



## SOIL SCIENCE

# Impact of coffee biochar on carbon, microbial biomass and enzyme activities of a sandy soil cultivated with bean

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**Abstract:** Biochar has been used to reuse the agro-industrial wastes and improve soil quality. Several studies have been carried out to show the impact of biochar on physical and chemical soil attributes. However, there are still gaps regarding the effects on microbial biomass and enzymatic activities that are important to determine sensitive indicators to evaluate changes in management practices. The objective of this study was to assess the effect of two biochars on the chemical, microbial biomass carbon, and the enzymatic activities in an Entisol cultivated with bean. We evaluate two types of coffee biochar: ground and husks, four doses (4, 8, 12, and 16 Mg ha<sup>-1</sup>) and control. All treatments received organic fertilization with cow manure. Husks biochar increase the soil pH, Ca, and K, also contributing to the reduction of toxic aluminum contents and raising the concentrations of P labile. The treatments that received ground biochar showed higher soil organic carbon, microbial biomass,  $\beta$ -glucosidase, and fluorescein diacetate. Biochar produced from coffee residues increased sandy soil quality. We showed the first report on the beneficial impact of coffee biochar on enzymatic and microbiological quality of sandy soil cultivated with the bean.

**Key words:** Entisols,  $\beta$ -glucosidase, *Phaseolus vulgaris*, fluorescein diacetate, coffee waste.

## INTRODUCTION

The waste generated by the agro-industry can be used as sources of organic matter for the soil or transformed into biocarbon, which can improve soil quality for a longer time than fresh organic matter. Biochar is a stable solid carbonaceous material of fine granularity with high carbon content (70% - 80%), and its properties depend on raw materials used on production process (Zhang et al. 2013). It is a pyrolysis product of organic matter such as crop residues, husk, manure, wood debris, various grasses and other

agricultural, and livestock at a temperature between 300 and 900 °C (Novotny et al. 2015).

In this sense, the coffee culture has great importance in the world, being one of the primary commodities. Waste generated by this crop can become a problem for the environment if it is not an efficient destination. This substrate can be harnessed as a low-cost option, besides helping to reduce accumulation in the surroundings (Meneghelli et al. 2016). One of the possibilities of use to these wastes is the production of biochar (Lima et al. 2018).

Biochar applied to the soil increases the efficiency of water retention and carbon sources, reduce greenhouse gas emissions ( $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$ ), stimulating biochemical conversion, improve microbiome (He et al. 2017) and the low fertility of sandy soils in dry regions, predominant in Northeast Brazil, as observed when applied to maize production (Lima et al. 2018). In plants, showed potential to improves the nutrient absorption, promoting growth, and crop production (Saxena et al. 2013, Tan et al. 2017).

The effects of biochar on microbial communities are essential because the microorganisms offer important functions in ecosystems acting as the primary regulators of biochemical soil processes (Huang et al. 2017). The biomass directly or indirectly determines the production of enzymes and any alteration in the microbial community of the soil can modify the enzyme activities (Raiesi & Beheshti 2014). Enzymatic are essential indicators of biochemical processes (Medeiros et al. 2017) because they are frequently involved in organic matter decomposition, synthesis, cycling nutrient availability and are also used as indicators of fertility and quality of the soil. In particular, enzymatic activities are related to soil or sediment functionality and can be widely used to assess microbial activity due to its response to changes in soil (Medeiros et al. 2015, Raiesi & Beheshti 2014).

Studies on the impact of different types of biochar on crop yield and soil attributes are well documented (Farhangi-Abriz & Torabian 2017, Foster et al. 2016). However, studies with the reuse of residues from the coffee industry to the production of biochar are still scarce, and it is necessary that they are evaluated, aiming at a new destination and use in agriculture, as well as the increase of bean and improving the

chemical and biological attributes of sandy soil with low fertility.

Here, we carried out this study to evaluate the effects of the two types of coffee biochar, in different doses, on the initial bean growth and the impact on the chemical attributes, soil organic C, microbial biomass C and on enzymatic activities in a sandy Entisol with beans.

## MATERIALS AND METHODS

The two biochars were produced by slow pyrolysis in a small kiln, which was based on a model widely used by Thai farmers (Prakongkep et al. 2015). Two different types of biomass were used to produce the biochars: coffee husk (CH) and coffee grounds (CC).

We used sandy Entisol collected on topsoil (0–20 cm) from native forest in São João, the neighborhood of Garanhuns (08° 48' 34,2" S, 36° 24' 29,3" W) at an elevation of 705 m above mean sea level. The soil was manually collected, sieved through a 2-mm sieve, and used in a pot experiment in a greenhouse.

Lima et al. (2018) showed the characterization of physical and chemical attributes of sandy Entisol before the installation of the experiment and biochar made of the coffee husk (CH), coffee grounds (CG) utilized in this study (Table I).

The experiment was carried out in under controlled greenhouse conditions; the design was completely randomized, distributed in a factorial scheme ( $2 \times 4 + 1$ ), with two types of biochar (CG and CH), 4 doses (4, 8, 12 and 16  $\text{Mg ha}^{-1}$ ) and control (without biochar), with ten repetitions. The fertilization consisted of cow manure (CM), according to recommendations of IPA (2008).

