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Article

Coffee waste as an eco-friendly and low-cost alternative for biochar production impacts on sandy soil chemical attributes and microbial gene abundance

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ABSTRACT: Biochar is a material produced by the pyrolysis of agro-industrial waste, which has become one of the most promising management tools to improve soil quality. The aim was to determine the effects of incorporating biochar from different coffee wastes in sandy soil, cropped with maize, on soil chemical and microbial attributes. The experiment followed a factorial design 2 × 3 + 1 with two types of biochar, including coffee ground (CG) or coffee husk (CH) in 3 doses (4, 8, and 16 t·ha^{-1}) and a control fertilized solely with bovine manure (3 t·ha^{-1}). The variables analyzed were soil organic carbon, chemical attributes, microbial biomass (C, N and P), soil basal respiration and microbial gene abundance (16S rRNA, 18S rRNA and *nif*H gene). Most chemical attributes were strongly increased by CH application, while CG at 8 t·ha^{-1} increased the soil C:N ratio (3.5 times), P (2.1 times) and K+ (7.9 times) and at 4 t·ha^{-1} increased the C content, microbial biomass C and N (3, 2.1 and 1.6 times, respectively). The application of CG biochar at 16 t·ha^{-1} showed trend to increase the abundance of bacteria, fungi and diazotrophic genes (11, 10 and 2%, respectively). Contribution of both coffee biochar types, but mainly CH, was more effective than the soil that received organic manure alone. Biochar from coffee wastes is a promising tool to improve sandy soil quality.

Key words: biocarbon, 16S rRNA, 18S rRNA, *nif*H.

INTRODUCTION

Excessive application of chemical fertilizers for improved plant growth and yield has become a serious problem around the world due to excessive losses to the environment leading to both eutrophication of water bodies and greenhouse gas emissions. On the other hand, intensive agriculture depletes soil nutrients, which must be furnished by the farmer. Therefore, there has been an increasing search for alternatives that reuse more waste, are accessible to producers and are environmentally friendly, such as biochar (Liu et al. 2018).

More than 200 million tons of biomass from agro-industry wastes are not exploited in Brazil and its usage would be advantageous (Martinez et al. 2019). Thus, biochar may serve as an economic alternative to producers by reducing dependence on chemical fertilizers, as well as reusing agro-industrial waste with potential applicability on several crops (Lima et al. 2018). Biochar is a carbonaceous-rich material produced from biomass waste by pyrolysis with a wide temperature range between 100 and 700 °C (Rangabhashiyam and Balasubramanian 2019).

The addition of biochar from different sources to acid soils improve soil quality and plant growth due to the alkalinity to neutralize soil acidity and a high pH buffering capacity (especially when pyrolyzed at high temperatures). Al^{3+} can be precipitated to less toxic compounds with the alkaline oxides, carbonates and silicates in biochar. The biochar shift in pH and the reduction in Al caused by biochar improves the availability of P, Ca and Mg, resulting in a balanced nutrient supply in the rhizosphere, thus enhancing the productivity of cultivated plants (Yu et al. 2019).

The application of biochar from different residues improves soil quality and crop performance, increasing carbon sequestration (Tan et al. 2017), soil porosity, soil aggregate stability, the activity of beneficial microorganisms and soil fertility (Pranagal et al. 2017). The improvements promoted by biochar on the chemical and physical attributes of the soil contribute to increase the microbial activity, carbon, nitrogen and phosphorus contents of nutrient cycling and increases the microbial colonization rate, since the pores of the biochar serve as habitat for microorganisms and protect against attack by predators (Singh et al. 2018). Some studies have also shown that biochar applied to the soil at different doses increase fungi, bacteria and microorganism populations involved with nitrogen cycling (Liu et al. 2019; Semida et al. 2019; Medeiros et al., 2021). For example, some remarkable increases in copy numbers or specific gene diversity were reported, leading to modifications in the composition and abundance of a microbial community (Zheng et al. 2016).

One possible agro-industrial residue for biochar production is the coffee industry, which has a wide availability of both grain and husk wastes with low cost and high nutrient content (Tsai et al. 2012; Lima et al. 2018). However, this possible reuse as biochar feed material is not yet known, as well as its impact on sandy soils.

This study evaluated the reuse of agro-industrial wastes and application of two biochar types from coffee (*Coffea arabica*) on sandy soil. The objectives of the present study were to demonstrate the impact of two types of biochar and cow manure applied to sandy soil on soil quality, specially examining 1) soil chemical attributes; 2) microbial biomass carbon, nitrogen and phosphorus (MBC, Nmic and Pmic); 3) soil basal respiration (SBR) and metabolic quotient (qCO_2); and 4) microbial gene abundance (16S rRNA, 18S rRNA and *nif*H).

MATERIAL AND METHODS

Soil collection and characterization

The soil used in the experiment was collected from the 0–20 cm layer of a native Brazilian tropical dry sub-humid forest of Pernambuco (latitude 8°48'34.2"S, longitude 36°24'29.3"W) at 705 m above mean sea level. According to the Köppen classification system, the climate in the region is hot and humid ('As'). The mean total annual rainfall is 782 mm and the mean annual air temperature is 23.2 °C. The topography of the area is flat to smooth-wavy. Its original vegetation cover was Caatinga, which is a tropical deciduous forest, with Fabaceae, Euphorbiaceae and Cactaceae (Barros et al. 2019) as the main genera. The soil is a lamellic eutric regosol, according to the classification system of the food and agriculture organization, with almost 90% sand and < 5% clay and it had 1.6% total C, 0.24% total N, pH of 5.1, extremely low values of cation exchange capacity (CEC) (3.98), specific surface area of 0.05, total porosity of 0.433 $m^3 \cdot m^{-3}$ and field capacity of 0.135 $m^3 \cdot m^{-3}$. Its mineral composition was quartz (90%), plagioclase and K-feldspar (5%), kaolinite, mica and traces of iron oxide (Lima et al. 2018).

