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Research article

Biochar alters the soil microbiological activity of sugarcane fields over time

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ABSTRACT: There are few long-term field studies on the effects of biochar on soil microbial abundance and diversity. This study aimed to evaluate doses of biochar in combination with mineral fertilizer on the activity and diversity of microorganisms in the soil of a sugarcane field. The experiment was carried out in a randomized block design, factorial 5 × 2, with four replications: five doses of eucalyptus (*Eucalyptus grandis* Hill ex Maiden × *Eucalyptus urophylla* S.T. Blake) wood biochar (0, 10, 20, 30, and 40 Mg ha^{-1}), with and without the application of nitrogen, phosphorus, and potassium (NPK) mineral fertilizer. Soil samples were collected from the sugarcane planting line and fertilized with biochar for two consecutive years. Regardless of the NPK fertilizer, over the two years of evaluation, the height growth of sugarcane plants and total organic carbon (TOC) increased linearly with biochar doses. For microbial biomass carbon (Cmic), soil basal respiration (SBR), metabolic quotient (qCO_2), microbial quotient (qMIC), and Shannon diversity index (H), the highest values were obtained where fertility correction (WFC) treatments were applied, regardless of the year of evaluation, in biochar doses between 20 and 30 Mg ha^{-1}. On the other hand, the highest CO_2 efflux values were obtained with zero doses of biochar, regardless of the NPK fertilizer applied, over the two years of evaluation. Therefore, the incorporation of biochar and NPK fertilizer into the soil contributes to increasing the soil's biological activity indicators and, consequently, the growth of sugarcane plants. It is essential to highlight the need for continuous assessments as the characteristics of biochar change over time.

Keywords: microbial biomass carbon, soil basal respiration, total organic carbon, metabolic quotient, microbial quotient

Introduction

Biochar is a solid carbonaceous material resulting from the thermochemical conversion of biomass under anaerobic conditions, a process known as pyrolysis (Chen et al., 2019). The physical and chemical properties of biochar contribute to improving soil quality and fertilizer efficiency (Novair et al., 2023) and to mitigating the effects of organic and inorganic contaminants, such as antibiotics (Zhang et al., 2020) and heavy metals - potentially toxic trace elements (Cheng et al., 2017; Li et al., 2020; Nobaharan et al., 2022), for example.

Biochar research is abundant, but only some long-term field studies have been carried out. Consequently, several questions surround the economic viability of applying biochar on a large-scale, the longevity of the benefits, and the potential ecological risks of biochar application (Ghodszad et al., 2021).

As regards soil properties, biochar can modify the structure of the microbial community involved in various physical and chemical soil processes (Brtnicky et al., 2021; Fan et al., 2020; Zhang et al., 2020). In addition, as microorganisms are considered indicators of soil quality, determining the biological activity of the soil is essential to an assessment of the functional diversity of the microbiota (Mendes et al., 2019).

The microbial biomass carbon (Cmic), which represents the living fraction of soil organic matter (SOM), composed of actinomycetes, bacteria, and fungi, is one of the most sensitive biological indicators of environmental changes (Vance et al., 1987). Another widely used indicator is soil basal respiration (SBR), sensitive to environmental disturbances (Anderson, 1982). SBR accounts for all metabolic functions that produce carbon dioxide (CO_2). Additionally, the soil metabolic quotient (qCO_2) is estimated by the ratio between SBR and Cmic, representing the ability of the microbiota to use the substrate present in the soil (Anderson and Domsch, 1993).

The measurement of total organic carbon (TOC) in the soil is also an indicator of quality and is related to aggregate formation and stability and, consequently, soil structure (Yeomans and Bremner, 1988). The microbial quotient (qMIC) is another biological indicator obtained by the ratio of Cmic and TOC associated with SOM (Sparling, 1992).

In this context, we hypothesize that the application of biochar contributes to the growth and reproduction of soil microbiota, which in turn alters microbial diversity. Thus, more detailed studies are needed to better understand the impact of biochar on the soil ecosystem under field conditions, which is constantly influenced by fluxes of matter and energy. This study aimed to evaluate doses of biochar in combination with mineral fertilizer on the activity and diversity of microorganisms in the soil of a sugarcane field.

