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## Impact of coffee biochar on soil carbon, microbial biomass and enzymatic activities in Semiarid Sandy soil cultivated with maize

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### ABSTRACT

The use of biochar can bring benefits such as long-term carbon sequestration and water use efficiency, being an alternative for semiarid regions. The objective of this study was to evaluate the impact of coffee biochar applied in sandy Entisol on maize growth, and soil chemical, microbial and biochemical attributes. The experimental design was completely randomized, distributed in a 2 x 4 + 1 factorial scheme, two types of wastes coffee ground (MCG) and coffee husks (MCH), four doses (4, 8, 12 and 16 Mg ha<sup>-1</sup>) and control (CONT), without biochar. All treatments received organic fertilization with manure. Coffee biochar applied to sandy soil cultivated with maize, as predicted, increased the total soil C content, mainly coffee husk biochar that increases twice the C content compared to the control. Nutrient and some enzyme activities of soils were improved with increasing coffee biochar doses. Microbial biomass of soils amended with the biochars MCH16 and MCH12 showed an increase of 100 and 116%, respectively. This study demonstrated a clear response of the soil nutrients, microbial community and enzyme activities related to types and doses of biochar. Therefore, the use of coffee biochar is recommended for increasing the quality of sandy soils in the Brazilian semi-arid.

Keywords: *Zea mays*, extracellular soil enzyme, soil total organic carbon, soil microbial biomass.

## Impacto do biochar de café sobre o carbono, biomassa microbiana e atividades enzimáticas de solo arenoso do semi-árido, cultivado com milho

### RESUMO

O uso do biochar pode trazer benefícios como o sequestro de carbono a longo prazo e a eficiência do uso da água, sendo uma alternativa para regiões semi-áridas. O objetivo deste estudo foi avaliar o impacto da aplicação de biochar oriundos de resíduos de café aplicado em solo arenoso sobre o crescimento de milho, atributos químicos, microbianos e bioquímicos. O delineamento experimental utilizado foi inteiramente casualizado, distribuído em esquema fatorial 2 x 4 + 1, sendo dois tipos de resíduos de café: borra (MCG) e casca de café (MCH), quatro doses (4, 8, 12 e 16 Mg ha<sup>-1</sup>) e controle (CONT), sem biochar. Todos os tratamentos receberam adubação orgânica com esterco bovino. O biochar de café aplicado em solo arenoso cultivado com milho, conforme previsto, aumentou o teor total de C no solo, principalmente o biochar de casca de café, que aumentou em dobro do teor de C, comparado ao controle. Os teores de nutrientes e algumas atividades enzimáticas dos solos foram aumentados com o aumento das doses de biochar de café. Solos que receberam os tratamentos MCH16 e MCH12 aumentaram a biomassa microbiana em 100 e 116%, respectivamente. Este estudo demonstrou uma clara resposta dos nutrientes do solo, comunidade microbiana e atividades enzimáticas relativos aos tipos e doses de biochar. Por isso, recomenda-se o uso do biochar de café para o aumento da qualidade de solos arenosos no semi-árido brasileiro.

Palavras-chave: *Zea mays*, enzima extracelular do solo, carbono orgânico total, biomassa microbiana do solo.

## Introduction

Caatinga is the third largest drylands ecoregion and are characterized by scarce rainfall concentrated at short intervals of time, strong sunshine and predominance of high temperatures (dos Santos et al., 2019). To improve the retention of water, structure and favors a greater aggregation and cohesion between soil particles under limited irrigation, the producers have applied organic matter. Alternatively, researchers have recommended the use of biochar produced from agroindustrial waste to compensate the limited water resources and to increase the water use efficiency (Lima et al., 2018).

Biochar is the result of thermochemical conversion through pyrolysis of biomass (Oliveira et al., 2017) subjected to high temperatures under limited O<sub>2</sub> conditions (Zhang et al., 2017). The product has minerals, volatile organic compounds and the presence of free radicals. These materials can change the soil microbial activity and enzymatic activity that catalyzes several processes related to biogeochemical cycles (Zhu et al., 2017).

In addition, the use of biochar provides other benefits to soil and environment such as long-term carbon sequestration, contributing to the mitigation of climate change effects (Bird et al., 2017). With high specific surface area and organic C content, the biochar can increase soil nutrient, cation exchange capacity, improves the fertility of soil, and crop production (Agegnehu et al., 2016).

Biochar applied to soil may result in changes of the structure of the microbial community (Dangi et al., 2020) and of many soil enzymes related to microbial properties (Foster et al., 2016). Studies reported that the addition of biochar increased soil enzyme activities due to microbial proliferation, and besides caused immobilization and protection against degradation and denaturation of enzymes (Khadem and Raiesi, 2017; Chen et al., 2020).

Reports showed that biochar application increased the content of enzymes related to N and P cycle and reduced the enzyme related C cycle (Yadav et al., 2019; Wu et al., 2020), although other reports were inconsistent (Song et al., 2016). Biochar properties may vary according to the source of biomass, type and application rate, soil type, and nutrient content (Li et al., 2017; Lima et al., 2018).

