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EFFECT OF BIOCHAR ON ENZYMATIC ACTIVITY IN A BRAZILIAN OXISOL

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ABSTRACT

The objective of this work was to assess the long-term effect of biochar application on enzyme activity in an Oxisol of Brazilian Cerrado. The experiment was conducted with a randomized block design consisting of the combination of two levels of fertilization (0 and 200 kg ha^{-1}, NPK 00-20-20 formulation) and five levels of biochar (0, 2, 4, 8 and 16 Mg ha^{-1}). The activity of urease, β-glucosidase, acid phosphatase, and total soil enzyme (hydrolysis of fluorescein diacetate - FDA) were evaluated in soil samples. The activity of urease and β-glucosidase were significantly increased by the application of biochar with and without chemical fertilization, respectively. Intermediate doses of biochar increased total soil enzyme activity. There was no effect of biochar on acid phosphatase activity. The application of biochar associated with chemical fertilization promotes urease, β-glucosidase and total soil enzyme activity.

Key words: Soil quality; Charcoal; Microbiology.

EFEITO OF BIOCHAR SOBRE A ATIVIDADE ENZIMATICA EM LATOSSOLO BRASILEIRO

RESUMO

O objetivo do trabalho foi avaliar o efeito de biochar sobre a atividade das enzimas em um Latossolo sob Cerrado no Brasil. O experimento foi conduzido em blocos casualizados consistindo de dois níveis de fertilização (0 e 200 kg ha^{-1} NPK 00-20-20) e cinco níveis de biochar (0, 2, 4, 8 e 16 Mg ha^{-1}). A atividade das enzimas urease, β-glucosidase, fosfatase acida e hidrolise de diacetato de fluoresceína – FDA, foi avaliada. As atividades da urease e β-glucosidase foram aumentadas pela aplicação do biochar com e sem fertilização química. As doses intermediarias do biochar aumentaram a atividade do FDA no solo. Não houve efeito do biochar sobre a fosfatase acida. A aplicação do biochar promove aumentada atividade das enzimas urease, β-glucosidase e a atividade enzimática total.

Palavras-chave: Qualidade do solo, carvão; Microbiologia

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INTRODUCTION

Growth of the cultivated area in the Brazilian Cerrado has placed this region as one of the most important agricultural regions in Brazil. Some factors have contributed to the rapid development of agriculture in the Cerrado, such as wide availability of land, predominance of favorable topography, and use of adapted crop varieties (Queiroz, 2009). Nevertheless, inadequate land management has brought about land degradation through soil erosion, organic matter losses, and compaction (Carvalho *et al.*, 2010), therefore reducing the productive potential of these areas.

On the other hand, the adoption of conservationist practices for increasing soil organic matter (SOM) content and soil microbial activity, through the introduction of cover crops and application of organic residues, are essential for the sustainability of production systems (Azadi *et al*., 2011). Recently, the use of carbonized biomass, known as biochar, formed by means of a pyrolysis process in which there is incomplete combustion (in a reduced oxygen environment) of plant biomass, has increased worldwide (Du *et al*., 2014; Mierzwa-Hersztek *et al*., 2016). Among the main benefits of biochar on soil properties are that this residue provides a suitable habitat for microorganisms, promoting their activity in the soil and affecting different microbial processes involved in nutrient cycling and dynamics of SOM (Jindo *et al*., 2012; Petter & Madari, 2012).

The activity of microorganisms can be measured through of the activity of enzymes that occurs in all intact and viable microbial cells (Gianfreda *et al*., 2005). Also, the activity of soil enzymes indicates biochemical functions and it may provide quantitative changes on SOM (Silva *et al*., 2012). Some important soil enzymes, such as dehydrogenase, hydrolysis of fluorescein diacetate and arylsulphatase, are involved in the biogeochemical cycles (C, N and S) and consequently, may reflect changes in the soil nutrient cycling (Karaca *et al*., 2011). Therefore, the activity of enzymes could be an useful indicator for evaluating the effect of biochar on soils under Cerrado.