Coffee biochar and doses were applied to the soil and mixed uniformly. We planted a commercial variety of bean (BRS style variety)

which is widely used by the farmers in the region. Seeds were treated with 3% sodium hypochlorite (NaOCl) and rinsed three times with sterile water to remove any residual effect of NaOCl, and sown into pots (four seeds per pot). Five days after emergence (DAE), thinning was performed, leaving one plant per pot.

The plants were irrigated every two days with distilled water to keep the soil at field capacity (FC). We evaluated the bean plant and collected soil samples 45 days after sowing. We repeated the experiment twice.

The heights (Heig45) of bean plants were measured from the plant neck to the apex of each pot. One pachymeter measured the plant diameters (Diam45) just below the cotyledon leaves. All parts of the plant were washed with distilled water, packed in individual Kraft bags and dried at 65–70 °C for 72 h to obtain shoot dry matter (SDM).

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>. Na<sup>+</sup>, P, and K<sup>+</sup> were extracted using Mehlich<sup>1</sup>, and K<sup>+</sup> 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 (TOC) was determined through thermal oxidation with potassium dichromate, according to Yeomans & Bremner (1988). We quantify the Microbial biomass carbon (MBC) content through the irradiation method (Mendonça & Matos 2005), followed by extraction with 0.5 M K<sub>2</sub>SO<sub>4</sub> and the carbon content in the extracts through the colorimetric method (Bartlett & Ross 1988).

The enzymatic activities evaluated were: the fluorescein diacetate (FDA) by hydrolysis method (Chen et al. 1988); the enzyme involved in C cycle, β-glucosidase (Bet) (3.2.1.21) by Eivazi & Tabatabai (1988); the enzyme involved in N cycle,

the urease (Ure) (EC 3.5.1.5) by Eivazi & Tabatabai (1977); and the enzymes involved in P cycle, acid and alkaline phosphatase (acid. Pho and Alk pho) (EC 3.1.3) by Kandeler & Gerber (1988). We used colorimetric analysis of the release products by each enzyme with soil sample subjected to normal conditions of incubation with a suitable substrate (Sigma Aldrich).

Statistical and multivariate analyses were conducted using the R language platform (v.3.4.3, 2017) based on the data of the biometric attributes of the plants, chemicals, and enzymatic activities of the soils. Multivariate exploratory analyses, including nonmetric multidimensional scaling (nMDS), analysis of similarity (ANOSIM), heatmaps, and Mantel tests were completed based on the tools of the *vegan* and *heat map* libraries. After adjusting the models and removing the outliers, the means, standard deviations, and variation coefficients were calculated using the *doBy* library. Homogeneity tests, analysis of variances (ANOVA), and comparisons between averages were completed with the balanced data, according to the tools contained in the *stats*, *AxpDes*, *multicomp*, and *Agricola* libraries.

## RESULTS

The Heig45 of bean plants was not affected by the interaction between biochar and doses applied to Sandy Entisol. However, the biochars delayed the elongation of the plants, mainly the coffee grounds (CG), which differed from the effect of the coffee husk (CH) biochar (Table II). Diam45 and SDM also reflected this behavior in all the treatments with biochar. The control treatment that did not receive biochar showed higher Diam45 and SDM of beans plants (Table II).

Despite these results, the determination of the quadratic model minimum point suggested

**Table I. Physico-chemical properties of Entisol and biochar from coffee husk (CH), and coffee grounds (CG).**

	pH	P	Ca	Mg	K	Al	Na	CEC	C	N	SSA
		mg Kg <sup>-1</sup>	cmol <sub>c</sub> Kg <sup>-1</sup>					%		m <sup>2</sup> g <sup>-1</sup>	
Soil	5.1	16.6	0.8	0.8	0.15	0.15	0.28	3.98	1.6	0.24	0.005
CH	10.31	470.65	0.14	0.12	22.17	0	0.06	22.54	67.11	2.05	244
CG	9.65	311.46	1.56	0.72	2.68	0	0.5	5.56	68.81	4.3	23.5

Source: Lima et al. (2018). CEC: cation exchange capacity, C and N: elemental carbon and nitrogen content, SSA: specific surface area.

**Table II. Initial growth of bean plants cultivated in sandy Entisol treated with biochar produced from the coffee ground (CG) and coffee husk (CH) residues applied to sandy Entisol in Northeastern Brazil.**

Biochar application (t ha <sup>-1</sup> )											
	4		8		12		16		Mean	Model:	
a. Height (cm)											
CH	38.3		38.5		37.0		40.7		38.6	a	y = 38.6
CG	37.0		<u>33.5</u>		36.7		37.2		36.1	b	y = 36.1
Mean	37.7		36.0		36.9		38.9		<u>37.4</u>	*	Control = 40.6
b. Diameter (cm)											
CH	<u>5.6</u>		<u>5.6</u>		<u>5.7</u>		<u>6.0</u>		5.7		y = 0.008x <sup>2</sup> - 0.137x + 6.087, R = 0.47
CG	<u>5.9</u>		<u>5.0</u>		<u>5.9</u>		<u>5.7</u>		5.6		
Mean	5.7		5.3		5.8		5.8		<u>5.7</u>		Control = 7.5
c. Shoot Dry Matter (g)											
CH	7.3	a	7.3	a	7.1	a	7.0	a	7.2		y = 7.2
CG	6.2	a	<u>4.1</u>	b	8.1	a	7.4	a	6.5		y = 0.022x <sup>2</sup> - 0.256x + 6.323, R = 0.37
Mean	6.8		5.7		7.6		7.2		6.8		Control = 7.5