The impacts of biochar amendment on soil physical and chemical attributes have been intensively studied in forest and agricultural soils, only few studies have focused its effects on soil microbial community and soil enzyme activities,

especially in sandy Entisols of Brazilian semiarid areas.

Therefore, this study is essential because the soil microbial communities and activities are fundamental component of ecosystems and play essential roles in ecological functioning associated with carbon, nitrogen, and phosphorus cycling (Gul and Whalen, 2016). Considering the factors listed above, the hypothesis that the application of different type and doses of biochar in a sandy Entisol cultivated with maize would increase the soil bases saturation and enzymes activity. The objective of the present study was to evaluate the effect of two type of coffee biochar on maize growth and the impacts on chemical, microbial biomass and enzyme activities in sandy Entisol from a Brazilian semi-arid area.

## Material and methods

### Production and characterization of biochars

The two biochars were produced by slow pyrolysis in a small kiln (Lima et al., 2018). Two different types of biomass were used to produce the biochars: coffee husk (CH) and coffee grounds (CG). The choice of these wastes was due to high availability in the region and in Brazil.

The values of P, C, N, Mg, Ca, K, Al and H + Al were determined for both types of biochars according to Embrapa (2009) and showed by Lima et al. (2018).

### Set-up experiment

The experiment was carried out in pots under controlled conditions (08° 48' 34,2" S, 36° 24' 29,3" W) at an elevation of 705 m above mean sea level (AMSL). The soil used was collected in a 0-20 cm layer in a tropical dry forest in São João, semi-arid region of Brazil (8° 52' 30" S; 36° 22' 00" O, with an altitude of 705 m) and characterized by Lima et al. (2018).

The experimental design was completely randomized (CRD), distributed in a 2 x 4 + 1 factorial scheme (coffee grounds-MCG and coffee husks -MCH), four doses (4, 8, 12 and 16 Mg ha<sup>-1</sup>) and one control (without biochar). Each treatment received organic fertilization, with cow manure.

Four maize seeds (commercial variety AG 1051, Agrocere) were planted and one week after emergence, thinning was performed, leaving one plant per pot. The pots were irrigated every two days with distilled water, in order to maintain the soil at field capacity, by weighing them. The maize

plants were evaluated at 45 days after sowing and soil was collected to evaluate the microbial and biochemical activities. Ten samples were collected in each repetition; the mixture was sieved in a 2 mm mesh.

#### *Soil Analysis*

The following chemical attributes were determined: pH in water (1: 2.5), available P, K<sup>+</sup>, Na<sup>+</sup>, Al<sup>3+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> (Embrapa, 2009). Na<sup>+</sup>, P and K<sup>+</sup> were extracted using Mehlich-I was quantified using the colorimetric method. The extractable inorganic P was quantified using the colorimetric method. Base saturation (V), an aluminum saturation and cation exchange capacity.

Total organic carbon (SOC) was performed according to the methodology of Yeomans and Bremner (1988). For the determination of microbial biomass carbon (MBC) the samples were submitted to irradiation according to the methodology described by Mendonça and Matos (2005). The biomass extraction was performed according to Vance et al. (1987) and Tate et al. (1988) using 0.5 M K<sub>2</sub>SO<sub>4</sub> extractor. CBM was determined by colorimetry (Bartlett and Ross, 1988).

The enzymatic activities were evaluated:  $\beta$ -glucosidase ( $\beta$ -GLU) (3.2.1.21), Urease (URE) (EC 3.5.1.5), acid and alkaline phosphatase (Pac and Palk) (EC 3.1.3) microbial activity performed by the fluorescein diacetate (FDA) hydrolysis method (Chen et al., 1988). Determinations of the enzymatic activities were based on colorimetric analysis of the release products by each enzyme when the soil sample was subjected to normal conditions of incubation with suitable substrate (Sigma Aldrich).

The  $\beta$ -glucosidase activity (C cycle), Phosphatase (P cycle), and Urease (N cycle) were determined according to Eivazi and Tabatabai (1988), Eivazi and Tabatabai (1977), and Kandeler and Gerber (1988), respectively.

#### *Plant analysis*

The heights of maize plants were measured from the plant neck to the apex of each pot. The plant diameters were measured with the pachymeter and measurements were taken just below the cotyledon leaves. The leaves, stems and grains were dried in a greenhouse with forced air circulation at 65 ° C until constant weight was reached, when the dry matter mass of the plant was measured.

#### *Data analysis*

The data were submitted to ANOVA analysis of variance, using STATISTICA 8.0 software (Statsoft Inc., Tulsa, USA), in order to investigate the significance of in the effects of biochars on the crop growth and the soil attributes. Significant differences were compared by the Tukey test at 5% probability. Regression curves were determined for each variable in order to determine the optimal biochar doses with the maximal efficiency.

### **Results and discussion**

#### *Soil chemical attributes after experiment*

The application of biochar from the coffee husk residue (MCH) increased ( $P < 0.05$ ) the soil chemical attributes. This biochar showed a significant increase of pH and P, K, C and N elements in the higher doses when compared to the residue of the coffee grounds (MCG) (Table 1).

