p = 0.0035$) and C_{mic} and organic matter ($p = 0.0034$).

Results in Table 1 show annual crops did not significantly alter soil parameters related to microbial activity. Areas of native Cerrado and corn showed the highest level of C_{mic} compared to other agroecosystems. Data are consistent with scientific literature, indicating reduced organic content and microbial biomass in agricultural ecosystems (Bini et al., 2013).

Reduction in C_{mic} can be attributed to two factors: quantity and quality of organic matter and species composition in native Cerrado areas. In these ecosystems, no human interference provides accumulation of organic residues on the soil surface over time. The greater abundance of roots and plant species promotes greater availability of organic substrates from rhizosphere (Zhu et al., 2014) and deposition of organic waste of varied composition. Thus, different substrates stimulate microbial activity in a continuous flow. These factors provide balanced conditions to microbiota performance and nutrient cycling.

Annual systems using no-tillage do not leave soil bare for long periods and retain part of organic matter on the soil surface after harvest (Baker et al., 2007) as in the soybean and corn fields. Therefore, we believe values of OM, TOC and TN did not differ from Cerrado soil as happened to sugarcane crop areas. Some authors evaluated management influence on soil quality and observed beneficial effects of no-tillage system on minimizing loss of fertility by using crop at the expense of removing native vegetation (Balota et al., 2014). According to the authors, high content of TOC improves soil physical conditions (stability of aggregates) protecting the microbial habitat, as well as providing compounds to be mineralized. Consequently, there is increased capacity of mineralization and immobilization of nutrients for microorganisms decomposing organic matter.

Soil hydrolase activities rapid respond to changes in land cover

Soil biochemical analysis showed enzymes α- and β-glucosidase, acid phosphatase, protease and glycine aminopeptidase were sensitive to changes in land cover (Fig. 1).

Corn and soybean showed the highest values of α-glucosidase and β-glucosidase and did not differ significantly in soils of native Cerrado (Fig. 1A and 1B). Soils collected in sugarcane crops showed lower activity of both enzymes compared to the native Cerrado areas (α-glucosidase: 2.8 μmol p-nitrophenol g^{-1} h^{-1}, β-glucosidase 37.7 μmol p-nitrophenol g^{-1} h^{-1}) and those under annual cultivation of corn (α-glucosidase: 8.4 μmol p-nitrophenol g^{-1} h^{-1} and β-glucosidase: 63 μmol p-nitrophenol g^{-1} h^{-1}) and soybean (α-

Table 1. Chemical characteristics and microbial biomass carbon of soils from sugarcane, soybean and corn crops (0-10 cm) in the last 15 years and native Cerrado soils in Goiás, Brazil.

Soil chemical characteristics [†]	Land use classes			
	Sugarcane	Soybean	Corn	Cerrado
P (Melich I) cmolc/dm^{-3}	12±8.2 [a]	17.4±9 [a]	22.8±15 [a]	10.7±10.2 [a]
K^{+} cmolc/dm^{-3}	80.1±78 [a]	53.1±52 [a]	58.1±57 [a]	63.3±62.3 [a]
Mg^{+2} cmolc/dm^{-3}	1.0±0.3 [a]	1.0±0.04 [a]	1.1±0.5 [a]	1.3±1.2 [a]
Ca^{+2} cmolc/dm^{-3}	3.3±0.8 [a]	4.1±2 [a]	4.3±1.5 [a]	4.2±3.9 [a]
OM %	2.2±0.5 [b]	2.5±0.5 [ab]	2.6±0.8 [ab]	3.3±1.3 [a]
TOC %	1.3±0.3 [b]	1.4±0.3 [ab]	1.6±0.5 [ab]	1.9±0.7 [a]
TN %	0.09±0.02 [b]	0.12±0.02 [ab]	0.14±0.1 [ab]	0.17±0.1 [a]
C_{mic} (µg g^{-1} C)	8.0±5.7 [c]	9.3±3.2 [bc]	11.0±6.0 [ab]	12.8±4.9 [a]

Values followed by the same letters in the same line are not statistically different (p>0.05). [†] OM: organic matter; TOC: total organic carbon; TN: total nitrogen; C_{mic}: microbial carbon biomass.