The effect of different types of biochar on enzyme activity has been assessed in some previous studies (Mierzwa-Hersztek *et al.,* 2016; Du *et al.,* 2014). Mierzwa-Hersztek *et al.* (2016) have evaluated the application of poultry litter biochar in temperate soils and did not find effect on the activity of enzymes. Previously, Du et al. (2014) assessed the consecutive application of crushed corncob biochar, during 4 years in Nothern China and found variable responses of soil enzymes. However, information about the influence of biochar on biological properties in tropical soils are scarce. Also, it is unclear the effect of biochar produced with plant species of Cerrado on the activity of soil enzymes. Here we hypothesized that application of biochar produced with species of Cerrado could affect differently the soil enzymatic activity in the Brazilian Cerrado. Therefore, the aim of this study was to evaluate the effect of rates of biochar produced with species of Cerrado on the activity of urease, β-glucosidase, acid phosphatase and hydrolysis of fluorescein diacetate in a Cerrado Oxisol.

MATERIAL AND METHODS

The study was conducted in Nova Xavantina, MT (14°35'36" S and 52°24'04" W, altitude of 310m). The soil was classified as Oxisol of sandy clay texture. Before implementation of the experiment, we determined the following chemical and physical characteristics of the soil (0-0.20 m), using the methodology of Embrapa (2006): pH (H_2O) 5.6, P (Mehlich method): 67 mg dm^{-3}, K^+: 61.5 mg dm^{-3}, Ca^{2+}: 1.4 cmol$_c$ dm^{-3}, Mg^{2+}: 0.4 cmol$_c$ dm^{-3}; Al^{3+}: 0.13 cmol$_c$ dm^{-3}, H^+ + Al^{3+}: 4.98 cmol$_c$ dm^{-3}, V%: 27%; CEC: 6.93 cmol$_c$ dm^{-3}, OM: 12.6 g kg^{-1}, Fe: 75.0 mg dm^{-3}, Mn: 49.0 mg dm^{-3}, Zn: 45.0 mg dm^{-3}, Cu: 1.7 mg dm^{-3}; Clay: 307 g kg^{-1}; Silt: 73 g kg^{-1}; Sand: 620 g kg^{-1}.

The study area was native Cerrado forest until 1985. After removal of the forest, soybean was cultivated under a no-tillage system using millet as a cover crop until 2006. Then the present experiment was initiated, using a randomized block design, composed of two levels of fertilizer application (0 and 200 kg ha^{-1} of 00-20-20 formula of NPK chemical fertilizer, the latter representing the presence of 40 kg ha^{-1} P_2O_5 and 40 kg ha^{-1} K_2O applied to the soil), and five doses of biochar

(0, 2, 4, 8 and 16 Mg ha^{-1}) randomly distributed, with four replications. Each plot was 10 m long and 4 m wide, totaling 40.00 m^2; the useful area for evaluations was 25.00 m^2 since the edges were discarded.

The biochar was made via slow pyrolysis in a cylindrical metal kiln using temperatures around 400–500 ºC. The biochar from Cerrado species was finely ground to a particle size of ≤ 2 mm and applied only once in September 2006, being manually distributed and incorporated by a rotary hoe to a depth 0-0.15 m. A single point surface area of biochar was determined by the Brunauer, Emmelet and Teller (BET) nitrogen absorption method (Brunauer *et al.,* 1938), using nitrogen gas sorption analysis at 77.3 K (-195.9 ˚C). The specfic surface area (SSA) of the biochar applied, with a bulk density of 0.3 g cm^{-3}, was 2.9±0.4m^{2}g^{-1}.

The elemental composition of it is presented in **Table 1**. After the incorporation of biochar, millet (*Pennisetum* sp.) was sown as a cover crop, which was desiccated two months later, followed by planting of soybeans (**Table 2**).

Table 1. Elemental composition (total values) of vegetable biochar used in this experiment.

Tabela 1. Composicao elementar (valores totais) do biochar utilizado neste experimento.