Many reports showed that biochar has the potential to improve soil quality by enhancing anion and cation exchange capacities, surface area, water retention capacity and affecting the bioavailability of nutrients, including P, Ca and N, but may vary according to its properties and soil reactivity (Lima et al., 2018).

The adsorption of cations and increase in pH are the main factors responsible for the increase of nutrient retention in soils modified with biochar (Hussain et al., 2017). Here, the two types of coffee biochar in different doses had positive effects in relation to the contents of Al and Na in MCG and P, K, C, N and the pH, probably due to the higher specific surface area and the higher amount of ash in the coffee husk biochar.

The available P increased after the addition of biochar, because abundant soluble P was formed during the biomass burning process, and could be released when the biochar was added to the soil. Studies have reported that biochar may increase P availability through surface adsorption and increase pH (Yang et al., 2017), as observed with coffee biochar, because it increased the pH and P available in our studies.

Similar results were observed by Colantoni et al. (2016) analyzing the characterization of biochars produced from granulated agricultural residues. The authors observed an increase of two elements, phosphorus and potassium. They also state that pyrolysis strongly influences the concentrations of the main elements of soil chemistry, as verified in the present study using coffee biochars.

Table 1. Effect of doses of biochar (4, 8, 12 and 16 Mg ha<sup>-1</sup>) on chemical attributes of Entisol treated with two biochars coffee grounds (MCG) and coffee husk (MCH), cultivated with maize.

	pH	P	Mg	Ca	K	Al	Na	H+Al	SB	CTC	V	C	N	C/N
TRAT.	1:2,5	mg kg <sup>-1</sup>	-----cmol <sub>c</sub> kg <sup>-1</sup> -----					-----%-----						
CM	4.3 aBC	7.1 aBC	1.06 aAA	0.63 aAA	0.09 aBC	0.20 aAA	0.09 aBC	4.3aAA	1.76aAB	6.27 aAA	28.4 aAB	1.08aBC	0.17aAB	6.6 aCC
MCG4	5.0 aA	8.3 aAB	1.06 aA	0.65 aA	0.12 bB	0.15 aAB	0.14 bAB	3.9 aA	1.83 aA	5.90 aA	31.0 aA	1.60 aA	0.20aA	8.1abBC
MCG8	5.1 bA	9.7 aAB	1.06 aA	0.75 aA	0.17 bAB	0.14 aB	0.13 aAB	4.3 aA	1.97 aA	6.36 aA	31.0 aA	1.62 aA	0.18aA	9.24 aAB
MCG12	5.2 bA	9.6 aAB	1.06 aA	0.64 aA	0.18 bAB	0.15 aAB	0.13 aAB	4.1 aA	1.88 aA	6.16 aA	30.6 bA	1.51aAB	0.16aA	9.47 aAB
MCG16	5.3 bA	9.9 aA	1.02 aA	0.58 bA	0.26 bA	0.15 aAB	0.15 aA	3.7 bA	1.86 bA	5.72 bA	32.5 aA	1.70 bA	0.16aA	10.82 aA
MCH4	5.2 bB	7.2 aC	1.02 aA	0.65 aA	0.34 aB	0.18 aA	0.13 aBC	4.2 aA	2.01 aB	6.40 aA	31.5 aAB	1.56 aB	0.15 bB	10.3aAB
MCH8	5.6 aA	8.0 aBC	1.02 aA	0.67 aA	0.43 aB	0.11 aB	0.15 aAB	4.0 aA	2.11aB	6.19 aA	34.2 aA	1.42aBC	0.15 bB	9.54 aB
MCH12	5.8 aA	10.1 aB	1.05 aA	0.72 aA	0.46 aB	0.11 aB	0.20 aA	4.1 aA	2.23 aB	6.42 aA	34.7 aA	1.82 aB	0.17 aB	10.52aAB
MCH16	5.9 aA	15.8 aA	1.08 aA	0.74 aA	0.65 aA	0.08 bB	0.03 bD	4.4 aA	2.48 aA	6.93 aA	35.7 aA	2.62 aA	0.23 aA	12.12 aA
CV%	3.84	12.33	5.70	16.32	22.1	18.65	19.46	5.43	8.50	3.55	7.93	14.02	10.26	10.40

Different lowercase letters indicate significant differences between types of biochar and different capital letters indicate significant differences among doses according to the Tukey test (P < 0.05). SB Base samples, CTC cation exchange capacity, V base saturation, C carbon, N nitrogen, C / N carbon / nitrogen ratio.

Total organic carbon and microbial biomass carbon

Biochar amendments impacted the SOC cultivated with maize, with increases of 21% (MCG16) and 31% (MCH16) at 16 Mg ha<sup>-1</sup> in relation to the zero dose (Figure 1A). All treatments in which the two type of coffee biochar were added showed increases in C content, mainly in the higher doses. However, the soils that received coffee biochar presented discrete increases of SOC. This impact occurred because with high doses of biochar increases the amount of stable carbon, increasing C storage. The stabilized

part of the SOC is quite susceptible to microbial decomposition (Yang et al., 2017), but, through a possible combination of physical protection and chemical complexation, the stable carbon may have been protected against decomposition (Hartley et al., 2016).