Means with different letters between lines differ statistically from each other by the Tukey test (p < 0.05). The absence of letters indicated statistically similar by the F test (p < 0.05) and the underlined averages differed significantly by Dunnett test (p < 0.05). Only the significant regressions by the F test (p < 0.05) were calculated, and the others returned a mean (constant) representative of the dosages evaluated. \* Comparison of the general means with that of the additional treatment (control) according to the F test (p < 0.05).

a progressive increase in Diam45 from 5.5 cm for increasing doses of biochar from 8.5 t ha<sup>-1</sup>. Similarly, there was an upward trend of plants from 5.6 g SDM to doses higher than 5.8 t ha<sup>-1</sup> of CG biochar. From the models, for the 45-day evaluation, plant growth was exceeded over control treatment at doses ranging from 16 to 25 t ha<sup>-1</sup> of biochar or at current doses of 6 to 9 t ha<sup>-1</sup> in later than 45 days after sowing (Table II).

On the other hand, the application of different coffee biochar in Sandy Entisol cultivated with bean increased the chemical attributes of the soil, mainly in the levels of P, K, and the pH (Table III). These variables have already begun to respond significantly from the 4 t ha<sup>-1</sup> dose of the two biochars. The levels of Ca, K, and pH of the soils with biochar CH were higher than the soils treated with biochar CG.

**Table III. Chemical attributes, microbial biomass carbon (MBC) and total organic carbon (TOC) of sandy Entisol treated with biochar produced from the coffee ground (CG) and coffee husk (CH) residues, cultivated with the bean plant in Northeastern Brazil.**

Biochar application (t ha <sup>-1</sup> )											
	4		8		12		16		Mean	Model:	
a. Na (cmolc kg <sup>-1</sup> )											
CH	0.092	b	<u>0.168</u>	a	<u>0.157</u>	a	<u>0.168</u>	a	0.146	$y = -0.001x^2 + 0.025x + 0.011, R = 0.85$	
CG	<u>0.157</u>	a	0.136	a	<u>0.157</u>	a	<u>0.190</u>	a	0.160	$y = 0.0008x^2 - 0.014x + 0.197, R = 0.97$	
Mean	0.125		0.152		0.157		0.179		<u>0.153</u>	Control = 0.090	
b. K (cmolc kg <sup>-1</sup> )											
CH	<u>0.30</u>	a	<u>0.42</u>	a	<u>0.63</u>	a	<u>0.79</u>	a	0.53	$y = 0.0006x^2 + 0.029x + 0.161, R = 0.99$	
CG	0.20	b	<u>0.29</u>	b	<u>0.34</u>	b	<u>0.40</u>	b	0.31	$y = -0.0004x^2 + 0.024x + 0.113, R = 0.99$	
Mean	0.25		0.35		0.48		0.59		<u>0.42</u>	Control = 0.15	
c. pH											
CH	<u>5.22</u>		<u>5.60</u>		<u>5.82</u>		<u>5.92</u>		5.64	a	$y = -0.002x^2 + 0.088x + 4.788, R = 0.99$
CG	<u>5.00</u>		<u>5.08</u>		<u>5.23</u>		<u>5.29</u>		5.15	b	
Mean	5.11		5.34		5.52		5.61		<u>5.39</u>		Control = 4.34
d. P (mg kg <sup>-1</sup> )											
CH	<u>3.74</u>		<u>4.14</u>		<u>4.62</u>		<u>5.57</u>		4.52		$y = 0.0035x^2 + 0.031x + 3.721, R = 0.98$
CG	<u>4.02</u>		<u>4.39</u>		<u>4.44</u>		<u>4.72</u>		4.39		
Mean	3.88		4.27		4.53		5.14		<u>4.45</u>		Control = 2.47
e. H+Al (cmolc dm <sup>-3</sup> )											
CH	3.92		3.59		3.26		3.42		3.55	b	$y = 0.007x^2 - 0.157x + 4.445, R = 0.95$
CG	3.96		3.55		3.88		3.82		3.80	a	
Mean	3.94		3.57		3.57		3.62		3.68		Control = 3.75
f. Ca (cmolc kg <sup>-1</sup> )											
CH	0.66	a	<u>0.72</u>	a	0.68	a	<u>0.75</u>	a	0.70		$y = 0.0002x^2 + 0.0026x + 0.655, R = 0.57$
CG	0.65	a	0.65	b	0.67	a	0.64	b	0.65		$y = 0.65$
Mean	0.65		0.69		0.67		0.69		0.68		Control = 0.66
g. Mg (cmolc kg <sup>-1</sup> )											
CH	0.99		0.97		0.98		1.05		1.00	b	$y = 0.0066x^2 - 0.155x + 4.400, R = 0.95$
CG	1.07		1.04		1.05		1.04		1.05	a	
Mean	1.03		1.01		1.01		1.05		1.02		Control = 1.02
h. Al											
CH	<u>0.150</u>		<u>0.150</u>		<u>0.150</u>		<u>0.133</u>		<u>0.146</u>	b	$y = 0.006x^2 - 0.149x + 4.220, R = 0.95$
CG	0.267		0.200		0.150		0.167		0.196	a	
Mean	0.208		0.175		0.150		0.150		0.171		Control = 0.22