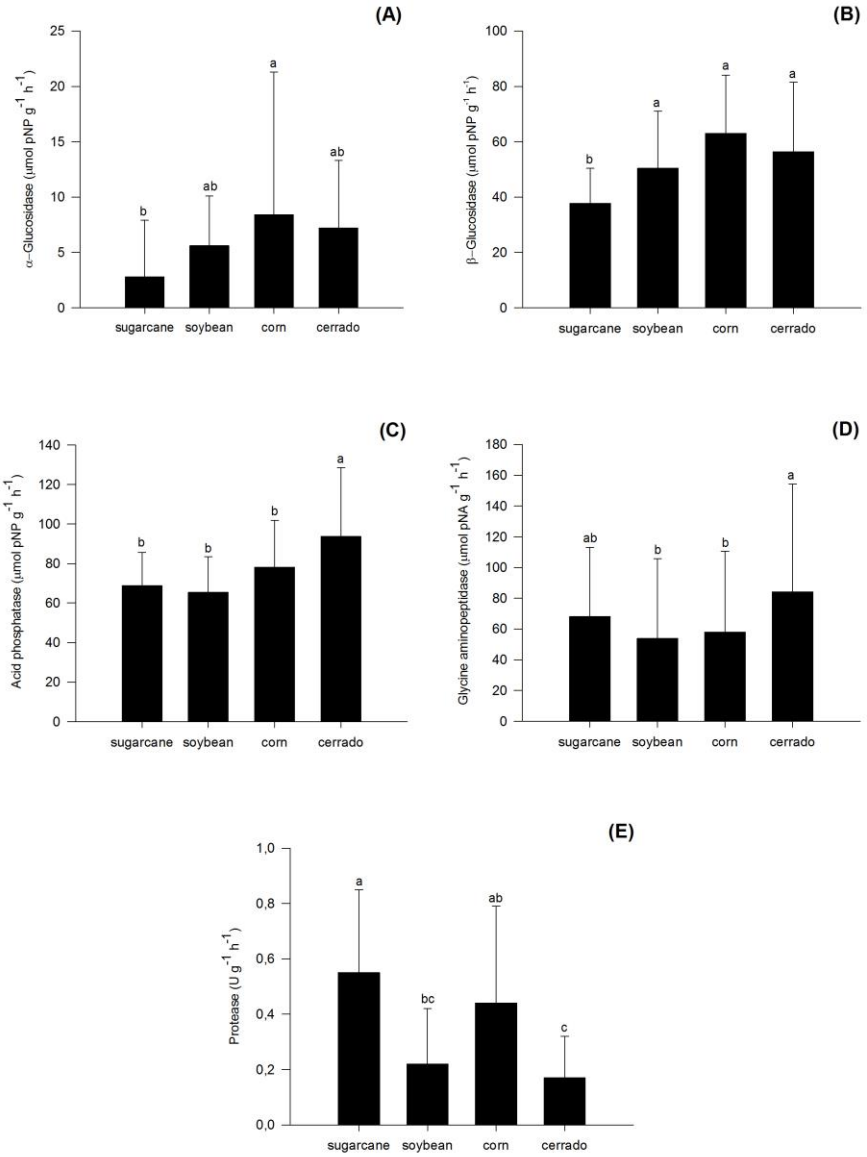

Fig 1. Activity of hydrolases under different land use classes. A: α-glucosidase; B: β-glucosidase; C: acid phosphatase; D: glycine aminopeptidase; E: protease. Different lowercase letters show significant variation among land use classes (p<0.05,ANOVA and Tukey test).

Table 2. Temperature, annual precipitation (mean) (last 15 years) and geographical location of soil sampling sites.