Biochar production and properties

Two common agro-industrial wastes (coffee husk [CH] and coffee grounds [CG]) from the coffee industry were used as biochar feedstock. Both were charred during 10 to 12 h under oxygen limited conditions in a slow pyrolysis process where temperature reached 530 °C. The final CH biochar contained 67.11% total C, 2.05% total N and had a pH of 10.31, CEC of 22.54, specific surface area (SSA) of 244, while the final CG biochar contained

68.81% total C, 4.30% total N and had a pH of 9.65, CEC of 5.56, SSA of 23.5. Other important biochar properties are listed in Lima et al (2018).

Experiment design

The experimental design was completely randomized, distributed in a factorial scheme (2 × 3 + 1) with two types of biochar (CH and CG) in 3 doses (4, 8 and 16 $t \cdot ha^{-1}$) and one additional treatment without biochar, with 10 repetitions. Pot experiments were performed in a greenhouse in Garanhuns, Pernambuco, Brazil (08°53'25"S and 36°29'34"W) with an elevation of 896 m and mesothermal tropical altitude (Cs'a) climate, according to Köppen, with annual average temperature and precipitation of 20 °C and 1,300 mm, respectively (Lima et al. 2018). All pots of 4 L (0.19 m of diameter at the top, 0.13 m diameter at the bottom and 0.15 m high) received 3 $t \cdot ha^{-1}$ of bovine manure. Phosphorus and potassium fertilization were carried out on the day of planting, nitrogen was applied 21 days after planting in all pots. Phosphorus and potassium fertilization were carried out on the day of planting, nitrogen was applied 21 days after planting in all pots, according to IPA Recommendation (Cavalcanti 2008) of Pernambuco state (0.08 g of N; 0.08 g of P and 0.06 g of $K \cdot pot^{-1}$ in the forms of urea, single superphosphate [SSP] and KCl). Four seeds of maize cultivar 1058 frequently used in the region were seeded per 5 kg pot and thinned to one plant per pot a week after emergence. The pots were irrigated every two days with distilled water to maintain soil at field capacity. After 45 days of sowing, the soil samples were collected for chemical and microbiological analysis and one part was stored in 2 mL microtubes inside the freezer for molecular analyses at –20 °C. The mixture of five subsamples formed a composite sample in each pot that was considered an experimental plot.

Soil chemical and microbiological attributes

Samples were air-dried, homogenized and sieved at 2 mm mesh screen. Soil chemical attributes were measured according to Silva (2009), with Ca^{2+}, Mg^{2+}, Al^{3+} extracted by KCl at 1.0 $mol \cdot L^{-1}$ and K^+, Na^+, P, by $Mehlich^{-1}$. Ca^{2+} and Mg^{2+} were determined by atomic absorption spectrophotometry, K^+ and Na^+ were determined by flame photometry, while P by colorimetry and Al^{3+} by titration.

Total organic carbon (TOC) was determined according to Yeomans and Bremner (1988). From the results obtained, the bases sum (BS) and the CEC were calculated. Soil carbon (C) and nitrogen (N) contents were determined by combustion with an CHNS-O elemental analyzer (Perkin Elmer PE-2400).

Carbon microbial biomass, Nmic and Pmic were estimated by irradiation-extraction according to Mendonça and Matos (2017). Carbon and nitrogen microbial contents were extracted with 0.5 $mol \cdot L^{-1}$ of potassium sulfate (K_2SO_4), 80 mL per sample, with pH adjusted to 6.5–6.8 and P was extracted with 0.5 $mol \cdot L^{-1}$ sodium bicarbonate ($NaHCO_3$). Soil basal respiration was quantified according to Alef and Nannipieri (1995) and the qCO_2 was calculated according to Anderson and Domsch (1993) as the relation between SBR and MBC.

Total genomic DNA was extracted from 0.5 g freeze-dried soil of each sample using a PureLink microbiome DNA purification kit (Life Technologies, Carlsbad, USA) according to the manufacturer instructions. The purity and integrity of extracted DNA were detected by 1% agarose gel electrophoresis at 100 V for 30 min in 0.5 × TBE buffer (Tris, Borate, EDTA) added to the SYBR Gold dye (Invitrogen, Breda, Netherlands). The DNA concentration was determined by fluorometry using a Qubit fluorometer (Life Technologies, USA) and the Quant-iT dsDNA BR kit (Life Technologies, USA). The extracted genomic DNA was stored at –20 °C for later use.

Total abundance of bacteria (16S rRNA gene), fungi (18S rRNA gene) and diazotrophs (*nif*H gene) were quantified by real-time polymerase chain reaction (qPCR). The quantifications were performed with a LightCycler 480 (Roche Applied Science), using the SYBR green I system. The reactions for the respective genes were performed in a 10 µL volume containing 5 µL Platinum Quantitative PCR SuperMix-UDG (Life Technologies - Invitrogen, Grand Island, NY, USA), 10 $\mu mol \cdot L^{-1}$ of the specific primers for each gene and 1 µL of DNA. Detailed information on the qPCR reaction procedures, primers and reaction conditions are described in Table 1.

Table 1. Primers and cycling conditions used to amplify the target genes.