Materials and Methods

The experiment was conducted at the Universidade Federal de Minas Gerais, Montes Claros, Minas Gerais, Brazil (16°41′2.03″ S, 43°50′19.28″ W, altitude 646 m). The climate of the region is classified, according to Köppen, as Aw, tropical with dry winters (Alvares et al., 2013). The chemical and physical attributes of the soil in the 0-20 cm depth layer were: pH (H_2O) = 6.3; TOC = 40.5 g kg^{-1}; P (Mehlich-1) = 3.10 mg dm^{-3}; K-exchangeable = 66.00 mg dm^{-3}; Ca-exchangeable = 6.5 $cmol_c$ dm^{-3}; Mg-exchangeable = 3.20 $cmol_c$ dm^{-3}; H + Al-total acidity = 2.20 $cmol_c$ dm^{-3}; and medium texture.

The experimental design followed a completely randomized block arrangement in a factorial scheme (5 × 2) with four replications. The treatments were five biochar doses (0, 10, 20, 30, and 40 Mg ha^{-1}) no soil fertility correction (NFC) with nitrogen, phosphorus and potassium (NPK) mineral fertilizer and with soil fertility correction (WFC) with NPK mineral fertilizer. For the WFC treatments, 13 kg ha^{-1} of N, 100 kg ha^{-1} of P_2O_5, and 33 kg ha^{-1} of K_2O were applied at planting. Additionally, 90 days after planting, 100 kg ha^{-1} of K_2O and 80 kg ha^{-1} of N were applied in coverage (top-dressing). Immediately after each top-dressing fertilization, the sugarcane field was irrigated. Top-dressing fertilization with K and N was repeated in subsequent years after the sugarcane harvest (Figure 1).

Biochar (BC) was obtained through slow pyrolysis, with a residence time of 48 h, from eucalyptus wood waste (branches) at 350 °C. After cooling, the biochar was crushed, sieved through a 5 mm mesh, and subsequently applied to the planting furrow, homogenized in the 0-20 cm depth layer. Biochar samples were taken to the laboratory to determine their chemical and physical properties (Table 1).

Sixty days prior to sugarcane planting, furrows of 20 cm depth were opened with a spacing of 1 m between rows, where the biochar was incorporated. After the biochar incorporation, the experimental area was irrigated to maintain soil moisture close to field capacity. Sugarcane was planted in July using the SP81-3250 variety, with ten viable buds per linear meter of planting furrow, making 100,000 plants ha^{-1}. Experimental units consisted of four planting rows with 30 plants each, making 120 plants in total per plot. The usable area included 48 plants in the central region of each experimental unit. Irrigation of the sugarcane field was carried out using conventional sprinkler irrigation, and there was no need for inputs to control pests and diseases.

In July/Aug of the second and third years after planting, the height of the plants was evaluated, and the sugarcane stalks were harvested. The leaves and tops of the plants were left on the soil surface as straw. Two days before harvest, soil samples were collected from the 0-20 cm depth layer along the planting line. Soil samples were kept at 4 °C until analysis (Figure 1).

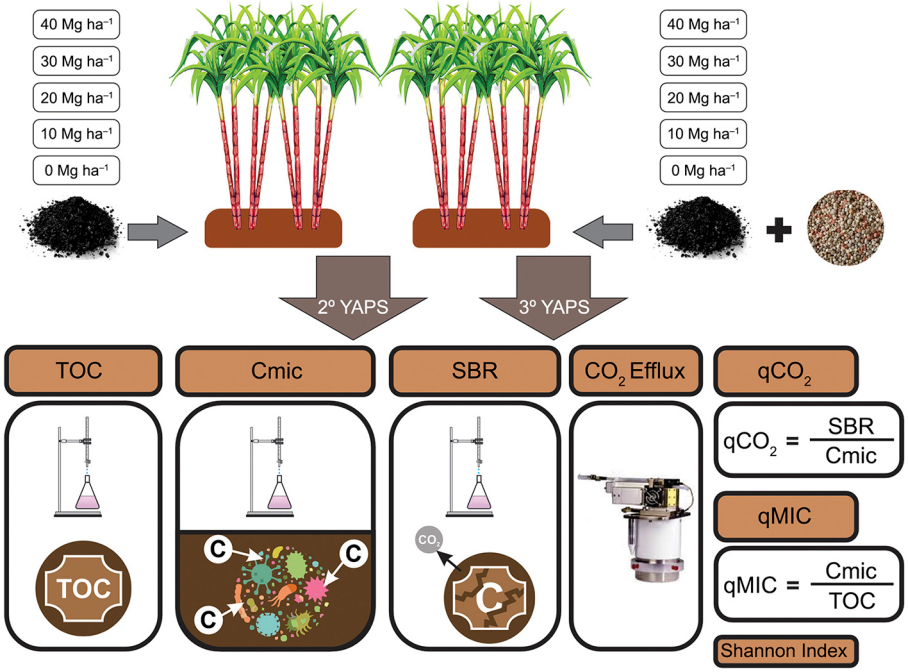

Figure 1 – Schematic representation of methodologies for determining total organic carbon (TOC), microbial biomass carbon (Cmic), soil basal respiration (SBR), carbon dioxide efflux (CO2 efflux), metabolic quotient (qCO2), microbial quotient (qMIC) and Shannon diversity index. Assessments were carried out in the second and third year after planting sugar cane (YAPS).