Studies have shown that the addition of biochar from wood to sandy soils improves soil structure and increased SOC due to the stability of structural components that improve when biochar is added to this type of soil (Hartley et al., 2016). In our experiment the C content increased twice compared to the control treatment (Table 1).

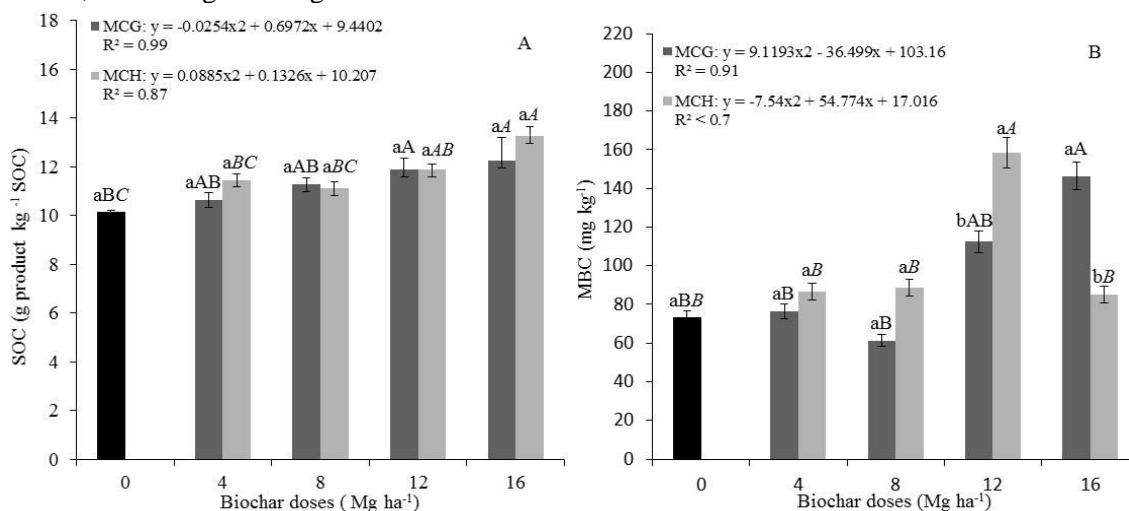


Figure.1 Effect of doses of biochar (4, 8, 12 and 16 Mg ha<sup>-1</sup>) on soil organic carbon (A) and microbial biomass carbon (B) of Entisol treated with two biochars coffee grounds (MCG) and coffee husk (MCH), cultivated with maize. Different lowercase letters indicate significant differences between types of biochar and different capital letters indicate significant differences among doses according to the Tukey test (P < 0.05).

The microbial biomass carbon was significantly affected by the biochar types and doses as well as their interactions. Under doses 12 and 16 both biochars showed the largest increases in microbial biomass, compared to the control treatment. The application of the coffee biochar to the sandy soil increased the microbial biomass carbon because biochar provides labile organic C and its pores can hold large quantities of air and oxygen at higher dosage being important for microbial use and growth (Ge et al., 2019). However, biochar may provide resistance to microbial decomposition due to low polarity and high aromaticity (Wang et al., 2020). Another factor responsible for the increase of microbial biomass in soils that have received biochar is the large surface area of the biochar (Lima et al., 2018) that offers favorable microhabitat for microbial communities (Ge et al., 2019).

Khadem and Raiesi (2017) analyzed the microbial performance in maize biochar applied to sandy and clay soils and showed a significant increase of microbial biomass in both types of soils, obtaining better results in sandy soils. The authors attributed the increase to higher availability of organic matter and labile C. In the present study, there was no relationship of the increase of SOC in relation to the increase of microbial biomass, but the highest doses of coffee biochar were those that presented higher levels of MBC, describing a relation of increase of the microbial biomass with the increase of biochar.

The MCH applied to Entisol cultivated with maize showed maximum MBC at dose 16 Mg ha<sup>-1</sup>, while the MCG obtained the maximum MBC content at dose 12 Mg ha<sup>-1</sup>.

#### Enzymatic activities

Extracellular enzymes activities differed ( $P < 0.05$ ) between biochar types and doses in all enzymes evaluated (Figure 2). The microbial activity assessed by fluorescein diacetate (FDA) was significantly affected by the biochar and doses. The MCH applied to Entisol showed the highest FDA at 16 Mg ha<sup>-1</sup> (Figure 2A). However, the MCH biochar amendments showed no increases of FDA compared to the control. The application of coffee biochar in sandy soil cultivated with maize altered the hydrolysis of fluorescein diacetate (FDA) that show the characterization and quantification of microbial activity (Jiang et al., 2016). Literature shows that high doses of biochar increase microbial

activity because this activity is influenced by increases of organic matter content by biochar application (Bera et al., 2016).

Another hypothesis to explain this increase in activity with increasing doses of biochar is due to the osmotic effect on soil microflora that may benefit the biochemical reactions. Studies by Bhaduri et al. (2016) using peanut husk biochar in soils also observed this relationship due to the osmotic effect that this amendment may impact on soil microorganisms. Another important factor is the increase in organic matter that provides higher substrate concentrations for the increase of microbial activity (Sarma et al., 2017).

