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Microbial biomass and organic matter in an oxisol under application of biochar

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ABSTRACT: The aim of this study was to investigate the short- and medium-term effect of biochar on soil microbial properties, organic carbon and nitrogen in an Oxisol from Brazil. The experiment was conducted in a randomized block design consisting of five levels of biochar (0, 2, 4, 8 and 16 Mg·ha^{-1}) with and without of synthetic fertilizer: 0 and 200 kg.ha^{-1} of synthetic fertilizer N-P-K (00-20-20). The following soil properties were determined: microbial biomass carbon (MBC) and nitrogen (MBN), microbial respiration (MR), metabolic quotient (qCO_2) and microbial quotient (qMIC), total organic carbon (TOC) and nitrogen (TN). The MBC, qCO_2 and carbon management index (CMI) were not altered by doses of fertilizer and biochar. The presence of biochar reduced the MBN by 11% and 5% with the application of 16 Mg·ha^{-1} compared to control in the third and sixth year, respectively. The qMIC was reduced exponentially with the application of biochar, but was within normal limits as a proportion of total organic carbon. There was an increase in TOC and TN with the application of biochar. The use of biochar did not cause significant negative changes in soil microbial properties or contribute to carbon sequestration in the soil.

Key words: microbial activity, soil organic carbon, metabolic quotient, Brazilian soil.

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INTRODUCTION

In tropical soils from Brazil, the maintenance and enhancement of carbon (C) stocks is challenging, since the decomposition of organic C by soil microorganisms is fast and stimulated by high temperatures and soil moisture (Santos et al. 2011; Leite et al. 2014). The adoption of suitable soil management practices becomes important to provide regular inputs of organic C and, therefore, increase C stocks. This strategy improves the soil chemical, physical and biological properties in the long-term. Also, the increase of labile-C stock is essential as this fraction promotes soil microbial activity over time (Guimarães et al. 2013). It is particularly important, as native vegetation has been replaced by pasture and cropland causing significant changes in the soil microbial properties and organic C dynamic (Carvalho et al. 2010). Recently, the use of carbonized biomass, known as biochar, in the soil has contributed to the stability of organic C and improved soil microbial properties (Hernandez-Soriano et al. 2016).

The improvement of soil microbial properties is important because soil microbes play several roles in essential ecosystem functions (Rodrigues et al. 2013). Specifically, soil microbial biomass (SMB) is responsible for organic matter dynamic and nutrients cycling (Balota and Auler 2011). Therefore, the study of SMB is particularly important because it is a useful ecological indicator that can be used to assess native soils as well as different soil management strategies for cropland (Araújo et al. 2008) and pastures (Lopes et al. 2010), such as the addition of biochar (Zhang et al. 2014). Also, the improvement of soil microbial properties and organic matter is important due to their influence on soil chemical and physical properties, and consequently, on soil productivity (Araújo et al. 2008).

Nonetheless, biochar may possess different properties as it can be obtained from various sources through a pyrolysis process that produces a stable and aromatic pyrogenic C (Petter and Madari 2012; Chintala et al. 2014). Although biochar can possess different properties, some studies have concluded that this product may improve soil properties and the soil organic matter (SOM) status (Liu et al. 2012; Biederman and Harpole 2013). Studies focusing on the effect of biochar amendments on SMB have shown contrasting responses, being positive (Luo et al. 2013; Zhang et al. 2014), negative (Dempster et al. 2012) or neutral (Castaldi et al. 2011). Contrasting results may occur because of the different characteristics of biochar, such as recalcitrance (Kuzyakov et al. 2009). Thus, knowledge gaps exist with respect to the effect of biochar amendment on soil microbial properties in tropical soils, given that biochar has a high C/N ratio and aromatic and molecular stability, which may make it more resistant to microbial degradation. Therefore, we evaluated the short- and medium-term effect of biochar amendments on soil microbial properties and organic C dynamics in a Cerrado Oxisol.

MATERIAL AND METHODS

Study area

The experiment was conducted in Nova Xavantina (14°34' S, 52°24' W, at 310 m altitude), Mato Grosso, Brazil, during the 2011/2012 and 2014/2015 periods in a dystrophic Oxisol. The regional climate is Aw according to the Köppen global climate classification, with two distinct seasons, consisting of a dry season (May to September) and a rainy season (October to April).