Primers PCR-DGGE	Primer sequence (5'-3')	Thermal cycling conditions
	Total bacteria (16S rRNA)	
341f-GC[1]	CGCCCGCCGCGCGCGGCGGGCGGGGCGGGG GCACGGGGGGCCTACGGGAGGCAGCAG	95 °C 10 min, 1 cycle; 95 °C 1 min, 57 °C 1 min, 72 °C 3 min, 30 cycles; 72 °C 10 min, 1 cycle
518r[1]	ATTACCGCGGCTGCTGG	
	Total fungi (18S rRNA)	
EF4[2]	AAGGG(G/A)TGTATTTATTAG	94 °C 5 min, 1 cycle; 94 °C 1 min, 55 °C 30 s, 72 °C 90 s, 34 cycles; 72 °C 5 min, 1 cycle
ITS4[3]	CAGGAGACTTCTACACGGTCCAG	
ITS1f-GC[4]	CCCCCGCCGCGCGCGGCGGGCGGGGCGGGG GCACGGGCCGCTTGGTCATTTAGAGGAAGTAA	94 °C 5 min, 1 cycle; 94 °C 30 s, 55 °C 30 s, 72 °C 30 s, 34 cycles; 72 °C 5 min, 1 cycle
ITS2[5]	GCTGCGTTCTTCATCGATGC	
	N-fixing bacteria (nifH)	
FGPH19[6]	TACGGCAARGGTGGNATH	95 °C 5 min, 1 cycle; 95 °C 1 min, 55 °C 1 min, 72 °C 2 min, 30 cycles; 72 °C 10 min, 1 cycle
PolR[7]	ATSGCCATCATYTCRCCG	
PolF-GC[7]	CGCCCGCCGCGCCCCGCGCCCGGCCCGCCG CCCCCGCCCCTCCGAYCCSAARGCBGACTC	94 °C 5 min, 1 cycle; 95 °C 1 min, 48 °C 1 min, 72 °C 2 min, 30 cycles; 72 °C 10 min, 1 cycle
AQER[7]	ACTATGTAGATYTCCTG	
Primers qPCR	**Primer sequence (5'-3')**	**Thermal cycling conditions**
	Total bacteria (16S rRNA)	
341f[1]	CCTACGGGAGGCAGCAG	95 °C 5 min, 1 cycle; 95 °C 10 s, 60 °C 10 s, 72 °C 30 s, 40 cycles
518r[1]	ATTACCGCGGCTGCTGG	
	Total fungi (18S rRNA)	
ITS1f[8]	TCCGTAGGTGAACCTGCGG	95 °C 15 min, 1 cycle; 95 °C 1 min, 53 °C 30 s, 72 °C 1 min, 40 cycles
5.8S[8]	CGCTGCGTTCTTCATCG	
	N-fixing bacteria (nifH)	
FGPH19[6]	TACGGCAARGGTGGNATH	95 °C 5 min, 1 cycle; 94 °C 1 min, 57 °C 45 s, 72 °C 1 min, 30 cycles; 72 °C 7 min, 1 cycle
PolR[7]	ATSGCCATCATYTCRCCG	

[1]Muyzer et al. (1993); [2]Smit et al. (1999); [3]White et al. (1990); [4]Gardes and Bruns (1993); [5]Anderson et al. (2003); [6]Simonet et al. (1991); [7]Poly et al. (2001); [8]Fierer et al. (2005).

The polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) profiles were performed using a vertical D-code system (Bio-Rad Laboratories, USA). The 8% DGGE polyacrylamide gels were prepared with a denaturing gradient of 15 to 55% for 16S rRNA, 20 to 55% for *nif*H and 30 to 45% for 18S rRNA. Denaturation of 100% consisted of the concentration of 7 M urea and 40% formamide. The gels for total bacteria and *nif*H were electrophoresed for 3 h at 200 V at 60 °C, while for total fungi, the gels were electrophoresed for 16 h at 100 V. After electrophoresis, the gels were stained with SYBR Gold (Invitrogen, Breda, The Netherlands) in 0.5 × TAE (Tris, acetate, EDTA) in the dark for 40 min and photographed under ultraviolet light using an E-BOX VX2 UV transilluminator. The gels were evaluated using the Gel Analyzer 2010 program. The structural similarity of the bacteria, fungi and diazotrophs communities was determined based on the presence or absence of amplicons detected after DGGE.

Statistical analysis

All statistical and exploratory analyzes were performed on the R language (build version 3.4.3, R Core Team (2020)).

Data were submitted to normality test, using the Shapiro–Wilk statistic, analysis of variance and, when appropriate, the means compared by Tukey's test at 5% of significance, while orthogonal contrasts were made between the factorial

treatments and the control. Dunnett's test at the 5% significance level was also used to compare the control with each biochar treatment. Canonical analysis of principal coordinates (CAP) ordination was calculated through the "vegan" library, a useful method of constrained ordination for ecology (Anderson et al. 2003). Biplot graphics were made through the ggplot2 library and the heatmaps graphics were built with the heatmaply library with correlations calculated by the Pearson coefficient for parametric data.

RESULTS AND DISCUSSION

Characterization of biochar and soil chemical attributes

The characterization of the different biochars (pH, P, Ca, Mg, K, Al, Na, cation exchange capacity, C, N, specific surface area, phosphorus adsorption isotherm, field capacity and wilting point) are shown in a previous paper (Lima et al. 2018). In summary, biochar soil mixtures properties were significantly affected by type of coffee waste and dose used (Table 2).

Table 2. Chemical attributes in a sandy soil that received different doses of biochar from CG and CH, cultivated with maize.

	pH [1]									
	4 t·ha^{-1}		**8 t·ha^{-1}**		**16 t·ha^{-1}**		**Mean**		**Model [4]**	**R²**
CG	**5.02** [2]	b	**5.08**	b	**5.27**	a	5.12		f(x) = 5.12	ns
CH	**5.62**	aB	**6.04**	aA	**5.17**	aAB	5.61		f(x) = 0.047x + 6.05	0.45
Mean	5.32		5.56		5.22		**5.37**	[3]		
Control							4.43			
	N (g·kg^{-1})									
	4 t·ha^{-1}		**8 t·ha^{-1}**		**16 t·ha^{-1}**		**Mean**		**Model**	**R²**
CG	**40.95** [4]		16.82		26.17		27.98	a	f(x) = 27.98	ns
CH	33.91		**69.06**		38.85		47.27	a	f(x) = 47.27	ns
Mean	37.43		42.94		32.51		37.63			
Control							42.39			
	C (g·kg^{-1})									
	4 t·ha^{-1}		**8 t·ha^{-1}**		**16 t·ha^{-1}**		**Mean**		**Model**	**R²**
CG	**82.02**		**83.86**		**76.57**		80.82	b	f(x) = 80.82	ns
CH	**153.10**		**122.13**		**122.15**		132.46	a	f(x) = 132.46	ns
Mean	117.56		103.00		99.36		**106.64**			
Control							51.08			
C:N ratio	**4 t·ha^{-1}**		**8 t·ha^{-1}**		**16 t·ha^{-1}**		**Mean**		**Model**	**R²**
CG	2.55	b	**5.25**	a	3.45	a	3.75		f(x) = 3.75	ns
CH	5.81	a	2.22	b	3.22	a	3.75		f(x) = 3.75	ns
Mean	4.18		3.74		3.34		**3.75**			
Control							1.51			
	Na^+ ($Cmol_c$·kg^{-1}·soil)									
	4 t·ha^{-1}		**8 t·ha^{-1}**		**16 t·ha^{-1}**		**Mean**		**Model**	**R²**
CG	**0.14**	a	**0.14**	a	**0.14**	a	0.14		f(x) = 0.14	ns
CH	**0.16**	a	0.02	b	**0.13**	a	0.10		f(x) = 0.10	ns
Mean	0.15		0.08		0.14		**0.12**			
Control							0.10			

continue...