Total Nitrogen (N)		6.6
Phosphorus (P_2O_5 citric acid)	g kg^{-1}	0.3
Phosphorus (P_2O_5 total)		1.0
K_2O		3.3
CaO		5.7
MgO		1.1
Sulfur (S)		0.4
Copper (Cu)		7.0
Zinc (Zn)		13.0
Molybdenum (Mo)	mg kg^{-1}	2.0
Cobalt (Co)		1.0
Boron (B)		5.0
Total Carbon (C)		490.6
Moisture	g kg^{-1}	50.0
Total mineral material		280.0
Ratio C:N		74.3

For the present study, soybean planting was done on 5 December 2012. Twelve seeds were distributed per meter, with the spacing of 0.45 m between rows and a depth of 0.02-0.03 m. In

Table 2. Timeline of crop sequence, fertilization and cultivation in the field experiment in Nova Xavantina, Mato Grosso State, Brazil.

Tabela 2. Cronologia das culturas, fetilizacao e cultivo no campo experimental em Nova Xavantina, Mato Grosso do Sul, Brasil.

	Sowing	Cover crop	Synthetic fertilizer	Crop	Synthetic fertilizer	Other practices
2006 (Y0)	Sep, 2006	Biochar applied - Sept 29, 2006*** *Pennisetum glaucum* 15 kg ha^{-1} seeds MT	Not applied	*Glycine max* (GM) ZT	Experiment****	Chemical control for pests**
2008 (Y2)	Mar, 2008	*Urochloa ruziziensis* 10 kg ha^{-1} seeds MT	Not applied	*Glycine max* (GM) ZT	Experiment****	Chemical control for pests**
2009 (Y3)	Apr, 2009	*Urochloa ruziziensis* 10 kg ha^{-1} seeds MT	Not applied	*Glycine max* (GM) ZT	Experiment****	Chemical control for pests**
2010 (Y4)	Sep, 2010*****	*Urochloa ruziziensis* 10 kg ha^{-1} seeds MT	Not applied	*Glycine max* (GM) ZT	Experiment****	Chemical control for pests**
2011 (Y5)	Sep, 2011*****	*Urochloa ruziziensis* 10 kg ha^{-1} seeds MT	Not applied	*Glycine max* (GM) ZT	Experiment****	Chemical control for pests**
2012 (Y6)	Apr, 2012	*Urochloa ruziziensis* 10 kg ha^{-1} seeds MT	Not applied	*Glycine max* (GM) ZT	Experiment****	Chemical control for pests**

ZT: zero tillage, the main crops were sown directly through the mulch of the cover crop, the synthetic fertilizer was applied in the same operation as sowing, into the furrow. *Glyphosate acid (1080 g ha^{-1}) and 2,4-D acid (242 g ha^{-1}); ** Trifloxystrobin + cyproconazole (66 + 28 g ha^{-1}), tebuconazole (100 g ha^{-1}) and methamidophos (420 g ha^{-1}); *** Biochar doses were applied once: 0, 2, 4, 8, 16 Mg ha^{-1}; **** P-K (20-20) synthetic fertilizer doses: 0 e 200 kg ha^{-1}; ***** In 2010 and 2011, due to insufficient precipitation in March and April the *U. ruziziensis* was sown only in September, when the rainy season began.

this same operation, the fertilization was done. The soybean cultivar used was TMG 108 RR®.

The evaluations for this study were performed in the sixth year following implementation (2012/2013 season), i.e. the effect on medium to long-term application of biochar. Soil sampling was conducted in February 2013, at the time of soybean flowering, at a depth of 0-0.10 m. In each plot, three simple samples were taken to form a composite, with the help of an auger, respecting the useful area of the plot. After collection, the samples were homogenized, milled and sieved (0.177 mm). For enzymatic activity, the samples were placed in plastic bags and transported to the laboratory where they were homogenized, sieved (2 mm) and refrigerated at 4°C, at field humidity, until the time of analysis.