**Table III. Continuation.**

Biochar application (t ha <sup>-1</sup> )											
i. CTC											
CH	6.01		5.85		5.68		6.14		5.92		y = 5.97
CG	6.19		5.73		6.08		6.04		6.01		
Mean	6.10		5.79		5.88		6.09		5.97		Control = 5.83
j. SB											
CH	1.95	a	2.11	a	2.28	a	2.59	a	2.23		y = 0.002x <sup>2</sup> + 0.006x + 1.889, R = 0.99
CG	1.92	a	1.98	a	2.05	b	2.07	b	2.01		y = 2.01
Mean	1.93		2.05		2.17		2.33		<u>2.12</u>		Control = 1.83
k. V%											
CH	32.43	a	<u>36.04</u>	a	<u>40.24</u>	a	<u>42.26</u>	a	37.74		y = -0.025x <sup>2</sup> + 1.338x + 27.331, R = 0.99
CG	31.00	a	34.80	a	33.79	b	34.27	b	33.47		y = 33.47
Mean	31.72		35.42		37.01		38.27		<u>35.60</u>		Control = 31.5
l. MBC (mg C <sub>mic</sub> kg <sup>-1</sup> soil)											
CH	170.6		247.8		278.3		246.9		235.9	b	y = -1.697x <sup>2</sup> + 42.97x + 56.34, R = 0.90
CG	217.3		<u>378.0</u>		<u>334.0</u>		386.1		328.8	a	
Mean	193.9		312.9		306.2		316.5		282.4		Control = 230.8
m. TOC (g kg <sup>-1</sup> )											
CH	12.41		10.75		12.41		13.74		12.33		y = 12.45
CG	12.51		12.64		11.34		13.76		12.56		
Mean	12.46		11.70		11.87		13.75		12.45		Control = 11.61
n. Conductivity (µS cm <sup>-2</sup> )											
CH	218.0	a	344.0	a	<u>455.3</u>	a	436.3	a	363.4		y = -2.265x <sup>2</sup> + 64.47x - 9.417, R = 0.98
CG	194.1	a	312.3	a	217.8	b	210.7	b	233.7		y = 233.7
Mean	206.0		328.2		336.6		323.5		298.6		Control = 255.0

Means with different letters between lines differ statistically from each other by the Tukey test (p < 0.05). The absence of letters indicated statistically similar by the F test (p < 0.05) and the underlined averages differed significantly by Dunnett test (p < 0.05). Only the significant regressions by the F test (p < 0.05) were calculated, and the others returned a mean (constant) representative of the dosages evaluated. \* Comparison of the general means with that of the additional treatment (control) according to the F test (p < 0.05).

Soils with CH also showed a significant reduction in Al<sup>3+</sup> concentrations and potential acidity (H<sup>+</sup>+ Al<sup>3+</sup>), regardless of the dose.

The SB and V% in soils with CH were significantly higher than the means of GC soils from 12 t ha<sup>-1</sup>, also significantly exceeding the control. The CTC and TOC did not present significant responses. Both biochars

significantly increase the Na<sup>2+</sup> levels in the soil but do not reach the levels that compromise the productivity of the bean crop (Table III).

These two types of biochar applied at different doses in sandy Entisol cultivated with beans had an impact on the enzymatic activities (Table IV). The Aci pho was higher in the soil with CG biochar than control and reached its

**Table IV. Enzymatic activities of sandy Entisol treated with biochar produced from the coffee ground (CG) and coffee husk (CH) residues, cultivated with the bean plant in Northeastern Brazil.**