Table 2. Continuation...

	K^+ ($Cmol_c \cdot kg^{-1} \cdot soil$)									
	4 t·ha^{-1}		**8 t·ha^{-1}**		**16 t·ha^{-1}**		**Mean**		**Model**	**R^2**
CG	**0.13**	B	**0.18**	AB	**0.26**	A	0.19	b	f(x) = 0.011x + 0.086	0.99
CH	**0.43**		**0.63**		**0.33**		0.46	a	f(x) = 0.46	ns
Mean	0.28		0.40		0.30		**0.33**			
Control							0.08			
	P (mg·kg^{-1})									
	4 t·ha^{-1}		**8 t·ha^{-1}**		**16 t·ha^{-1}**		**Mean**		**Model**	**R^2**
CG	**8.11**	a	**9.55**	b	**10.21**	a	9.29		f(x) = 9.29	ns
CH	**8.04**	a	**15.25**	a	**7.13**	b	10.14		f(x) = 10.14	ns
Mean	8.08		12.40		8.67		9.71	A		
Control							7.06	B		
	$H^+ + Al^{3+}$ ($Cmol_c \cdot kg^{-1}$)									
	4 t·ha^{-1}		**8 t·ha^{-1}**		**16 t·ha^{-1}**		**Mean**		**Model**	**R^2**
CG	**3.93**	aAB	**4.21**	aA	**3.66**	bB	3.93		f(x) = -0.029x + 4.21	0.43
CH	**3.88**	aB	**4.48**	aA	**4.32**	aAB	4.23		f(x) = 0.028x + 3.96	0.31
Mean	3.91		4.35		3.99		**4.08**			
Control							4.37			
	Ca^{2+} ($Cmol_c \cdot kg^{-1} \cdot soil$)									
	4 t·ha^{-1}		**8 t·ha^{-1}**		**16 t·ha^{-1}**		**Mean**		**Model**	**R^2**
CG	**0.65**		**0.79**		**0.58**		0.67	a	f(x) = 0.67	ns
CH	**0.67**		**0.72**		**0.63**		0.67	a	f(x) = 0.67	ns
Mean	0.66		0.76		0.60		0.67			
Control							0.62			
	Mg_{2+} ($Cmol_c \cdot kg^{-1} \cdot soil$)									
	4 t·ha^{-1}		**8 t·ha^{-1}**		**16 t·ha^{-1}**		**Mean**		**Model**	**R^2**
CG	**1.074**		**1.067**		**1.005**		1.049	a	f(x) = 1.05	ns
CH	**1.011**		**1.073**		**0.992**		1.026	a	f(x) = 1.03	ns
Mean	1.043		1.070		0.999		1.037	A		
Control							1.055	A		
	Al^+ ($Cmol_c \cdot kg^{-1} \cdot soil$)									
	4 t·ha^{-1}		**8 t·ha^{-1}**		**16 t·ha^{-1}**		**Mean**		**Model**	**R^2**
CG	**0.150**	a	**0.133**	a	**0.167**	a	0.150		f(x) = 0.15	ns
CH	**0.100**	b	**0.083**	b	**0.194**	a	0.126		f(x) = 0.0087x + 0.044	0.79
Mean	0.125		0.108		0.181		**0.138**			
Control							0.217			
	TOC (g·kg^{-1})									
	4 t·ha^{-1}		**8 t·ha^{-1}**		**16 t·ha^{-1}**		**Mean**		**Model**	**R^2**
CG	**10.65**		**11.30**		**12.25**		11.40	a	f(x) = 11.40	ns
CH	**11.69**		**10.94**		**13.27**		11.96	a	f(x) = 11.96	ns
Mean	11.17		11.12		12.76		**11.68**		f(x) = 0.142x + 10.353	0.87
Control							10.11			

(1) The analysis of variance was performed at the 5% significance level, where the absence of letters indicates that there were no significant differences (ns). Means followed by different lowercase letters between columns and uppercase letters between lines differed, using the Tukey's test at the 5% level of significance.
(2) The means in **bold** differed from the control according to the Dunnett test at the level of 5% of significance.
(3) Overall mean in **bold** differed from the control according to the Helmet's contrast at the 5% significance.
(4) Regression analysis was performed for the quantitative variables (increasing doses of the biochar) with different variances and the corresponding linear model [*f(x)*] was shown for each biochar, followed by the adjusted R^2. In the case of data with equal variances (ns), the means were considered similar and the global mean (constant) was calculated.

Soil pH, C, C:N, Na^+, K^+, P and TOC contents all increased with the application of biochar, while the H + Al and Al^{3+} decreased, with higher values found for CH than for CG biochar (Table 2) for pH, P and C:N ratio, while Al^{3+} was lower.

Amoah-Antwi et al. (2020) showed that, usually, biochar increases soil pH and can increase Ca^{2+} and Na^+ availability and reduce the solubility of metals, including Al, as also found by Gul et al. (2015). Pandian et al. (2016) demonstrate that the soil pH increase is due to alkaline pH from the biochar (here, CH = 10.31 and CG = 9.65). Biochar pH is linked both to pyrolysis temperature and feedstock, as wood-based biochar tends to have higher pH than from crop waste and manure (Gul et al. 2015). According to Tomczyk et al. (2020), higher pH with increasing temperature has been associated with increases in ash content and oxygen functional groups that occur during pyrolysis. As the two types of biochar (CH and CG) were produced in high pyrolysis temperature (> 500 °C), the resulting alkaline pH promoted a decrease in soil acidity, when compared to the control (Table 2). The high pH of biochar is explained by the varying concentrations of alkaline ash in its composition, which are added to the soil as oxides of Ca, Mg, K, hydroxides and carbonates (Han et al. 2020).