We determined the potential activities of three soil enzymes: urease, β -glucosidase, acid phosphatase, and total enzymatic activity (based on hydrolysis of fluorescein diacetate - FDA), according to the methods described by Tabatabai (1994). These methods are based on the colorimetric determination of p-nitrophenol (yellow color) formed after the addition of colorless substrate specific for each enzyme measured. For each soil sample analytical replicates were performed in the laboratory. The soil enzymatic activity is expressed in μg p-nitrophenol h^{-1} g^{-1} dry soil. For the determination of urease, β -glucosidase, acid phosphatase, and the total enzymatic activity (hydrolysis of fluorescein diacetate) the substrates used were 0.2 M urea solution, 0.05M p-nitrophenol-β-D-Glucopyranoside (0.05 M PNG), 0.05 M p-nitrophenol phosphate (0.05 M PNP) and 100 μl 2.4 M – diacetylfluorescein, respectively. For total enzyme activity absorbance was read at 490 nm. The results were analyzed using the statistical programs Sisvar 5.1 (Ferreira, 2011) and SigmaPlot 10.0 (SPSS, 2001) using multiple regression in which the independent variables were the chemical fertilizer (NPK) and biochar and dependent variables were the enzyme activity.

RESULTS AND DISCUSSION

The application of 16 Mg ha^{-1} of biochar associated with the application of chemical fertilizers induced higher urease activity (**Figure 1A**). This result is due to the highest input of N and also biomass that stimulated urease activity with higher availability of easily decomposed organic N (Saya-Cork *et al.,* 2002). In addition, biochar can serve as substrate (N-biochar) and microhabitat for soil microorganisms (Jindo *et al.,* 2012), protecting urease against the action of other compounds naturally present in the soil and maintaining the activity for a longer period of time.

For β-glucosidase, the significant effect of biochar was found in the treatments without chemical fertilization (**Figure 1B**). There was a significant difference when comparing the means as a function of fertilization. In the treatments without chemical fertilization, the response curve behaved in a linear way, demonstrating an increase in β-glucosidase activity with increasing doses of biochar. These results differ from those obtained by Bailey *et al.* (2011), who found no significant variability in the activity of β-glucosidase with the application of biochar. The main reason for this different result may be related to the difference in the time between application of biochar and determination of β-glucosidase activity. In our study, the activity of β - glucosidase was determined in long-term (7 years), while Bailey *et al.* (2011) evaluated β - glucosidase activity in short-term (less than 1 year). This fact determines the magnitude of response of β-glucosidase to the application of biochar, since the release of phenolic compounds derived from pyrolysis of the biochar reduces over years. According to Warnock *et al.* (2010), phenolic compounds have the power to inhibit the enzyme β-glucosidase. Hence the importance of evaluating the dynamics of biochar in the long term.

The greater availability of plant residues as a function of chemical fertilization may have minimized the direct effect of biochar on the β-glucosidase activity, since these residues are readily available for the decomposition process. Non-pyrolysed plant residues can afford higher β-glucosidase activity compared to those in which there was burning of vegetation, due to the aromatic structures present in the pyrolyzed materials. These results indicate that in soils with higher levels of organic matter, the values of β-glucosidase activity are higher, as evidenced in this