Biochar

The biochar was derived from the plants species *Qualea grandiglora*, *Qualea parviflora*, *Qualea multiflora* and *Tachigali vulgaris* found in Cerrado stricto sensu. It was produced in cylindrical furnaces by slow pyrolysis, in temperatures ranging from 200 to 450 °C at the initial and final stages of carbonization, respectively. It was subsequently grounded to particle size ≤ 2 mm, applied only once in September 2006, by manual distribution, and incorporated to a 0 to 15 cm depth using a rotary tiller. The surface area of biochar was determined by means of sorption isotherms/physical desorption of nitrogen on the sample surface by the method of Brunauer, Emmelet and Teller (BET) (Brunauer et al. 1938) at 77.3 K (–195.9 °C). The specific surface area was 4.6 ± 0.4 $m^2 \cdot g^{-1}$, with a density of 0.4 $g \cdot cm^{-3}$. The chemical composition of biochar is shown in Table 1.

A sample of biochar underwent NMR analysis of ^{13}C (^{13}C-NMR) in order to verify the functional groups. The samples were analyzed by variable-amplitude cross polarization (STC) ^{13}C solid state nuclear magnetic resonance (NMR), using a Varian 500 MHz spectrometer at frequencies of 125 and 500 MHz for ^{1}H and ^{13}C, respectively. The experiments were performed using a rotation magic angle (MAS) →

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

Total Nitrogen (N)[1]	g·kg^{-1}	6.6
Phosphorus (P_2O_5citricacid)	g·kg^{-1}	0.3
Phosphorus (P_2O_5 total)	g·kg^{-1}	1.0
K_2O	g·kg^{-1}	3.3
CaO	g·kg^{-1}	5.7
MgO	g·kg^{-1}	1.1
Sulfur (S)	g·kg^{-1}	0.4
Copper (Cu)	mg·kg^{-1}	7.0
Zinc (Zn)	mg·kg^{-1}	13.0
Molybdenum (Mo)	mg·kg^{-1}	2.0
Cobalt (Co)	mg·kg^{-1}	1.0
Boron (B)	mg·kg^{-1}	5.0
Total Carbon (C)[1]	g·kg^{-1}	490.6
Moisture	(%)	5.0
Total Mineral Material	g·kg^{-1}	2800
Ratio C:N	-	74.3

[1] Determined by the Dumas method using an elemental analysis; the other elements were determined using a methodology for analysis of soils.

of 14 kHz, with cross-polarization time of 1 ms, acquisition time 15 ms, recycle delay of 500 ms and high power two-phase modulation and pulse proton decoupling of 70 kHz. The cross-polarization time was chosen after the experiments of variable dwell time and late CP recycling experiments were chosen to be greater than five times the longest spin relaxation ^{1}H-structure (T^1H) as determined by inversion recovery experiments. The spectra were processed with Gaussian apodization (gf = 0.004 s). There was virtually no presence of aliphatic structures (Alkyl generally ~100-0 ppm) or O (~70 ppm) and di-O-Alkyl (~105 ppm) that would come from the pulp, and there was no indication in the spectrum of methoxyl groups (~56 ppm) from lignin. The spectrum showed a clear signal of aromatic groups (C=C, ~130 ppm), responsible for the stability of the material. Although in lower proportion than the aryl groups, there was a presence of phenolic groups (~150 ppm) in the spectrum, responsible for the chemical reactivity of biochar (Petter et al. 2016).

Analysis of biochar and soil

The properties of both biochar and soil were assessed according to different methods. The pH was determined using the electrode method (Thomas 1996) soil and water 1:2.5 ratio. P, Ca, Mg and K were extracted by diluted concentration of strong acids (0.05 mol·L^{-1}HCl + 0.0125 mol·L^{-1} H_2SO_4; Mehlich I). Phosphorus was determined by the colorimetric method (Silva 2009), Ca and Mg were determined by atomic spectroscopy and K by flame emission spectrometry (Wright and Stuczynski 1996). Aluminum was extracted using potassium chloride solution and titrated with sodium hydroxide, according to Bertsch and Bloom (1996) modified by Silva (2009). Potential acidity (H + Al) was determined by extracting with 0.5 mol·L^{-1} calcium acetate solution at pH 7.1 to 7.2, and titrating with 0.025 mol.L^{-1}NaOH, using 10 g·L^{-1} phenolphthalein as indicator according to Silva (2009). Cation exchange capacity was obtained through the sum of Ca, Mg and K (Faithfull 2002). Specifically, soil texture was measured with a standard hydrometer that had the Bouyoucos scale (Gee and Bauder 1996), and the texture was identified as sandy loam. SOM was determined by the Walkley-Black method (Nelson and Sommers 1996).