Biochar application (t ha <sup>-1</sup> )										
	4		8		12		16		Mean	Model:
a. Acid phosphatase (μmol p-nitrophenol-phosphate g <sup>-1</sup> soil h <sup>-1</sup> )										
CH	2.51	b	<u>1.84</u>	b	<u>1.76</u>	b	2.24	a	2.09	y = 2.09
CG	4.15	a	2.88	a	<u>5.15</u>	a	<u>1.81</u>	a	3.50	y = -0.032x <sup>2</sup> + 0.530x + 2.087, R = 0.34 *
Mean	3.33		2.36		3.46		2.03		<u>2.79</u>	Control = 3.64
b. Alkaline phosphatase (μmol p-nitrophenol-phosphate g <sup>-1</sup> soil h <sup>-1</sup> )										
CH	<u>11.01</u>	a	3.51	a	<u>1.56</u>	a	5.40	a	5.37	y = 0.177x <sup>2</sup> - 4.009x + 24.223, R = 0.99
CG	4.15	b	2.69	a	3.41	a	5.52	a	3.94	y = 0.055x <sup>2</sup> - 0.991x + 7.181, R = 0.99
Mean	7.58		3.10		2.49		5.46		4.66	Control = 4.91
c. Urease (μmol NH <sub>4</sub> -N g <sup>-1</sup> dwt 2h <sup>-1</sup> )										
CH	1.05	a	1.05	a	<u>2.32</u>	a	<u>2.60</u>	a	1.76	y = 0.004x <sup>2</sup> + 0.057x + 0.636, R = 0.87
CG	0.38	b	0.76	b	<u>1.21</u>	b	<u>1.13</u>	b	0.87	y = -0.007x <sup>2</sup> + 0.214x - 0.393, R = 0.96
Mean	0.71		0.91		1.77		1.87		1.31	Control = 0.60
d. β-Glucosidase (μg -nitrophenol-β-D-gluco-pyranoside g <sup>-1</sup> soil h)										
CH	14.21		16.91		<u>20.75</u>		16.70		17.14	y = -0.059x <sup>2</sup> + 1.365x + 11.518, R = 0.41
CG	19.15		16.44		21.81		18.24		18.91	
Mean	16.68		16.68		21.28		17.47		18.03	Control = 16.19
e. FDA (μg g <sup>-1</sup> soil hydrolysates)										
CH	24.01	b	21.50	b	<u>38.84</u>	a	<u>40.60</u>	a	31.24	y = 0.066x <sup>2</sup> + 0.346x + 19.781, R = 0.78
CG	32.82	a	29.66	a	<u>35.60</u>	a	<u>42.84</u>	a	35.23	y = 0.162x <sup>2</sup> - 2.347x + 39.222, R = 0.97
Mean	28.41		25.58		37.22		41.72		<u>33.23</u>	Control = 27.11

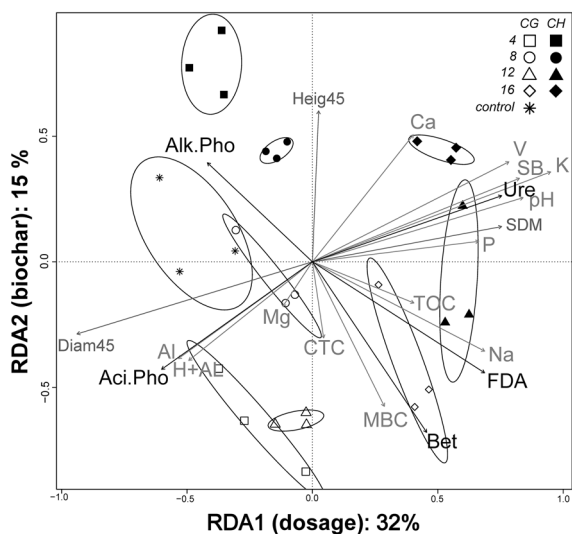
Means with different letters between lines differ statistically from each other by the Tukey test (p < 0.05). The absence of letters indicated statistically similar by the F test (p < 0.05) and the underlined averages differed significantly by Dunnett test (p < 0.05). Only the significant regressions by the F test (p < 0.05) were calculated, and the others returned a mean (constant) representative of the dosages evaluated. \* Comparison of the general means with that of the additional treatment (control) according to the F test (p < 0.05).

apex for dosage between 8 and 12 t ha<sup>-1</sup> by quadratic model. Soils with 4 t ha<sup>-1</sup> of CH showed a significant increase in Alk pho activity, which progressively decreased to 11.3 t ha<sup>-1</sup>. Ure activity in soils with CH was significantly higher than in soils with GC, and all were superior to the control from applications of 12 t ha<sup>-1</sup>. Bet did not respond to the types of coffee biochar but returned a significant quadratic model pointing the apex of 19.4 p-nitrophenol-phosphate μg g<sup>-1</sup> soil h to 11.6 t ha<sup>-1</sup> of any biochar. The FDA was continually

increasing until it became significantly different from the control from 12 t ha<sup>-1</sup> (Table IV).

In this sense, the doses of the biochars applied to sandy Entisol cultivated with beans contributed more to the analysis of RDA than the types of biochar and separated the soils that received 12 t ha<sup>-1</sup> from the others (Figure 1). The CH biochar contributes to the increase of pH, the sum of bases (SB) and its saturation in the soil (V%), mainly Ca and K, highlighting the improvements in the levels of labile P and the activity of the Ure with the apex in 16 t ha<sup>-1</sup>.





**Figure 1.** Redundancy analysis (RDA) among enzymatic activity, chemical and microbiological attributes of sandy Entisol cultivated with beans and treated with biochars of coffee grounds (CG) and coffee husks (CH) in Northeastern Brazil. *Bet*=  $\beta$ -Glucosidase; *Ure*= urease; *Alk. Pho*= alkaline phosphatase; *Aci. Pho*= acid phosphatase; *FDA*= fluorescein diacetate; *MBC*= microbial biomass carbon; *TOC*= total organic carbon; *Heig45*= Height; *Diam 45*= Diameter; *SDM*= shoot dry matter.