Sample Site	T† (º C)	Precipitation (mm year^{-1})	Soil Use Class	Geographic Coordinates	
				Latitude	Longitude
Itumbiara (P1)	24.5	1119	Sugarcane	18°23'28.9"	49°19'30.8"
			Soybean	18°20'11.6"	49°06'56.9"
			Corn	18°20'42.4"	49°06'39.7"
			Cerrado	18°20'09.6"	49°06'55.6"
Goiatuba (P2)	23	1368	Sugarcane	18°03'01.2"	49°37'33.0"
			Soybean	18°01'33.5"	49°31'44.2"
			Corn	18°09'58.4"	49°16'50.8"
			Cerrado	18°05'32.8"	49°34'13.0"
Morrinhos (P3)	23.3	1346	Sugar cane	17°53'15.8"	49°14'39.7"
			Soybean	17°55'42.6"	49°10'42.2"
			Corn	17°55'20.6"	49°11'02.3"
			Cerrado	17°55'42.0"	49°09'58.8"
Edéia (P4)	24.1	1422	Sugarcane	17°36'15.1"	50°06'17.2"
			Soybean	17°23'07.4"	49°46'19.0"
			Corn	17°25'47.0"	49°54'09.7"
			Cerrado	17°45'20.6"	50°09'28.0"
Inhumas (P5)	23.1	1516	Sugarcane	16°21'23.4"	49°26'56.0"
			Soybean	16°16'15.4"	49°32'27.3"
			Corn	16°19'40.3"	49°27'56.9"
			Cerrado	16°15'39.2"	49°33'04.6"
Anicuns (P6)	23.6	1535	Sugarcane	16°24'09.2"	49°52'06.8"
			Soybean	16°20'04.4"	50°00'04.6"
			Corn	16°22'44.4"	49°57'54.4"
			Cerrado	16°25'09.5"	49°52'06.0"

† T: temperature (Lapig-Maps, 2015; Climate-data, 2015).

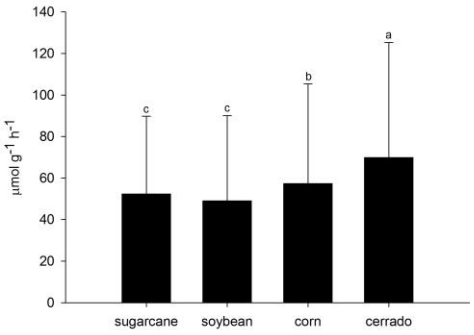

Fig 2. Total enzyme activity under land use classes in Brazilian Cerrado, Goiás, Brazil. Different lowercase letters show significant variation among land use classes ($p<0.05$, MANOVA and Hotelling's T^2 test).

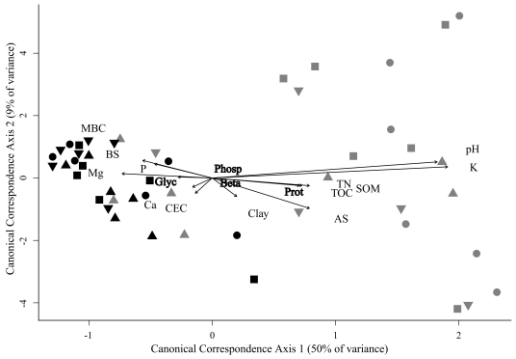

Fig 3. Canonical-correlation analysis (CCA) of soil chemical attributes for enzymatic activity under different land use classes in the Cerrado biome. Symbols show land use sites as follows: inverted triangle = Cerrado, circle = corn, square = soybean, triangle = sugarcane. Black symbols represent data collected during rainy season; gray symbols represent data on dry season in Cerrado biome. Biochemical variables: Beta = Beta glucosidase, Glyc = Glycine aminopeptidase, Phosp = Acid Phosphatase, Prot = Protease, MBC = Microbial Biomass Carbon. Chemical variables: AS = Aluminum saturation, BS = Base saturation, Ca = Calcium, CEC = Cation Exchange Capacity, K = Potassium, Mg = Magnesium, P = Phosphorus, SOM = Soil organic matter, TOC = Total organic carbon, TN = Total nitrogen. Physical variable: Clay.