Table 1 – Chemical and physical properties of eucalyptus wood waste biochar.

	pH	H	O	C	N	P	Ca	Mg	S	
		mg kg^{-1}								
Mean	6.1	24.6	226	567	5.0	80.0	13.8	12.4	2.6	
CI	0.1	1.8	11.8	20.1	0.4	6.1	6.1	11.9	6.4	
	Cu	Zn	Fe	Mn	Si	Ni	Pb	Cd	Density	Ash
	mg kg^{-1}								g cm^{-3}	%
Mean	51.5	270	1.43	56.9	798.6	5.20	3.03	4.50	0.45	10.00
CI	3.8	18.1	0.37	6.16	19.6	0.53	0.45	0.53	0.03	1.02

Mean (n) = 3; CI = confidence interval.

On the third, fourth, fifth, and sixth days after harvesting, the soil CO_2 efflux was evaluated on site using an infrared gas analyzer (IRGA) model LCpro-sd, coupled to an ADC Soil Hood chamber. Measurements per block were taken, one block per day, between 08h00 and 12h00, with the aim of mitigating the influence of temperature.

Soil Cmic was assessed using the fumigation-chloroform extraction method (Vance et al., 1987). Cmic was calculated using Eq. (1):

$$Cmic = (Fc - NFc) \times kc \quad (1)$$

where: Fc (fumigated) and NFc (non-fumigated) represent the CO_2 released from fumigated and non-fumigated soil samples, respectively, and kc a constant (0.33) representing the proportion of carbon from dead microbial biomass that is converted into CO_2 during the incubation period (Sparling, 1992).

The oxidation method was used to determine the TOC content of the soil (Yeomans and Bremner, 1988). Soil basal respiration (SBR) was determined according to Anderson (1982), which involves measuring the amount of CO_2 generated under aerobic incubation at 25 °C for seven days. The qCO_2 was estimated by the ratio between SBR and Cmic (Anderson and Domsch, 1993), while qMIC was estimated by the ratio between Cmic and TOC (Sparling, 1992).

H was determined using Biolog Ecoplate microplates (Bloem et al., 2006), where each microplate consisted of three sets of 31 different carbon substrates (carboxylic acids, carbohydrates, polymers, amino acids, and starches), together with a control (no substrate). Each sample was introduced into a microplate and incubated at 28 °C for 48 h. Microbial growth was assessed through spectrophotometry at 590 nm. The ability to utilize a carbon source was determined using Eq. (2) (Ibekwe and Kennedy, 1998):

$$WE = 100 \times \frac{(WA - W0)}{W0} \quad (2)$$

where: WE is the color development index, WA the absorbance of each well and W0 the blank absorbance. The condition for the reaction to be positive is that the WE should be greater than 100.

According to Eq. (3), the H comprises the richness of substrates and the intensity with which the microbiota used them (Wasilewska, 1995; Zak et al., 1994):

$$H = -\sum p_i \ln p_i \quad (3)$$

where: H is the Shannon diversity index and p_i the ratio between the utilization activity of a given substrate and the utilization activity of all substrates.

Data were submitted to analysis of variance (ANOVA) by F test ($p < 0.05$), and regression analysis was carried out in case of significance between biochar doses. Pearson correlation between the variables studied was determined. All statistical analyses were carried out using the R software, version 3.4.2.

Results

Sugarcane plants' height growth increased linearly with biochar dose increase (Figure 2A). In the absence of biochar, the differences between the NFC and WFC treatments were more significant than between the highest doses of biochar. According to the regression equations applied to height growth as a function of biochar doses (Figure 2A), in the first sugarcane harvest, plant height was 2.21 and 2.64 m; in NFC and WFC treatments, respectively, with zero dose of biochar. With a dose of 40 Mg ha^{-1} of biochar, the heights were 2.94 and 2.95 m, respectively, under the NFC and WFC treatments. In the second sugarcane harvest, plant height was 1.14 and 2.45 m with the zero dose and 1.72 and 3.01 m with the 40 Mg ha^{-1} dose under the NFC and WFC treatments, respectively (Figure 2A).