The activity of *Bet*, *TOC* and *FDA* followed the growth of *MBC* in soils with biochar of coffee grounds (CG). The *Aci pho* activity was higher in the control soil and those treated with CG in the initial doses (4 and 8 t ha<sup>-1</sup>), following the concentrations of Al<sup>3+</sup> and potential acidity (H<sup>+</sup> + Al<sup>3+</sup>). Both showed a negative correlation with pH and soil bases, showing that biochars progressively decreased *Aci pho* activity as they contributed to the increase of SB, V% and soil P in the soil (Figure 1).

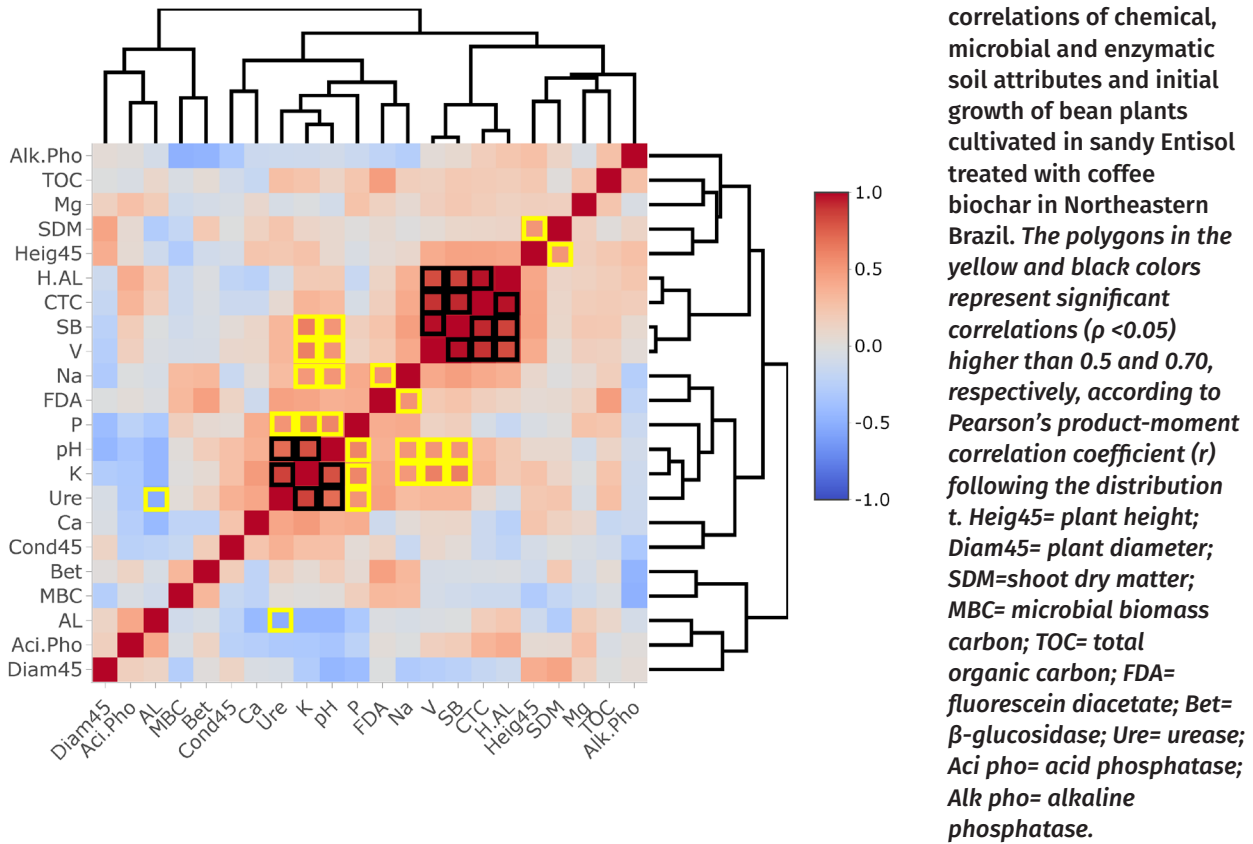
The variables of chemical, microbiological, and enzymatic activities showed significant linear correlations ( $\rho < 0.05$ ), mainly the positive ones between the *Ure* activity with the concentrations of K (0.86) and the pH (0.70) of the soil. Both variables also showed significant correlations with labile P contents, but with lower

intensity (0.5 < r < 0.7). The only relevant negative correlation occurred between the *Ure* activity and the Al<sup>3+</sup> levels in the soil (-0.51). *CTC*, *SB*, and *V%* presented significant mutual relationships all above 0.9. Both also correlated strongly with the potential acidity (H<sup>+</sup> + Al<sup>3+</sup>). Significant moderate correlations occurred between K and pH, both positive for Na, SB, and V% (0.5 < r < 0.7). We observed similar relations between Na and *FDA* (0.53) and between *Heig45* and dry matter (*SDM*) of bean plants (0.52) (Figure 2).