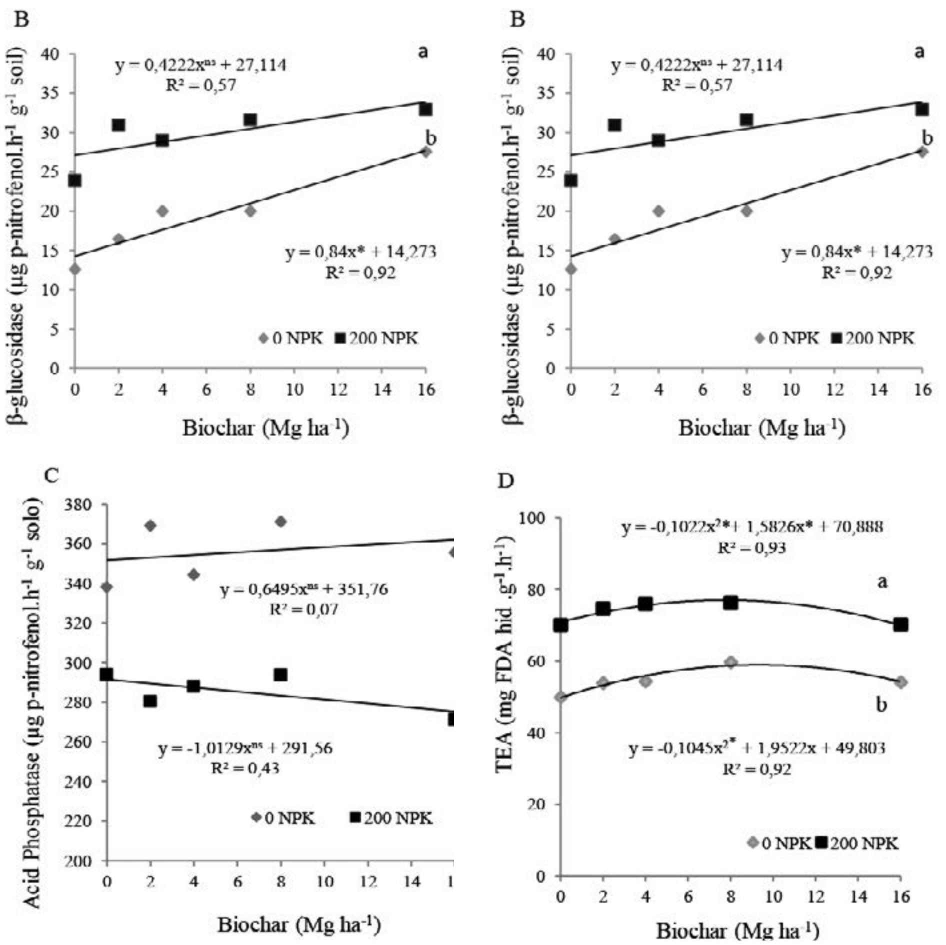

Figure 1. Values of the enzymes urease (A), β-glucosidase (B), acid phosphatase (C), and total enzyme activity (D) at a depth of 0-0.10 m in Oxisol, for different doses of biochar, with NPK fertilization of 0 and 200 kg ha^{-1}, in Nova Xavantina, MT, 2012. *Significant at 5% probability by "t" test for biochar. ^{ns} not significant.

Figura 1. Valores das enzimas urease (A), β-glucosidase (B), fosfatase acida (C) e atividade enzimática total (D) nas profundidades 0-0.10 m em um oxisol, para diferentes doses de biochar, com fertilização NPK de 0 e 200 kg ha^{-1}, em Nova Xavantina, MT, 2012. *Significante a 5% de probabilidade pelo teste "t" para biochar. ^{ns} não significante.

study, thereby showing a positive correlation of TOC with the activity of β-glucosidase (Turner *et al*., 2002).

There was no significant effect of application of biochar on acid phosphatase activity, which was influenced only by chemical fertilization (**Figure 1C**). The fertilization reduced the activity of acid phosphatase compared to treatments without fertilization. According to Gatiboni *et al.* (2008), the greater the availability of soluble P in the soil, after chemical fertilization, decreases the activity of acid phosphatase, since this enzyme is released by plants and microorganisms when there is a low availability of soluble P in soil.

Regardless of the presence or absence of chemical fertilization, biochar significantly affected total enzymatic activity (**Figure 1D**). In both treatments, the response curve showed a quadratic response, with the highest average recorded in the presence of chemical fertilization. These results agree with Lammirato *et al*. (2011) who also found quadratic response of total enzymatic activity due to the application of biochar. This behavior with elevated quantities of biochar may be associated with increased release, even if slowly, of soluble C-compounds which can be aromatic, aliphatic or carboxylic, and which have the potential ability to inhibit the enzyme activity in the soil solution (Smith *et al.,* 2010).

CONCLUSION

In conclusion, the application of biochar produced with species of Cerrado promoted increased activity of urease and β-glucosidase and total enzyme activity. As these enzymes are involved in important nutrient cycles and are indicators of soil microbial activity, biochar may be a suitable option to ameliorate the land degradation and improve the soil quality.

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