glucosidase: 5.6 μmol p-nitrophenol g^{-1} h^{-1} and β-glucosidase: 50.4 μmol p-nitrophenol g^{-1} h^{-1}). Differences among Cerrado areas, annual crops and sugarcane areas follow same pattern of soil organic matter, total nitrogen, total organic carbon and C_{mic}. This is because of glucosidases are related to carbon cycle, in which starch and cellulose degradation depends, among other factors, on the cellular respiration rate (Mizuta et al., 2015). Increased organic matter in Cerrado, corn and soybean soils promotes a rich environment in nutrients, increasing resources supply for microbial growth. This is evident through the increased activity of glucosidases produced by microorganisms and high C_{mic} values in these soils. According to Alves et al. (2011) and Nannipieri et al. (2012), system of land use influences metabolic activity of microorganisms and soil enzyme activity. Management systems little disturbing soil can maintain or increase organic matter on the surface (Wang et al., 2013). In the studied areas, except for sugarcane crop areas, grain producers associated tillage techniques to crop rotation. According to Roldan et al. (2005), waste produced in the soil tillage by plowing is smaller than residue produced by no-tillage system. Moreover, the use of plows and harrows affects aggregate stability and reduces glomalin concentration. The main acid phosphatase activity occurred in soils of native Cerrado (Figure 1C), which was expected by the results of soil P content analysis (Table 1). This difference is usually associated with the availability of inorganic phosphate in the soil, which tends to be higher in agricultural areas due to the use of fertilizers (Nannipieri et al., 2010). Furthermore, low concentration of phosphorus in the soil is essential for activating transcription factors and phosphatase gene expression (Leal et al., 2007). Thus, phosphatase activity is lower when under great abundance of inorganic phosphate in the soil (Allison et al., 2007). Fertilization increased inorganic phosphate supply and reduced enzymatic activity in the soil. This is supported by Nannipieri et al. (2010), since phosphatase activity was lower in soils containing high phosphate concentration and controlled by addition of fertilizers. Finally, Alves et al. (2011) found agricultural practices can disturb soil microbial community and consequently affect biogeochemical processes. Soil of native Cerrado showed higher activity of glycine aminopeptidase (Fig. 1D) relative to that observed in agricultural soil samples. Several authors have shown changes in land use led to alterations in soil peptidase activity (Pandey et al., 2014; Zhang et al., 2015). Thus, impact of removing native vegetation coupled with loss of microbial biomass (Table 1) can be co-responsible for differences in classes of land use.

Replacement of native vegetation to agricultural systems causes increased soil temperature, erosion and soil compaction besides reducing input and diversity of natural resources. This directly affects soil microbial activity and causes changes in microbial processes associated with nitrogen cycle by damaging ecosystem sustainability (Babujia et al., 2010). Important to be considered, high mineralization rates promote high content of immobilized N and reduce losses of this element by leaching or denitrification (Fagotti et al., 2012). Enzymes related to nitrogen cycle are dependent on the quantity and quality of substrates (Enowash et al., 2009). Diversity of species in Cerrado soils favors the entry of organic waste of varied chemical composition and increases organic C and N stocks in the soil, which in turn stimulate enzymatic activity and increase microbial biomass (Bini et al., 2013; Kuwano et al., 2014). There is evidence that microbial community is sensitive to N, P and K fertilization, which can influence on the microbial and enzyme activity to a short or long term (Geisseler and Scow, 2014). Soil protease showed distinct pattern of activity compared to that of glycine aminopeptidase, although both may be associated with similar substrates (Fig. 1E). While glycine aminopeptidase showed higher values in native Cerrado soils (146.06 μmol p-nitroaniline g^{-1} h^{-1}) (Fig. 1D), protease activity showed its lowest value (0.07 U g^{-1} h^{-1}) in the same ecosystem (Fig. 1E). This enzyme may have been stimulated by the quality of protein substrates in crop areas compared to that of natural environments (native Cerrado). The addition of organic matter promotes increased protease activity (Kandeler et al., 1999), but it is also known proteolytic enzymes depend on the concentration of protein substrates and variations in soil temperature and pH (Geisseler and Horwath, 2008; Rejsek et al., 2008). Hence, higher pH values (5.7) of sugarcane crop areas compared to native Cerrado (pH 4.9) may have improved conditions for enzymes. In addition, bacteria and fungi (major producers of proteases in soils) (Gupta et al., 2002) may have not been affected by nitrogen fertilization (Treseder, 2008). Therefore, combined effect of pH and soil fertilization has made possible proteases action in perennial crop areas. Fig. 2 shows combined assessment of enzyme activities related to the cycles of carbon (α-glucosidase and β-glucosidase), phosphorus (acid phosphatase) and nitrogen (aminopeptidase and protease glycine) for evaluating influence of land use on biological activities. Results confirm the hypothesis that enzymatic activity is higher in native Cerrado soil areas (69.9 μmol product g^{-1} h^{-1}).