Experimental design

The experimental site was under native Cerrado vegetation until 1985. After that, soybean was cultivated under a no-tillage system, using millet as a cover crop. In 2006, the experiment was started in a randomized four blocks design, composed of five levels of biochar: 0, 2, 4, 8 and 16 Mg·ha^{-1} (equivalent to 0, 1, 2,4 and 8 Mg·ha^{-1}C) with and without of synthetic fertilizer N-P-K (00-20-20) 0 and 200 kg·ha^{-1}. The plots were 10 m long and 4 m wide (40 m^2); the useful area for evaluation was 25 m^2. In this study, soybean was planted in December 2008 and 2011(only 2 of the 5 crops, were part of the experiment). The crop residues after harvest season were deposited on the soil, removing only the grains. The soybean cultivation was in the rainfed system, and during the seasons 2008/2009 and 2011/2012 there were periods of water stress. The plants were spaced 45 × 3 cm. Table 2 shows plant production data during the six years after the biochar application.

Soil organic C, N and microbial properties

Soil sampling was done in February 2012 (third year) and 2015 (sixth year) during soybean flowering, at a 0 to 15 cm depth. The crop residue deposited on the soil surface was removed before sampling. In each plot, three simple samples were taken to form a composite sample. Soil samples were immediately stored in sealed plastic bags and transported in →

Table 2. Soybean dry biomass after six years of biochar application in the soil.

Biochar (Mg·ha^{-1})	Dry biomass (kg·ha^{-1})*						%
	2006/2007	2008/2009	2009/2010	2010/2011	2011/2012	Accumulated	
0	1.820	3.573	8.690	11.823	13.543	13.543 c**	-
4	1.873	3.730	8.968	12.354	14.783	14.783 b	+9.15
16	2.153	4.239	10.077	14.039	16.992	16.992 a	+25.4

*Dry biomass aerial part without the grains; ** different letters mean significant differences at 5% probability by Tukey.

an ice box to the laboratory, where analyzes have already started. A portion of the soil samples (300 g) was stored in bags and kept at 4 °C for microbial analysis, and another portion (200 g) was air-dried, sieved (0.177 mm mesh) and homogenized for chemical analyses.

Total organic C (TOC) and nitrogen (TN) were determined by the Dumas method (dry combustion at high temperature) (Chintala et al. 2013) using an elemental analyzer (Perkin Elmer 2400 Series II CHN/O). The levels of soil microbial biomass C (MBC) and N (MBN) were determined according to the methods developed by Joergensen and Brookes (1990) and Brookes et al. (1985), respectively, with 0.5 mol·L^{-1} K_2SO_4 extraction of the organic C and total N contents from fumigated and un fumigated soils. The coefficients of extraction (0.38 and 0.45) were used to convert the differences in C and N contents between the fumigated and unfumigated soils to microbial C and N, respectively. The soil basal respiration was monitored through daily measurement of CO_2 evolution under aerobic incubation at 25 °C for 7 days, with the moisture in the samples corrected for 70% of field capacity (Anderson 1982). The microbial quotient was calculated by the ratio of MBC to TOC (Sparling 1992) and the respiratory quotient (qCO_2) was determined by the ratio of soil basal respiration to MBC (Anderson and Domsch 1993).

Statistical analysis

The results were analyzed with the statistical program Sisvar 5.1, using regressions where the independent variable was biochar and the dependent variables were the soil properties (TOC, TN, MBC, MBN, respiration, and microbial metabolic quotient). The significance of the angular coefficients by t-test was used as a prerequisite in the selection of the regression equation. The regression analyses allowed verifying the isolated effect of biochar as an independent variable on the parameters analyzed.

RESULTS AND DISCUSSION

Although this study included two levels of P and K, the results have shown a direct and significant effect of applying biochar on soil biological properties. In this case, the main discussion is related with the biochar effect soil microbial biomass and activity. Thus, the application of biochar did not show a positive or negative response on MBC, which suggests that the application of biochar, in the long-term, did not influence the increase or decrease of microbial biomass C (Fig. 1a). On the other hand, the increase in biochar rates resulted in a reduction on MBN (Fig. 1b) indicating that biochar residues residue may promote losses of N from microbial biomass.