## DISCUSSION

The different source and doses of coffee biochar applied to sandy Entisol no affected bean growth that corroborated with Sanger et al. (2017) that applied biochar to the sandy soil cultivated with winter wheat, winter rye and maize during 3-year, but were not affected the crop yields, only improved availability of plant nutrients in the first year. However, other studies have shown that the influence of biochar on plant growth is due to the porous characteristic of biochar, which provides adequate habitats and substrates, increasing the microbial activity for the degradation of the minerals present in the soil (Saxena et al. 2013, Zhang et al. 2014, Rawat et al. 2019). These microorganisms not only mobilize biodegradable substances but also provide micronutrients and other beneficial elements that promote plant growth (Mukherjee & Zimmerman 2013). For example, Saxena et al. (2013) evaluated the effect of biochar on bean production and observed that biochar impacts on French beans growth. Here, the biochar from coffee residues no impact in bean growth evaluated with 45 days, probably due to the co-application of biochar with plant growth promoting rhizobacteria that help the growth of the French bean.





In this sense, Park et al. (2011) confirmed that biochar affected plant growth and that the efficiency varies with the type and amount applied. Prapagdee & Tawinteung (2017) used biochar obtained from the cassava stem, on the growth, uptake, and translocation of nutrients in green bean plant (*Vigna radiata* L.). The authors showed that biochar improves the soil quality of bean, being efficient for the plant development, such as height, and seed production, corroborating in part with the present study that applied coffee biochar on sandy soil that is poor in nutrients and low soil organic carbon (Lima et al. 2018) that showed no effect only in soil attributes.

The application of coffee biochar did not increase the SDM of bean plants cultivated in sandy Entisol. However, forms of biochars are

generally intended to increase crop yields, and there is evidence that this can be successful and there may also be short-term adverse effects on yields (Galinato et al. 2011). These effects may be related to degradation or soluble organic phytotoxic compounds that are associated with pyrolysis carbonization (Borchard et al. 2014). Here, the small increase of the SDM of bean plants that received CH biochar was due to sequester C in the decomposition of TOC and because of a better establishment of plants due to the improvement of environmental humidity with the addition of biochar (Biederman et al. 2017).

The small difference compared the two coffee biochars was related to the short time of evaluation or the low doses of biochar applied to the soil. In this sense, Reed et al. (2017) analyzing the effect of long-term wood-derived biochar on

soil quality and productivity, also observed that their applied biochar doses were insufficient and therefore did not show significant changes in productivity. Probably the administered dose of biochar was inadequate or the time of evaluation was not adequate for the biochar to influence the characteristics of the plants in an indirect way that is, for example, through the efficiency of the use of water (Lima et al. 2018), the soil structure and the microorganisms (Lehmann et al. 2011).

On the other hand, the coffee biochars showed a significant increase in soil chemical attributes and pH due to the numerous ions present on the surface of the biochar or by the association of the soil with biochar that can mobilize the microbial activity that degrades the soil components making the nutrients available to the plant (Liu et al. 2017). The pyrolysis leads to the accumulation of alkaline substances on the surface of the biochar, which increases soil pH and is linked to the availability of nutrients, such as phosphorus among others (He et al. 2017). The biochar applied to acid soils, exert the function of correction, as observed in the present study that showed the highest doses, the most elevated pH, especially in soil that received CH biochar.

The soil pH, P, K<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup> are essential for plant growth, and the interaction of these variables with soil (physical, chemical and microbiology attributes) can positively or negatively affect plants. In this sense, the addition of biochar in the soil can alter the dynamics of these variables. The higher content of K<sup>+</sup> and Ca<sup>2+</sup> is by the presence of these elements in the biochar added and may be related to the negatively charged surface of the biochar that attracts cations as K<sup>+</sup> and Ca<sup>2+</sup> through electrostatic interactions (Godlewska et al. 2017).

The pH was the variable that responded more to the variation of the treatments with different doses and types of coffee biochar applied to the Entisol cultivated with beans in multivariate analysis. The variable pH is an essential feature of soils in nutrient availability and plant growth. Most plants have an ideal pH range, where maximum growth and yield can usually be achieved between 6 and 6.5. In this way, raising the pH allows the plants to grow to their full potential when other requirements such as availability of water and macronutrients are met. When high pH biochar changes soil pH, calcium levels increase and reduce aluminum toxicity (Agegnehu et al. 2017). Biochars are, in most cases, alkaline, thus contributing to the liming and sorption effects of cations in soils (Qui et al. 2017).

The volume and size of the pores, the specific surface area, and the particle size of the biochar are vital parameters in the definition of the physical and sometimes chemical properties of the biochar, as observed by Lima et al. (2018) that used the same biochar of the present study. In this sense, the C amount in biochar is related to its porosity and to the temperature of the pyrolysis that was relatively high (> 500 °C). Besides, the presence of limited amounts of oxygen serves to oxidize the carbon in CO<sub>2</sub>, thus reducing the carbon content of the biochar (You et al. 2017). Therefore, the highest C amount in CG biochar is due to its lower porosity (it has more upper activation contact).

The coffee biochar incorporated into the soil stimulated the increase in the SOC in the higher doses. This increase is due to higher doses of biochar that provide more elevated amounts of stable carbon to the soil, increasing SOC (Zhou et al. 2017). Hartley et al. (2016) applied wood biochar in sandy soils and showed improvements on the SOC in all soils treated with biochar due to the stability of the

structural components that improves through the application of the biochar interacting with soil minerals.