These findings reaffirm biological parameters can be used to indicate environmental changes resulting from land use. This study showed hydrolase activity has significant variation considering the different land use classes. These results demonstrate replacement of native vegetation in agricultural systems causes impacts on the soil ecosystem. Deforestation promotes loss of diversity of microorganisms responsible for biochemical processes and nutrient cycling in the soil (García-Orenes et al., 2013). Each enzyme behaves differently depending on the environmental conditions. Acid phosphatase and glycine aminopeptidase had low activity in agricultural systems. Furthermore, α- and β-glucosidase had high activity in native Cerrado areas and annual crop soils. Protease showed less activity in soil under natural conditions. Nevertheless, perennial crop (sugarcane) causes more chemical changes and impacts negatively to the activity of some enzymes. Annual crops combined with no-tillage system and crop rotation could contribute positively to improve chemical and biological attributes in deforested areas (Bini et al., 2014). Consequently, soil biochemical functioning and its cycling capacity and mineralization compounds are also enlarged, thereby promoting gains in soil fertility. According to Roldan et al. (2005), no-till is more effective for improving soil biochemical quality and reducing nutrient losses. This type of soil management stimulates enzyme activity and carbon cycling, helping to improve agroecosystem sustainability. The rapid response of those hydrolases and transformation generated by human action may early indicate on the soil conservation status, i.e. quality of soil under agricultural use.

Interactions between chemical and biochemical attributes indicate soil quality

The influence of physical and chemical predictors on enzyme activities summarized by CCA explained 59% of data set variation (50% for axis 1 and 9% for axis 2). Fig. 3 shows soil components relationships analyzed altogether. Seasonality effect over physical and chemical parameters of soil only became clear when this perspective of analysis was used. Protease had greater activity in the dry period, glycine aminopeptidase had greater activity in the rainy season, but alpha, beta-glucosidase, and acid phosphatase did not seem to be influenced. Land use sites are grouped according to variables in the rainy season, while there is great dispersion of sites in the two-dimensional space in the dry period. Main variables included K (R^2 = 0.58, p <0.01), pH (R^2 = 0.55, p <0.01) and AS (R^2 = 0.13, p = 0.05), which positively influenced most data set in the dry period and negatively influenced data set in the rainy season.

Materials and methods

Sampling site

The study was conducted in sites containing sugar and alcohol mills, private farms with soybean and corn crops and native Cerrado areas in the state of Goiás, Central-West Brazil (Table 2). We selected six sites of natural ecosystems (native Cerrado) and 18 agroecosystems considering type of vegetation cover (named as land use classes) and time of land use upper 10 years (six areas of soybean, six areas of sugarcane and six areas of corn crops). According to Köppen, climate is Aw corresponding to a rainy tropical climate. Data on surface temperature and precipitation to each sampling site were obtained in LAPIG-Maps platform. Data are described in Table 2.

Experimental design

Soils were collected on early December 2014 and January 2015 (rains) and on late June and July 2015 (drought). Samples were collected along a grid in each plot (5.0 x 5.0 m) to a depth of 10 cm. We collected three individual random samples within each plot and homogenized them to form one composite sample. This procedure was performed at all sampling sites, totaling six composite samples for sugarcane, six for soybean, six for corn and six for native Cerrado. Native Cerrado soils were collected in areas adjacent to or at least close to cropping sites.

Sample preparation and chemical characterization

Soils were sieved (<2 mm) for removing roots, twigs, stones and gravel, and packed into polyethylene bags at 4 °C. Soil moisture content was determined by drying 5 g of soil at 105 °C for 48 h. Chemical analyses and biological activity were carried out within 15 days of collection using soil dry mass as reference. Nutrient content (Ca^{2+}, Mg^{2+}, K^{+} and P), organic matter (OM), total organic carbon (TOC) and total nitrogen (TN) were estimated according to the methodology of EMBRAPA (2011).