The biochar applied to sandy Entisol cultivated with maize decrease the enzymatic activity beta-glucosidase (carbon cycle). The Biochar that presented the highest BETA activity was the one from MCG at dose 4 Mg ha<sup>-1</sup> (Figure 2B). On the other hand, the two types of coffee biochars showed different behavior regarding the beta-glucosidase activity that is associated with the C mineralization present in the soil. In this study, MCG applied to the soil increased the activity of this enzyme due to the amount of C after application that increased with increasing doses of biochar which also favored MBC content, indicating preferential habitat with increased nutrient concentration (Bera et al., 2016), of the MCH applied.

Other factor that may be involved in the relationship of the addition of biochar to beta-glucosidase activity was the alteration of the soil pH that received this amendment, since the pH change is one of the factors that alter the functioning of the enzymes. Luo and Guo (2016) verifying the alteration of beta-glucosidase activity using biochar produced from bamboo residues, showed that this may have happened due to the changes in soil characteristics, pH, soluble phenolic materials and availability of nutrients, which probably led to changes in the microbial community and enzymatic activities.

The biochar application from the coffee residue showed a positive trend on the increase of the urease activity at the highest doses of biochar, with increases of 54.22% of the urease in treatments that received MCH at 12 Mg ha<sup>-1</sup> compared to the control treatment (Figure 2C). Urea hydrolysis by urease may reflect on soil microbial processes and alter the N available in the soil, specially if the soil received N enrichment because affected the response of soil enzyme

activities, as the decrease the urease activity (Jia et al., 2020). The present study showed an increase in the urease activity in the MCH12 treatment, since the biochar that has high molecular weight makes an association with the enzyme (Bera et al., 2016), making that high concentrations of biochar may have higher activities due to protection. In addition, organic manure is rich in enzymes and provides nutrition and environment for the growth of microorganisms and the production of enzymes (Li et al., 2019). So, when biochar is associated with nitrogen compounds, the growth stimulation occurs synergistically.

Similar results in which the URE enzymatic activity was more accentuated with increases doses of biochar were observed by Chen et al. (2020) who studied the rate of biochars addition produced from dry rice hull that influence the enzymatic activity and the microbial community of the soil. This increase is related to the substrate that presents organic and inorganic compounds and possibly affect the enzymes that are related to N.

The coffee biochar applied to the sandy soil with pH 4.3, cultivated with maize, showed a reduction of Pac and increased Palk activity (Figure 2D and 2E). The enzyme phosphatase is involved in the phosphate cycle and is particularly known to have a rapid response to soil management (Al Marzooqi and Yousef, 2017). This increase is related to the physicochemical interactions of biochar with the soil enzyme and the amount of the activity will depend on soil pH (Elzobair et al., 2016), as observed in the present study.

Lima et al. (2018) evaluating the adsorption of the same biochars used in the present study, verified that the coffee substrate used showed an increase of P in the MCG from 2 to 6 mg kg<sup>-1</sup>, whereas MCH increased from 3 to 8 mg kg<sup>-1</sup>. Another factor is the increase in pH caused by the high concentration of biochar (Aamer et al., 2020). So, the increase in alkaline phosphatase activity at lower doses of biochar is due to the pores in the biochar and the availability of nutrients that improves the absorption of P.

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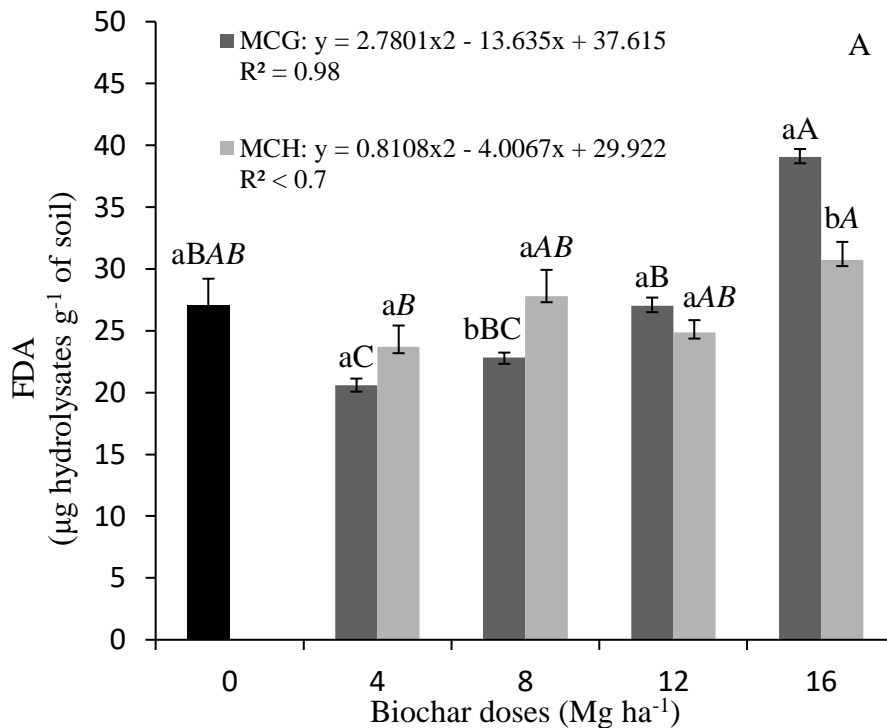