Soil microbial biomass may respond positively or negatively to the application of chemical fertilizers, according to their N and C sources; to organic fertilizers due to their chemical and organic properties, in particular the C/N ratio. Soil microbial biomass C did not vary according to the different treatments with biochar, in the long-term, which may be related to the characteristic of biochar, e.g. molecular structure, high aromaticity and C/N ratio. It happened probably because the fraction of C easily degradable from biochar is degraded shortly and in the medium to long-term there is no more degradable C for soil microbial biomass, as also reported by Shenbagavalli and Mahimairaja (2012), who did not find an effect of biochar on soil microbial biomass after 90 days of application.

The decrease in MBN observed with biochar amendment suggests that biochar does not act as an N source for soil microbial biomass. Thus, the application of biochar probably promoted N immobilization and consequently resulted in N limitation for microbial growth. Our finding agrees with Zhang et al. (2014) who found that biochar amendments decreased microbial biomass N by 7 to 10% as compared to unamended soil.

Some previous studies have also shown the long-term stability of biochar since soil microbial biomass is not able →

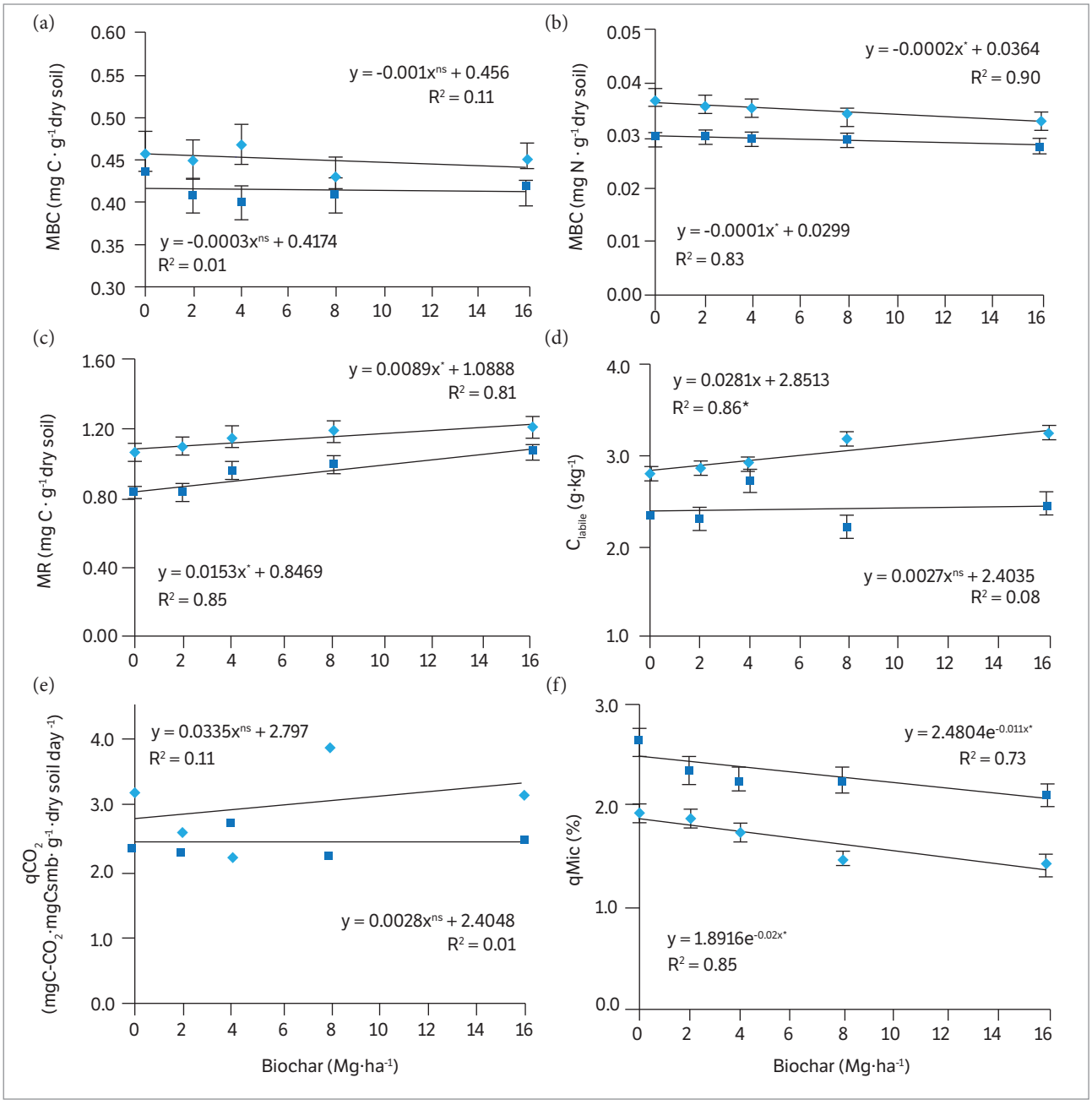

Figure 1. (a) Carbon and (b) nitrogen of the soil microbial biomass, (c) microbial respiration, (d) labile carbon, (e) metabolic quotient and (f) microbial quotient at a depth of 0 to 15 cm in an Oxisol in the third (♦) and sixth (■) year after the application of biochar in Nova Xavantina, Mato Grosso, Brazil. ^{ns} non significant; * significant at 5% probability by Student "t" test for biochar. (Mean values n = 4 replicates and standard error).

to easily degrade its aromatic C (Lehmann et al. 2011; Petter et al. 2012; Chintala et al. 2015). There would be some possible reasons for these results: i) other chemical and physical properties of biochar contribute to its stability, such as specific surface area (4.6 $m^2 \cdot g^{-1}$), presence of functional groups and aromatic structure, ii) specifically, the oxidation of aromatic structures of biochar over time showed a linear relationship between the dose and the components of oxidized biochar, suggesting that just three years after biochar application, a small proportion was already oxidized in the form of poly-condensed aromatic structures with carboxylic functionality. However, a large proportion of the applied biochar is maintained unchanged in the humin fraction as reported by Bruun and El-Zehery (2012). Also, the higher amount of fresh labile biomass from biochar may explain the largest degradation of labile-C during the first years of

application. This can also be evidenced by increased levels of labile-C in the third year and no effect in the sixth year after application.