Here, the applied biochar increased the main cations and nutrients (Ca^{+2}, K^+, Na^+, C, N, P) and their N content was reduced, as found in previous reports (Lima et al. 2018; Martins Filho et al. 2020)[5]. A point to consider is that, while both CG and CH are coffee by-products, both are obtained from different coffee processing stages. While CG has already been used for actual coffee preparation and thus submitted to water extraction, this is not the case for CH. It is likely that the water extraction during coffee preparation for CG biochar reduced nutrient availability in comparison to that from CH biochar. For example, CH biochar at 8 $t \cdot ha^{-1}$ increased P (2.1 times) and K^+ (7.9 times) compared to the control. The biochar effect on P availability has been previously reported (El-Eyuoon and Amin 2020).

Coffee husk biochar at 4 $t \cdot ha^{-1}$ increased C content by 67% compared to the control, reinforcing the understanding that biochar is a C rich product that contains aromatic compounds, representing an excellent alternative for C sequestration in agriculture soils (Sohi et al. 2010; Zimmerman et al. 2011).

Lima et al. (2018) stated that CH biochar at 16 $t \cdot ha^{-1}$ was the treatment that sequestered most C in the soil and that it is an effective alternative for C sequestration in agricultural systems as a consequence of the higher stability of C compounds in biochar compared to other organic waste compounds, as also found with biochar in an alkaline soil from a semi-arid region (El-Eyuoon and Amin 2020).

The CG biochar at 8 $t \cdot ha^{-1}$ increased the soil C:N ratio (3.5 times), which was also found to limit N availability and to change the soil dissolved organic C and N substrates and soil microbial community composition and gene abundance in other research (Van Zwieten et al. 2014). The underlying mechanisms for these effects might vary with biochar feedstocks (Lima et al. 2018).

Soil microbial biomass (C, N and P), basal respiration and microbial quotient

Microbial biomass carbon for CH at 4 $t \cdot ha^{-1}$ was 210% higher than for the control soil (Table 3), likely due to the higher labile C and soil organic matter and nutrient availability (Zhou et al. 2017), even for a short time scale, because biochar reportedly provides a suitable habitat for microbial growth (Khadem and Raiesi 2017). This is considered to be due to biochar pores protecting microbes from predators and to C organic matter and nutrients supplying the substrate necessary for their proper development (Wang et al. 2017).

However, MBC was significantly higher for soils receiving CH biochar than CG at all doses, which might be due to its higher specific surface (Lima et al. 2018). The type of waste, pyrolysis temperature, type and soil conditions are variable, which have been shown to influence MBC increments (Zhou et al. 2017).

5. Martins Filho, A. P., Medeiros, E. V., Lima, J. R. S. L., Duda, G. P., Silva, W. M., Antonino, A. C. D., Silva, J. S. A. S., Oliveira, J. B. and Hammecker, C. (2021) Impact of coffee biochar on carbon, microbial biomass and enzyme activities of a sandy soil cultivated with bean. Anais da Academia Brasileira de Ciências. In press.

Even the lower dose of CH biochar (4 t·ha^{-1}) increased MBC and the C:N ratio, but Nmic was not influenced by any treatments. Some studies have reported similar variations on microbial biomass that can be explained by the soil type, biochar feed material and biochar doses (Zheng et al. 2016; Medeiros et al., 2021). Here, sandy soils were used, as did Yadav et al. (2019), who reported an increase in C:N ratio and microbial biomass at the highest doses used, with increases in the N mineralization process due to the high demand of this nutrient for microbial growth.

The Nmic did not change after incorporating the biochar into the soil. As biochar application does not increase the total nitrogen content of the soil (Table 1), microorganisms were unable to immobilize nitrogen in their biomass. This corroborates the study by Dempster et al. (2012), who evaluated the influence of incorporating biochar on the microbial activity of sandy soils.

Table 3. Carbon microbial biomass, Nmic, Pmic, SBR and qCO_2, total abundance of bacteria (16S rRNA gene), fungi (18S rRNA gene) and diazotrophs (*nif*H gene) in a sandy soil that received different doses of biochar from CG and CH, cultivated with maize.

	MBC (µg·g^{-1}·soil) (1)						
	4 t·ha^{-1}	**8 t·ha^{-1}**	**16 t·ha^{-1}**	**Mean**		**Model (4)**	**R²**
CG	82.02	83.86	76.57	80.82	b	f(x) = 80.82	ns
CH	**153.10** (2)	122.13	122.15	132.46	a	f(x) = 132.46	ns
Mean	117.56	103.00	99.36	106.64	(3)		
Control				72.73			
	N_{mic} (µg·g^{-1}·soil)						
	4 t·ha^{-1}	**8 t·ha^{-1}**	**16 t·ha^{-1}**	**Mean**		**Model**	**R²**
CG	41.01	16.57	26.29	27.96	a	f(x) = 27.96	ns
CH	33.91	69.06	38.75	47.24	a	f(x) = 47.24	ns
Mean	37.46	42.82	32.52	37.60			
Control				48.78			
	P_{mic} (µg·g^{-1}·soil)						
	4 t·ha^{-1}	**8 t·ha^{-1}**	**16 t·ha^{-1}**	**Mean**		**Model**	**R²**
CG	0.97	2.94	2.21	2.04	a	f(x) = 2.04	ns
CH	1.60	**0.62** (4)	2.13	1.45	a	f(x) = 1.45	ns
Mean	1.29	1.78	2.17	1.75			
Control				2.96			
	SBR (mg·C-CO_2·kg^{-1}·day^{-1})						
	4 t·ha^{-1}	**8 t·ha^{-1}**	**16 t·ha^{-1}**	**Mean**		**Model**	**R²**
CG	**31.00**	**34.27**	**28.96**	31.41	a	f(x) = 31.41	ns
CH	**26.91**	**28.96**	**24.88**	26.92	b	f(x) = 26.92	ns
Mean	28.96	31.62	26.92	29.16			
Control				26.53			
	qCO_2 (mg·C-CO_2·g^{-1}·Cmic·day^{-1})						
	4 t·ha^{-1}	**8 t·ha^{-1}**	**16 t·ha^{-1}**	**Mean**		**Model**	**R²**
CG	39.01	41.48	38.05	39.51	a	f(x) = 39.51	ns
CH	17.91	27.49	20.33	21.91	b	f(x) = 21.91	ns
Mean	28.46	34.48	29.19	**30.71**			
Control				52.45			
	Log of 16S rDNA gene copy numbers						
	4 t·ha^{-1}	**8 t·ha^{-1}**	**16 t·ha^{-1}**	**Mean**		**Model**	**R²**
CG	8.18	9.08	9.28	8.85	a	f(x) = 8.85	ns
CH	9.31	8.48	8.42	8.74	a	f(x) = 8.74	ns
Mean	8.74	8.78	8.85	8.79			
Control				8.34			

continue...