Figure.2 Absolute A) FDA B) Beta-glucosidase, C) Urease, D) Acid phosphatase and alkaline phosphatase activities of Entisol treated with two biochars coffee grounds (MCG) and coffee husk (MCH), cultivated with maize. Different lowercase letters indicate significant differences between types of biochar and different capital letters indicate significant differences among doses according to the Tukey test (P < 0.05).

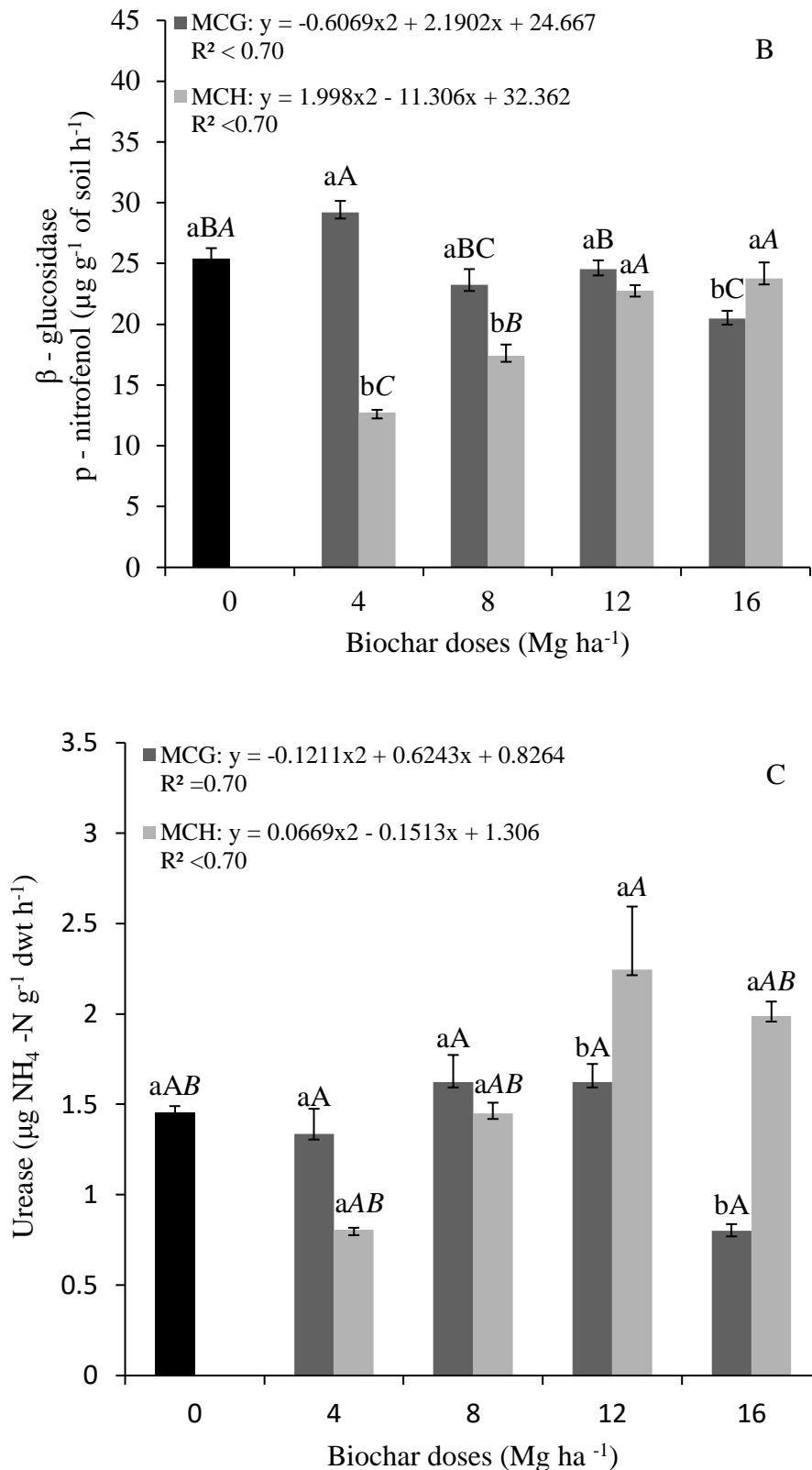


Figure.2 (continue) Absolute A) FDA B) Beta-glucosidase, C) Urease, D) Acid phosphatase and alkaline phosphatase activities of Entisol treated with two biochars coffee grounds (MCG) and coffee husk (MCH), cultivated with maize. Different lowercase letters indicate significant differences between types of biochar and different capital letters indicate significant differences among doses according to the Tukey test ( $P < 0.05$ ).