The application of biochar increased soil respiration (Fig. 1c), while it did not influence labile-C in the sixth year (Fig. 1d). However, there was an effect of biochar on the C-labil levels until the third year (Fig. 1d), which is possibly due to labile-C coming from the biochar pyrolysis process in the form of condensable compounds. Although the soil respiration increased with biochar amendment, it did not influence the respiratory quotient (qCO_2), which did not vary in the evaluated years (Fig. 1e). Therefore, the observed high soil respiration possibly reflected the biological activity of the soil in response to biochar amendment. On the contrary, the application of biochar promoted a decrease in the microbial quotient (qMic) and the response was explained through an exponential model (Fig. 1f).

The greater soil respiration, as soil biological activity, may support the explanation that labile-C was degraded (coming from the biochar pyrolysis process in the form of condensable compounds) by soil microorganisms during the first three years. Also, the aromaticity and molecular stability of biochar explain the absence of soil respiration response during the sixth year, since the labile-C was already degraded within the first years. The linear effect of biochar rates on microbial respiration seems to be related with some stress factor responsible for the loss of C from the soil and, thus, it did not favor an increase in MBC. In the evaluation years there were periods of water stress, which may have contributed to these results.

The qCO_2 represents the specific rate of respiration of the microbial biomass, and generally is used to indicate if the microbial population is oxidizing carbon from its own cells (maintenance respiration), i.e., reflecting a stress condition (Islam and Weil 2000). However, the qCO_2 can also serve as a predictor of the changes in the decomposition of organic matter (Anderson and Domsch, 1993). usually the fresh carbon amendments, such as C from crop straw and manure, selects for fast growing microbes, increasing the qCO_2. On the other hand, biochar contains recalcitrant C sources and, thus, its effects on qCO_2 may be insignificant as also reported by Liu et al. (2016), who observed that biochar amendments did not affect the qCO_2 across different Chinese agricultural soils.

The reduction of qMIC was not proportional to the dose of biochar applied, which is positive in the long-term because, despite the reduction in qMIC, the values remained above 2%, considered within the normal range by Jenkinson and Ladd (1981), which is 1 to 4% of TOC. High qMic values indicate elevated availability of organic C for soil microbes and active organic matter (Sampaio et al. 2008).

The application of biochar resulted in an increase in TOC content after three and six years (Fig. 2), while TN was influenced ($p < 0.05$) by applying biochar only in the sixth year after application (Fig. 3). Specifically, the application of 16 $Mg \cdot ha^{-1}$ of biochar resulted in TN 10% higher than the control without biochar, equivalent to 150 $kg \cdot ha^{-1}$ in absolute values.

The increase in TOC content agrees with Petter et al. (2012), who observed linear and significant increase in TOC after application of biochar in Cerrado soil. The molecular stability of biochar had great influence on the TOC, since the decomposition of this material is slower and its presence, together with the annual higher input of plant residues to the soil, likely contributed significantly to the increase in TOC inventories.