Measurement of enzyme activities

We evaluated activities of five extracellular enzymes involved in cycling of carbon (α- and β-glucosidase), nitrogen (protease and aminopeptidase) and phosphorus (acid phosphatase) in soils. Monitoring of enzyme activity was performed in triplicate by colorimetric methods using specific substrates for each enzyme. The activity of α-glucosidase (EC 3.2.1.20), β-glucosidase (EC 3.2.1.21) and acid phosphatase (EC 3.1.3.2) was determined according to the method described by Baldrian et al. (2005). Soil samples (0.05 g dry mass) were incubated with specific substrate (p-nitrophenyl-α-D-glucopyranoside, p-nitrophenyl-β-D-glucopyranoside, p-nitrophenyl phosphate) at 40 °C for 60 min. We used the method of Allison and Vitousek (2005) with some modifications for determine glycine aminopeptidase activity (EC 3.4.11). Soil samples (0.1 g dry mass) were incubated at 37 °C for 60 min using 900 μL p-nitroanilide (pNA) (0.005 mol L^{-1}) prepared in 0.05 mol L^{-1} sodium acetate buffer (pH 5.0). The p-nitroaniline released during reaction was measured at 405 nm. Substrate volume was replaced by sodium acetate buffer (0.05 mol L^{-1}, pH 5.0) as control. We used the method of Ladeira et al. (2010) with modifications to evaluate protease activity (EC 3.4.2.21). Soil (0.05 g dry mass) was combined with 1.0 mL azocasein (0.2%, w/v) dissolved in Tris-HCl buffer (0.2 mol L^{-1}, pH 8.5). and this mixture was incubated at 37 °C. Reaction was stopped after 10 min by adding 0.5 mL 15% trichloroacetic acid (TCA w/v) and the mixture was centrifuged for 5 min (4000rpm/2000xg). To the supernatant was added 0.5 mL of NaOH solution (1.0 mol L^{-1}) and color developed was measured at 420 nm wavelength. Control differed by adding 5 mL TCA before substrate. With exception of protease, enzyme activity was determined by reference to a calibration graph and expressed in micromoles of product formed per gram of dry soil per hour of reaction (μmol g^{-1} h^{-1}). Protease activity was expressed in enzyme units (U) per gram per hour of reaction (U g^{-1} h^{-1}), defined as the amount of enzyme required to produce 0.1 increase in absorbance at 420 nm after 60 min assay. Total enzyme activity was also calculated for all soil sample sites. We used the sum of enzyme activity values related to the cycling of carbon (α- and β-glucosidase), phosphorus (acid phosphatase) and nitrogen (aminopeptidase glycine) grouped by land use (native Cerrado, sugarcane, soybean and corn). As we could not standardize protease activity to the same enzyme unit (μmol g^{-1} h^{-1}), we have decided to exclude this enzyme from analysis.

Soil microbial respiration

Microbial biomass carbon (C_{mic}) was determined according to the irradiation-incubation method (Ferreira et al., 1999). Results were expressed as microgram of carbon per gram of soil (μg g C^{-1}) and calculated as the difference between carbon released by irradiated and non-irradiated soil using conversion factor (Kc) = 0.45 (De-Polli and Guerra, 1996).

Statistical analyses

Two-way ANOVA was used to test the influence of sugarcane, soybean and corn, as well as periods of rains and drought on the hydrolase action. Data were analyzed

according to treatments in a factorial arrangement 4 x 2 x 6, with four types of land use (sugarcane, soybean, corn and native Cerrado), two periods (rainy and dry seasons) and six sampling sites (Table 1). The Tukey test was used to calculate differences among average activity of each enzyme in the four land use classes, two periods of the year and six sampling sites, considering $p < 0.05$.

Data on total enzyme activity were analyzed by multivariate analysis (one-way MANOVA) to check for the influence of land cover on the set of four enzyme activities. Means of total enzyme activity were compared *a posteriori* (Hotelling's T^2 test) considering $p < 0.05$.

The relationship between biological parameters (enzyme activity and C_{mic}) and chemical parameters (Ca^{2+}, K^{+}, Mg^{+2}, P, OM, TOC, TN) was identified by Pearson's correlation analysis, using the average of values of such attributes for four land use classes. Analyses were performed using the Assistat 7.7 software and graphs were plotted in the Sigmaplot version 12.0. We used canonical-correlation analysis (CCA) to compare the entire dataset for soil chemical properties and seasons, defined as environmental variables, and enzymatic activities of the different tillage systems, considered as dependent variables (Sant'Ana et al., 2015; Calil et al., 2016). We analyzed on the importance of predictor variables on enzyme activity through Pearson correlation analysis ($p<0.05$) between variables and axes summarized by CCA.

Conclusion

Despite land cover changes and tillage systems affecting soil biological activity and biochemistry, no alteration was detected by traditional soil analysis. Soil hydrolases may be useful in the early quality monitoring of these ecosystems as good soil quality indicators. Using this tool, we observed monoculture crop did not contribute to renewal of soil biological properties, which was determined by changes in the organic components of this system. Additionally, we observed effect of other attributes (seasonality or soil chemical components) on soil quality only when entire dataset was analyzed. This finding shows the importance of considering soil components under a global perspective to observe multiple relationships of those agents in this ecosystem.

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