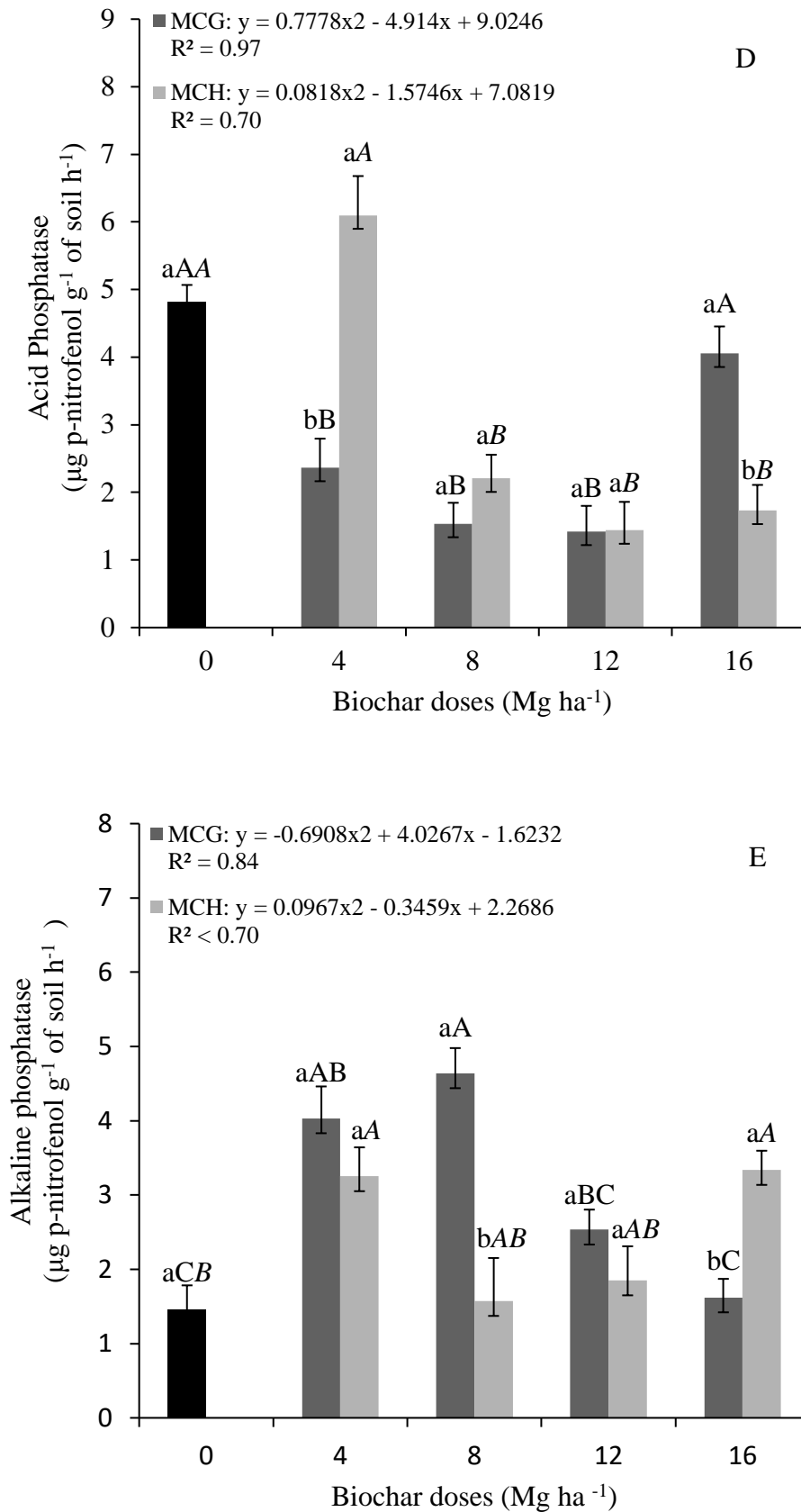


Figure.2 (continue) Absolute A) FDA B) Beta-glucosidase, C) Urease, D) Acid phosphatase and alkaline phosphatase activities of Entisol treated with two biochars coffee grounds (MCG) and coffee husk (MCH), cultivated with maize. Different lowercase letters indicate significant differences between types of biochar and different capital letters indicate significant differences among doses according to the Tukey test ( $P < 0.05$ ).

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Maize growth

The coffee biochar added to the soil did not significantly increase the height of the maize plant. However, the MCG at 4 Mg ha<sup>-1</sup> showed a slight increase due to the addition of the substrate (coffee grounds) that releases the nutrients to the soil and may also be related to the strong adsorption property of the biochar, which favors nutrient availability for the plant (Hupfauf et al., 2016). In addition, the coffee biochar applied to sandy soils increases water retention and soil nutrient availability (Lima et al., 2018).

Likewise, the biochar applied did not have a positive effect on the plant diameter, this may also be linked to the time of the experiment or dose

concentration of the biochar, as they were not suitable to have a significant effect on this characteristic.