In the two years of evaluation for TOC, there was no difference between NFC and WFC treatments (Figure 2B). On the other hand, TOC contents increased linearly with biochar doses. In the first year, TOC contents at zero and 40 Mg ha^{-1} of biochar were 30.5 and 35.8 g kg^{-1}, respectively. In the second year, TOC contents were 26.5 and 43.0 g kg^{-1}, at zero and 40 Mg ha^{-1} of biochar, respectively (Figure 2B).

For the soil Cmic, SBR, CO_2 efflux, qCO_2, qMIC, and H variables, there was an effect generated by the interaction between the doses of biochar and soil fertility correction (NFC and WFC), in both evaluation years (Table 2). The Cmic, SBR, and qCO_2 values were adjusted to a

quadratic model, regardless of the year of evaluation and application of NPK (Table 2). The highest Cmic values were observed under the NFC treatments, 389.51 mg microbial C kg^{-1}, in the first year, and under the WFC treatments, 408.09 mg microbial C kg^{-1}, in the second year (Table 2). Regardless of the NPK application, over the two years of evaluation, the highest Cmic values were obtained between the doses of 21.67 to 25.38 Mg ha^{-1} of biochar (Table 2).

For SBR, the highest values were obtained under the WFC treatments in the first and second years, 0.19 and 0.20 mg C-CO_2 kg^{-1} soil h^{-1}, respectively. Biochar doses to obtain the highest SBR values ranged from 22.75 to 23.50 Mg ha^{-1} (Table 2). Similarly, the highest qCO_2 values were obtained under the WFC treatments in both the first and second years, 0.47 and 0.50 mg C-CO_2 g^{-1} Cmic h^{-1}, respectively (Table 2). The doses to obtain the highest qCO_2 values ranged from 25.67 to

Table 2 – Microbial biomass carbon (Cmic), soil basal respiration (SBR), CO2 efflux, metabolic quotient (qCO2), microbial quotient (qMIC), and Shannon diversity index (H) as a function of biochar doses in treatments without and with nitrogen, phosphorus, and potassium (NPK) fertilizer, in two years of evaluation. Year I = 2018; Year II = 2019.

Variable	Year	NPK	Equation	R^2	Xmax[1]	Ymax[1]
Cmic mg C microbial kg^{-1}	I	without	y = 250.32 + 10.363**x – 0.2384**x^2	0.87	21.73	389.51
		with	y = 261.55 + 11.81**x – 0.2725**x^2	0.97	21.67	362.94
	II	without	y = 267.70 + 8.995**x – 0.205**x^2	0.82	21.94	366.37
		with	y = 288.01 + 9.4621**x – 0.1864**x^2	0.91	25.38	408.09
SBR mg C-CO_2 kg^{-1} soil h^{-1}	I	without	y = 0.0689 + 0.0057**x – 0.0001**x^2	0.79	28.50	0.15
		with	y = 0.0788 + 0.0094**x – 0.0002**x^2	0.93	23.50	0.19
	II	without	y = 0.0813 + 0.0059**x – 0.0001**x^2	0.80	29.50	0.17
		with	y = 0.1002 + 0.0091**x – 0.0002**x^2	0.93	22.75	0.20
CO_2 efflux µmol m^{-2} s^{-1}	I	without	y = 3.345 – 0.094**x + 0.002**x^2	0.78	0.00	3.35
		with	y = 3.425 – 0.13**x + 0.0023x^2	0.91	0.00	3.43
	II	without	y =3.5986 – 0.072**x + 0.0014**x^2	0.92	0.00	3.60
		with	y = 3.7493 – 0.1396**x + 0.0024x^2	0.88	0.00	3.75
qCO_2 mg C-CO_2 g^{-1} Cmic h^{-1}	I	without	y = 0.2782 + 0.0069*x – 0.0001*x^2	0.62	34.50	0.39
		with	y = 0.3001 + 0.0154**x – 0.0003**x^2	0.69	25.67	0.47
	II	without	y = 0.3115 + 0.0081*x – 0.0002*x^2	0.60	20.25	0.39
		with	y = 0.3483 + 0.0154**x – 0.0004*x^2	0.62	19.25	0.50
qMIC %	I	without	y = 0.8612 + 0.0214**x – 0.0006**x^2	0.82	17.83	1.05
		with	y =0.8232 + 0.0426**x – 0.0011**x^2	0.94	19.36	1.24
	II	without	y = 1.1288 – 0.0095**x	0.90	0.00	1.13
		with	y = 1.0449 + 0.0286**x – 0.0008**x^2	0.90	17.88	1.30
H	I	without	y = 1.1257 + 0.0994**x – 0.0021**x^2	0.91	23.67	2.30
		with	y = 1.7036 + 0.0598**x – 0.0014**x^2	0.75	21.35	2.34
	II	without	y = 1.3621 + 0.0826**x – 0.0012**x^2	0.99	34.42	2.78
		with	y = 2.535 + 0.0213**x	0.82	40.00	3.39