The larger accumulation of TN in the sixth year is attributed to the slow release of N from biochar that increased over six years. Despite a high C/N ratio (74:1) and molecular stability, partial oxidation of biochar may have contributed to the retention of N (N-biochar) originating from decomposition of organic material contributed over the years, since N release is slow, as mentioned by De La Rosa and Knicker (2011). According to these authors, as biochar degrades, N is slowly available in a form that plants can use, thus contributing to the reduction of N losses.

The increase in TN is reported in the literature as related to the improvement of soil quality. Therefore, it is possible to verify that the soil management program used in this study (soybean + biochar) resulted in the maintenance of microbial activity at normal levels, similar to other agricultural systems, such as crop-livestock integration (Souza et al. 2009). These authors reported that the addition of organic waste caused changes in the soil, such as increased aggregation and, consequently, greater protection of organic matter, as well as a reorganization of soil structure, promoting lower N losses. In addition, the direct organic matter protection provided by biochar through fresh organic matter sorption →

the biochar surface and also protection provided inside the biochar porous system could have contributed to these results.

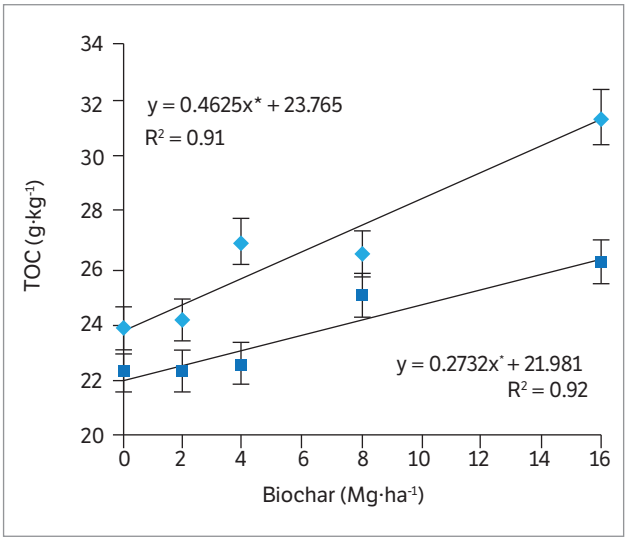

Figure 2. TOC content at 0 to 15 cm in depth in an Oxisol in the third (♦) and sixth (■) year after the application of biochar in Nova Xavantina, Mato Grosso, Brazil. ns non significant; * significant at 5% probability by Student "t" test for biochar. (Mean values n = 4 replicates and standard error).

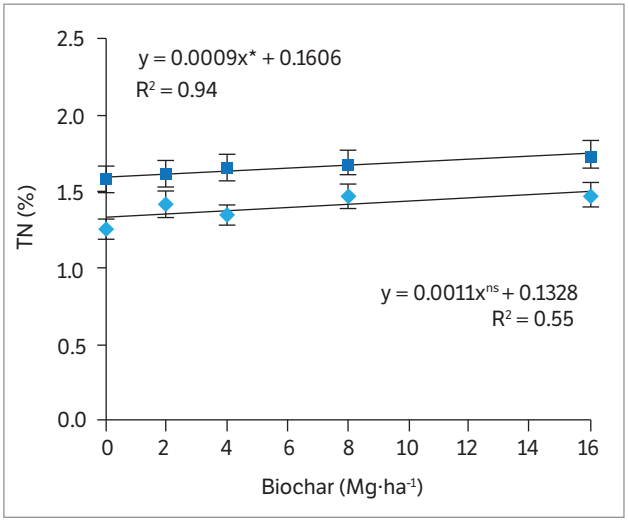

Figure 3. Total stocks of nitrogen at a depth of 0 to 15 cm in an Oxisol in the third (♦) and sixth (■) year after the application of biochar in Nova Xavantina, Mato Grosso, Brazil. ns non significant; * significant at 5% probability by Student "t" test for biochar. (Mean values n = 4 replicates and standard error).

CONCLUSION

Microbial properties have shown different responses after six years of biochar application. Soil microbial biomass C was not influenced, while microbial biomass N decreased six years after biochar application. Although microbial biomass was not positively influenced by biochar, the application of this product increased the soil organic carbon content. It is an important finding since biochar application could improve the soil microbial properties and organic matter status. This study did not find an optimal biochar rate for using in soil, but further studies should be done to find the optimal rate and also verify the effect of biochar on soil properties and plant yield in long-term.

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