Foster et al. (2016) analyzing the impact of biochar and fertilizer on soil nutrients and microbial enzymatic activities in a maize crop system, showed that the dry matter did not obtain increases on yield, stating that it would need a longer period to have a better definition on the influence of biochar on maize yields, as well as on the present work. Therefore, more studies are needed to evaluate the cultivation time and several crop cycles to evaluate if the coffee biochar really has no effect on maize growth in sandy soil (Figura 3).

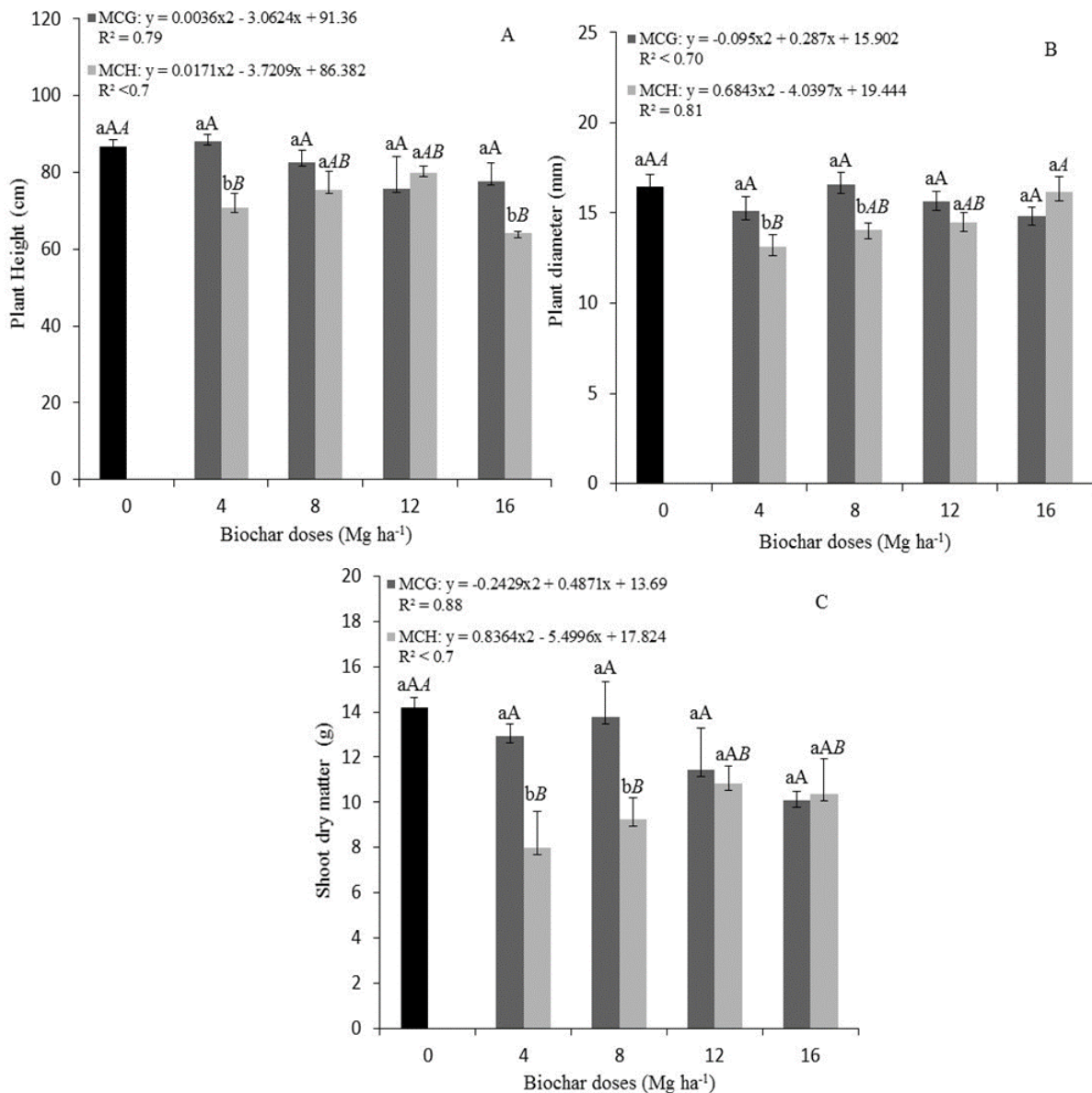


Figure 3. Effect of doses of biochar (4, 8, 12 and 16 Mg ha<sup>-1</sup>) on plant height (A), plant diameter (B) and shoot dry matter (C) of maize grown in a Entisol. Different lowercase letters indicate significant differences between types of biochar and different capital letters indicate significant differences among doses according to the Tukey test (P < 0.05).

## Conclusions

Coffee biochar applied to sandy Entisol cultivated with maize, as predicted, increased the total soil C content, mainly coffee husk biochar that increases twice the C content when compared to the control. This study clearly demonstrated that soil nutrients, microbial community and enzyme activities increased in response to the type and the doses of biochar. Therefore, the reuse of coffee residue to biochar production is recommended for increase the Sandy soil quality in the Brazilian semi-arid areas. We recommend the application of coffee husk biochar to increase the Sandy Entisol quality.

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