The increase MBC in this study was due to greater availability of soil organic matter (substrate) or by the amount of volatile matter, the labile or extractable carbon and the nutrients present in the biochar available to the soil (Zhou et al. 2017). The microbial growth in the soil is potentially promoted after the addition of biochar in the short term, although the microbial biomass was smaller about the carbon. This element presents a potential for the performance of this biochar with the habitat suitable for microbial growth (Lehmann et al. 2015). The increase of MBC is due to the addition of biochar, which has a large surface area that provides favorable microhabitat for soil microbial communities (Khadem & Raiesi 2017). Xu et al. (2016) observed an increase in MBC in the highest concentrations of corn biochar related to the control due to C labile of biochar that increased the microbial frequency of the soil that will have a possible nutrient cycle, corroborating with the present study.

Biochar may modify soil microorganism conditions, including abiotic factors such as available C, nutrients, pH, toxic matter and water content; and biotic factors such as different habitats may lead to changes in the structure of the microbial community. In general, the porous structure of biochar may provide habitat for microbial communities, which protect them from predators, and soluble organic carbon and other nutrients adsorbed by biochar may provide substrates necessary for its development (Wang et al. 2017).

Several enzymes hydrolize fluorescein diacetate (FDA) that indicated the quantification of active cells and to characterize soil microbial activity. Their increase in response to the higher doses of the different types of coffee biochars in

sandy Entisol cultivated with bean suggests that such treatments may stimulate the microbial community due to the high content of organic matter present in the soil (Bera et al. 2016). Sarma et al. (2017) found increases of FDA in soil due application of biochar that contributed to the rise of organic matter that is due to the activity of the FDA, as in the present study.

The coffee biochar added to soil cultivated with beans increased Bet activity (C cycle) that is an enzyme involved in carbon mineralization from the degradation of exogenous organic matter (Kader et al. 2017). This enzyme is highly associated with the availability of carbon substrate that is quickly mineralized and is a driver for changes in enzymatic activity that may increase due to the addition of biochar (Luo & Gu 2016). Biochar changes the enzymatic activity through the substrate, and this change varies with the nature of the residues and adsorbs the substrate-enzyme to hydrolyze it (Sun et al. 2014). Günal et al. (2018) evaluated the effects of different types of biochar and showed an increase in the activity of the  $\beta$ -glycosidase enzyme in sandy loam soils when compared to clayey soils with the use of bean and rice husk residues that corroborate our findings with coffee biochar applied to sandy soils.

Here, Ure activity increased in the higher doses of CH biochar. The increases of Ure activities is due to the presence of nitrogen compounds contained in the biochar which acts as a substrate (Huang et al. 2017) because biochar acts as a storehouse of carbon and nutrients, which favors soil microbial growth (Lehmann et al. 2011). Wang et al. (2015) analyzed the effect of the application of corn straw biochar on the soil Ure activity, showed significant increases in this enzyme because biochar presented organic and inorganic compounds that affected that related to the N cycle.

The Pac present in the extracellular substances (EPS) and the bacterial cell wall is a group of enzymes responsible for catalyzing a vast variety of phosphomonoesters and transphosphorylation reactions in acidic pH medium (Behera et al. 2017). Biochar has an essential influence on microorganisms that are associated with soil nutrient transformations and may be susceptible to changes after the addition of biochar, which has several effects on soil chemical properties, such as pH (Lehmann et al. 2011). According to Jin et al. (2016), evaluating the influence of manure biochar on soil properties showed that Pac activity decreased with the addition of substrate, occurring variations caused by biochar influences on pH and soil phosphorus composition.

The coffee husk biochar at the dose of 4 Mg ha<sup>-1</sup> showed higher Alk pho due to the availability of nutrient and the absorption of phosphorus that is present in the substrate. Higher doses of coffee biochar should have inhibited Alk pho activity (Abujabhah et al. 2016) because the biochar has a direct relationship with the microorganisms that transform the nutrients present in the soil (Lehmann et al. 2011), and may have had some interference in the production of this enzyme in Entisol cultivated with beans. Al Marzooqi et al. (2017) analyzed the influence of the biochar derived from *Salicornia bigelovii* (green salt) showed that the increase of Alk pho was due to the physicochemical interactions with the biochar. Jin et al. (2016) showed similar results analyzing the influence of biochar produced from swine manure, showed that the Alk pho increase related to microbial proliferation and increased availability of nutrients.

## CONCLUSIONS

Coffee husk biochar increased pH, Ca, and K, also contributing to the reduction of toxic aluminum contents and raising the concentrations of P labile. We recommend the application of CH biochar at a dosage of 12 to 16 t ha<sup>-1</sup>. Enzymatic activities and chemical attributes of soils treated with higher doses (12 to 16 t ha<sup>-1</sup>) of husk and grounds coffee biochars tend to be similar due to the rapid increase in P contents, bases, and β-glucosidase. At lower doses, the amount of readily available nutrients is smaller and depends on the type of substrate, providing a more considerable distinction between the effects of husk and coffee grounds biochars. We showed the first report on the beneficial impact of coffee biochar on enzymatic activities and microbiological attributes of an Entisol cultivated with the bean.

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