Table 3. Continuation...

	Log of 18S rDNA gene copy numbers									
	4 t·ha^{-1}		8 t·ha^{-1}		16 t·ha^{-1}		Mean		Model	R^2
CG	6.05	aB	6.52	aAB	6.87	aA	6.48		f(x) = 0.065x + 5.875	0.93
CH	6.58	a	6.68	a	6.18	b	6.48		f(x) = 6.48	ns
Mean	6.32		6.60		6.53		6.48			
Control							6.20			
	Log of *nif*H gene copy numbers									
	4 t·ha^{-1}		8 t·ha^{-1}		16 t·ha^{-1}		Mean		Model	R^2
CG	4.41	B	4.76	A	4.73	AB	4.63	a	f(x) = 0.065x + 5.875	0.93
CH	4.46		**4.40**		**4.22**		4.36	b	f(x) = 6.48	ns
Mean	4.43		4.58		4.47		4.50			
Control							4.62			

(1) The analysis of variance was performed at the 5% significance level, where the absence of letters indicates that there were no significant differences (ns). Means followed by different lowercase letters between columns and uppercase letters between lines differed, using the Tukey's test at the 5% level of significance.
(2) The means in **bold** differed from the control according to the Dunnett test at the level of 5% of significance.
(3) Overall mean in **bold** differed from the control according to the Helmet's contrast at the 5% significance.
(4) Regression analysis was performed for the quantitative variables (increasing doses of the biochar) with different variances and the corresponding linear model [*f(x)*] was shown for each biochar, followed by the adjusted R^2. In the case of data with equal variances (ns), the means were considered similar and the global mean (constant) was calculated.

The Pmic presented the lowest value with the addition of the 8 t·ha^{-1} dose of CG compared to the control (Table 2). At this same dose, the available P was significantly higher, demonstrating that the microbial biomass was able to mineralize the P from the biochar and make it available in the soil. Thus, the phosphorus from the biomass is released in the soil, making it available to the plants. The increase in Pmic can be related to the properties of the biochar, which, after being incorporated into the soil, improved the environment for colonization by microorganisms (Zhai et al. 2015). However, the current maize experiment showed an increase in the available P content, with P from microbial biomass reduced by biochar. These microbial biomass variations can be explained by the type of soil used in the experiment and by the type and rate of application of the biochar (Zhang et al. 2014; Zhu et al. 2017).

Castaldi et al (2011) found that biochar incorporation facilitates C, N and P mineralization, which is affected by biochar particle size, since thin particles are mineralized faster than thicker ones. Another factor is the feedstock lignin and complex compounds content, since the higher these are, the slower the mineralization tends to be, which might explain the observed differences between the CH and CG biochar.

The CG biochar at 8 t·ha^{-1}, although there is no significant difference, numerically increased the SBR rate by 30% compared to the control. Lima et al. (2018) have shown that this increase in C-CO_2 fluxes varies according to the feedstock used, porosity and specific surface area.

Soil microbial abundance

The abundance of total bacteria, gene 16S rRNA, showed no statistical difference between the number of copies found in the treatments and the biochar doses compared to the control and soil with manure. With respect to the fungi community represented by the 18S rRNA gene, in the soil that received the manure treatment, the number of copies of the gene was lower than the number of copies found in the absolute control. The highest dose of biochars (16 t·ha^{-1}) influenced the abundance of 18S genes, with CH being higher than CG. The results showed that all doses of CG differed statistically from the control for the population of diazotrophs, with a lower number of copies of the gene in question in soils treated with CG. Besides, the highest doses of both biochars differed, with soils treated with CH having a higher number of copies of the respective gene compared to CG (Table 3).

Coffee ground biochar at 16 t·ha^{-1} showed a trend toward higher microbial levels, though not significant, with respect to the abundance of bacteria, fungi and diazotrophic genes (increase of 11, 10 and 2%, respectively), likely due to the supply of degradable carbon (Manirakiza et al. 2019). Biochar influences soil microbial abundance by interfering with the chemical and physical properties of the soil and can provide a habitat for microbial growth (Khadem and Raiesi 2017) due to the structures of its pores, protecting microbes from the action of predators.

Multivariate analysis

On the CAP coordinates plot (Fig. 1), the PC1 and PC2 axis explained 35 and 16% of the variation of bacterial community; 40 and 20% of fungal community and 30 and 23% of diazotrophic community numbers, respectively. The analysis revealed a similar microbial composition for each biochar type, independent of dose since the plots separated the communities according to biochar type.

Principal coordinates canonical analysis explained 51% of the total variation and correlated to the DGGE distance matrix for the 16S rRNA gene (Fig. 1a). The lower doses of CH were positively correlated with copy numbers of 16S rRNA, 18S rRNA, *nif*H, Pmic, qCO_2 and Nmic, while CG showed influence on Ca^{+2}, Na^{+}, Al^{+3} and SBR. These variables were negatively correlated with copy numbers for the 16S rRNA gene. The impact of biochar on increased microbial abundance has been documented in both pot and field experiments (Jones et al. 2012, Chen et al. 2016; Liu et al. 2019).

Biochar amendments serve as a habitat and protection for microorganisms against predator's action. Here, bacterial abundance genes were grouped according to biochar types and doses, demonstrating that biochar can be considered a consumable carbon source that changes according to feedstock (Chen et al. 2016). Bacterial abundance was largely related to the increase in soil organic matter and soil pH, as also shown by Liu et al. (2019).

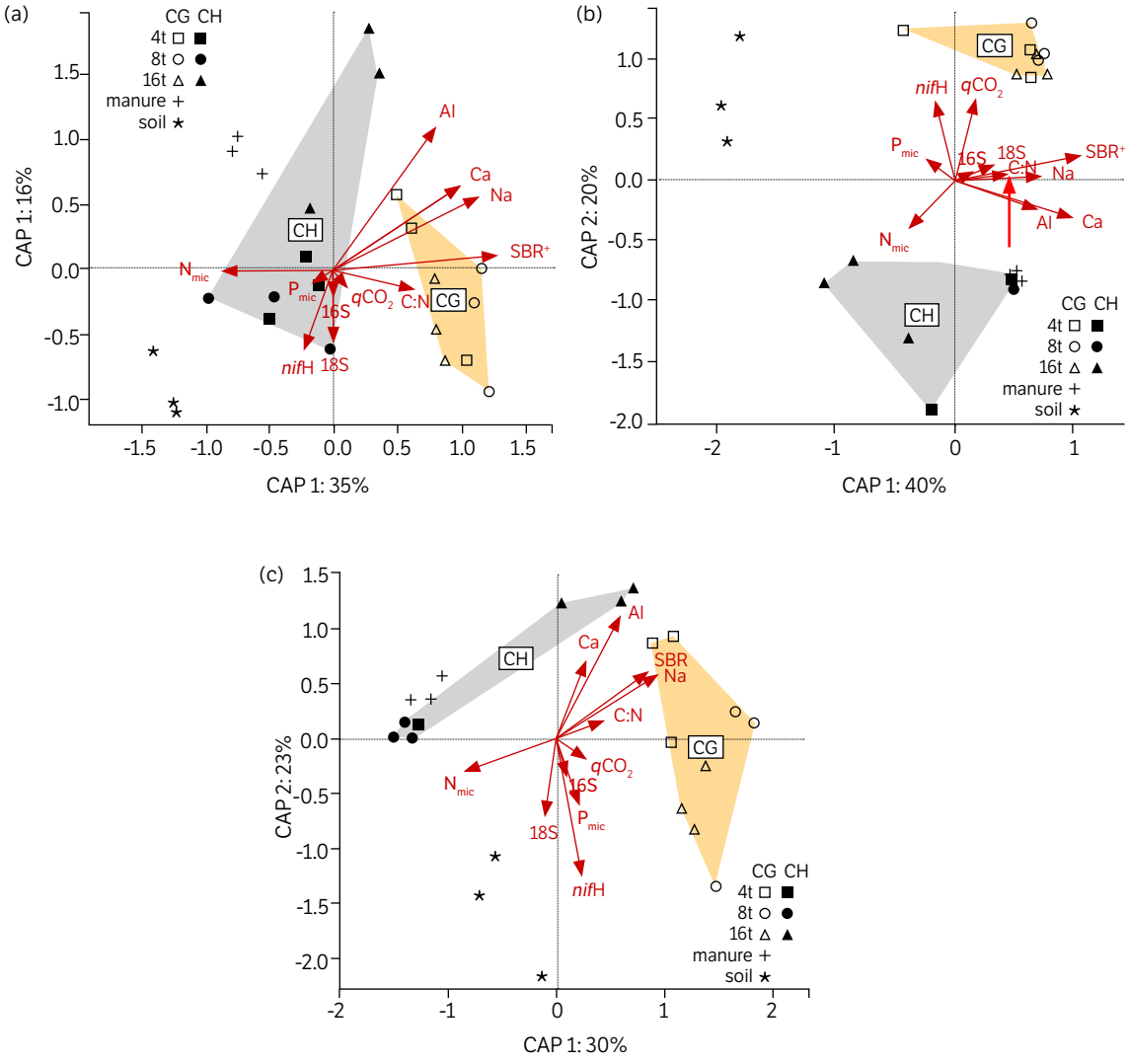

Figure 1. Canonical analysis of principal coordinates between structures of a) bacterial, b) fungal and c) diazotrophic communities and the attributes of a sandy soil that received different doses of biochar from CG and CH, cultivated with maize.

CG = coffee ground; CH = coffee husk; MBC = microbial biomass carbon; Nmic = microbial biomass N; Pmic = microbial biomass P; SBR = soil basal respiration; qCO_2 = metabolic quotient.

Soil fungal communities from CH and CG biochar-treated soil were distanced from each other, with few overlaps, and from the control treatment. Fungal communities are next to the Nmic at 16 t·ha^{-1} of CH. All CG doses were more favorable to the 18S rRNA gene copy number, correlating positively with SBR.

While some studies reported that the fungal community was not affected and no change in the community structure with biochar application (Chen et al. 2016), Jones et al. (2012) found a possible inhibition effect on fungal growth and Chen et al. (2013) reported that fungal communities experienced structural changes with increased diversity. In the current study, CG biochar led to a slight increase in the abundance of fungi by only 10% at the higher dose. This can be attributed to the product porosity and raw composition used in the biochar production, since the fungi have the capacity to degrade more recalcitrant materials (Gul et al. 2015).

The canonical analysis explained 53% of the total variation for the *nif*H gene (Fig. 1c). The highest CG dose approached the *nif*H copy numbers, Pmic, qCO_2 and 16S rRNA gene and the lower doses approached the C:N ratio, Na^+ and SBR. Lehmann et al. (2011) demonstrated that bacteria respond more rapidly to pH changes, while fungi tolerate a broad pH range and are not as affected by the modifications that biochar exerts on the soil. Thus, the pH of the biochar can exert influence on the soil microbial communities' abundance, confirming these findings. Soil with CG biochar with its lower pH increased diazotrophs abundance, while CH biochar with its higher pH decreased this community with increase the dose. The increased diazotroph abundance obtained with CG biochar was likely due to its pH (5.27) being close to the optimal (5.5–6.0) range for biological nitrogen fixation, also resulting in increased nutrient availability and labile organic C that can be directly utilized by soil diazotrophs community (Liu et al. 2019).

Correlation heatmap analysis

The correlation heatmap analysis (Fig. 2) revealed significant linear correlations ($p \leq 0.05$) among the variables, mainly the positive ones between TOC and the largest number of variables, including C, P, pH, H+Al, Mg^{2+}, Ca^{2+}, SBR, Al^{3+} and Na^+.

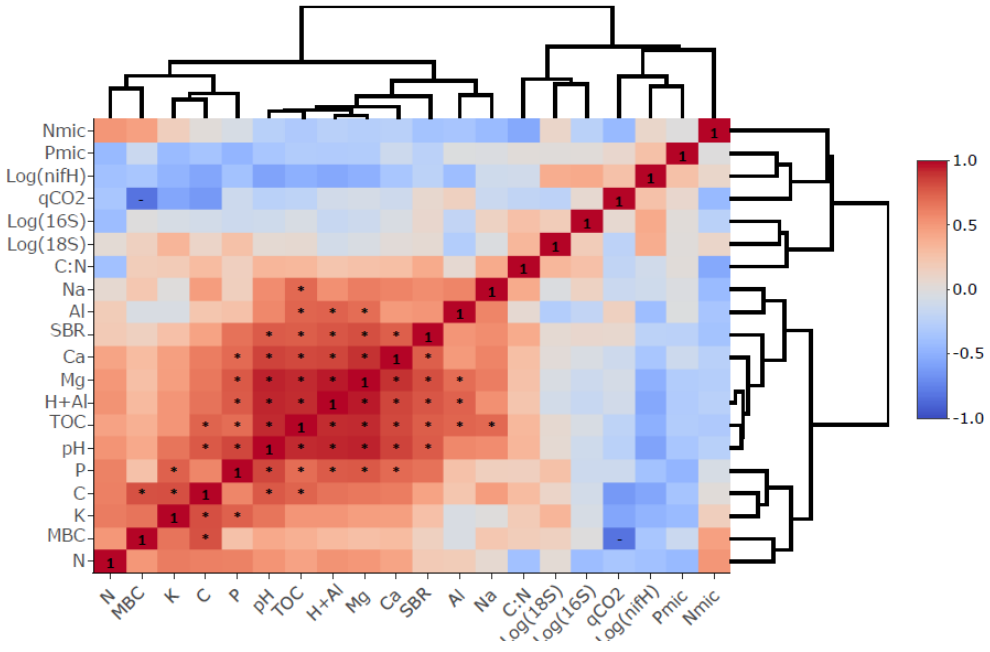

Figure 2. Heatmap correlations between variables of sandy soil that received different doses of biochar from CG and CH, cultivated with maize.

MBC = microbial biomass carbon; Nmic = microbial biomass N; Pmic = microbial biomass P; SBR = soil basal respiration; qCO_2 = metabolic quotient, Log (16S) = Log of 16S rRNA gene copy numbers; Log(18S) = Log of 18S rRNA gene copy numbers; Log(*nif*H) = Log of *nif*H gene copy numbers. The polygons with asterisks (*) and dashes (-) show, respectively, significant positive and negative correlations ($\rho < 0.05$) with modules greater than 0.7, according to statistics based on Pearson's product-moment correlation coefficient (r) following the t distribution.

Increased C content in sandy soils due to coffee biochar was accompanied by an increase in MBC. Use of residues rich in organic matter has been a widely used practice worldwide, due to its large-scale availability and nutrient content. The stabilized part of the C found in the soil is quite susceptible to microbial decomposition and through a possible combination of physical protection and chemical complexation, the stable carbon may have been protected against decomposition (Martins Filho et al. 2020).

This study showed positive effects from the reuse of coffee waste to produce biochar as an eco-friendly and low-cost alternative to increase sandy soil quality by different plausible mechanisms. Here, the CH biochar was the most efficient in increasing the chemical and microbiological attributes of sandy soil. A previous study using both coffee biochar (CH and CG), Lima et al. (2018) showed that CH biochar presented a high specific surface area that contributed to a higher water holding capacity on the wet end of the retention curve, compared to CG.

The addition of biochar from coffee waste to acid soils improve soil quality and plant growth due to its alkalinity and high pH buffering capacity. A shift in pH and reduction in Al caused by biochar amendments will improve the availability of P, Ca and Mg, resulting in a balanced nutrient supply in the rhizosphere, thus enhancing the productivity of cultivated plants (Yu et al. 2019).

This study demonstrated that soil nutrients and microbial biomass increased in response to the type and doses of biochar. Thus, the reuse of coffee residues (mainly husk) for biochar production is recommended to increase sandy soil quality in Brazilian semi-arid areas, which is plausible due to the large amount of waste that the coffee industry produces. Harnessing such waste for field experiments can be an eco-friendly alternative to improve soil health. However, additional research is necessary to investigate some of those variables where no clear significant differences were observed in order to better understand the potential benefits and underlying mechanisms from biochar amendment applications in different culture cycles and in different crops.

CONCLUSION

The present study provided an insight into the effects of biochar produced from coffee waste as a low-cost alternative to promote sandy soil quality. This strategy can help small farmers to improve their productivity due the numerous benefits provided by biochar to improve soil health and the environment, as well as to its ability to sequester carbon in the soil. Here, the treatments that received different biochar types and doses considerably altered the soil quality. Application of lower doses of CH biochar increased the C content, MBC and Nmic (3, 2.1 and 1.6 times, respectively). Application of higher doses of CG biochar showed a slight increase in the abundance of bacteria, fungi and diazotrophic genes (increase of 11, 10 and 2%, respectively). However, this pot experiment only lasted for one crop season, therefore further study is needed to focus on the field and long-term impacts of biochar soil applications. Despite this, a positive effect from the reuse of coffee waste to produce biochar as an eco-friendly and low-cost alternative to increase sandy soil quality was found.

AUTHORS' CONTRIBUTION

Conceptualization: Lima J. R. S., Medeiros E. V. and Hammecker C.; **Methodology:** Medeiros E. V., Lima J. R. S., Fracetto G. G. M., Fracetto F. J. C. and Duda G. P.; **Investigation:** Silva C. C. G., Martins Filho A. P. and Costa D. P.; **Writing – Original Draft:** Medeiros E. V., Costa D. P., Silva C. C. G. and Lira Junior M. A.; **Writing – Review and Editing:** Medeiros E.V., Costa D. P., Silva C. C. G. and Lira Junior M. A.; **Funding Acquisition:** Medeiros E. V. and Lima J. R. S.; **Resources:** Fracetto G. G. M., Fracetto F. J. C., Duda G. P., Silva C. C. G. and Martins Filho, A. P.; **Supervision:** Medeiros E. V., Hammecker C. and Lima J. R. S.

DATA AVAILABILITY STATEMENT

Data will be available when requested.

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