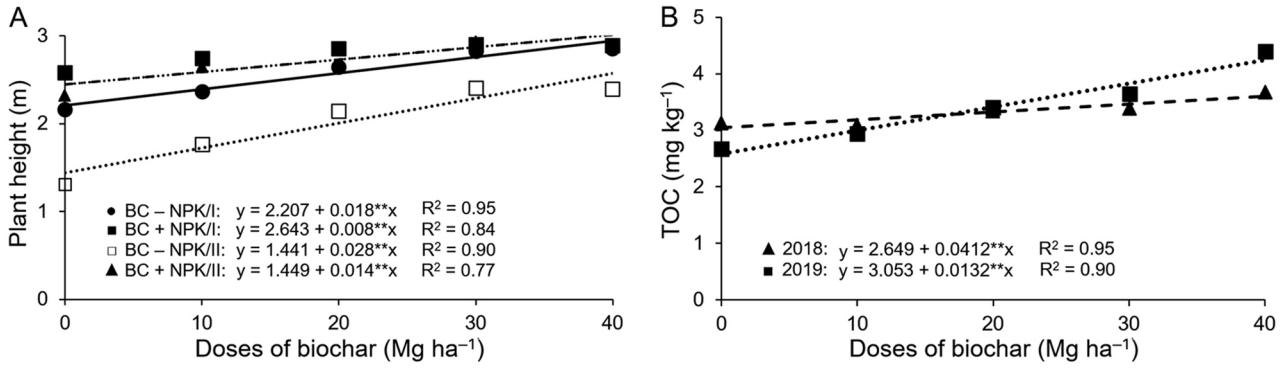

Figure 2 – A) Growth in plant height of sugarcane and B) soil total organic carbon (TOC) content as a function of biochar doses in treatments with biochar without mineral fertilizer in the first year (BC – NPK/I) and the second year (BC – NPK/II) and in treatments with biochar and mineral fertilizer in the first year (BC + NPK/I) and the second year (BC + NPK/II). Year I = 2018; Year II = 2019. NPK = nitrogen, phosphorus, and potassium and BC = biochar.

34.50 Mg ha^{-1} in the first year, while in the second year of evaluation the doses ranged from 19.25 to 20.25 Mg ha^{-1} (Table 2). The CO_2 efflux values were adjusted to a quadratic model in the two years evaluated, regardless of the application of NPK (Table 2).

According to the fitted equations, the highest CO_2 efflux values were obtained at zero biochar doses and ranged from 3.35 to 3.75 µmol m^{-2} s^{-1}. The lowest values, 2.24 µmol m^{-2} s^{-1} (without NPK) and 1.59 µmol m^{-2} s^{-1} (with NPK), were obtained with the corresponding doses of biochar 23.50 and 28.26 Mg ha^{-1}, respectively, in the first year of evaluation. The lowest values were obtained in the second year with doses of 25.71 and 29.08 Mg ha^{-1} and corresponded to 2.67 and 1.72 µmol m^{-2} s^{-1}, respectively, for the NFC and WFC treatments (Table 2).

In the first year of evaluation, under both the NFC and WFC treatments, the qMIC values were adjusted to a quadratic model as a function of biochar dose (Table 2). The highest values, under the NFC and WFC treatments, 1.05 % and 1.24 % were obtained at doses of 17.83 and 19.36 Mg ha^{-1}, respectively. In the second year, under the NFC treatments, the qMIC values linearly decreased with biochar doses, while in the WFC treatments, the values were fitted to a quadratic model. Under the WFC treatments, the highest qMIC value was 1.30 %, with the dose corresponding to 17.88 Mg ha^{-1} of biochar. On the other hand, in the NFC treatments, the highest value, 1.13 %, was obtained with a zero dose of biochar (Table 2).

The H fit a quadratic model, except for WFC treatments, in the second year of evaluation (Table 2). In the first year, the values were 2.30 and 2.34, with doses of 23.67 and 21.35 Mg ha^{-1}, respectively, under the NFC and WFC treatments. In the second year, under the WFC treatments, the highest value, 3.39, was obtained with the dose of 40 Mg ha^{-1} of biochar, while under the NFC treatments, the highest value, 2.78, was obtained with the dose of 34.42 Mg ha^{-1} of biochar (Table 2).

The Cmic correlated positively with SBR, qCO_2, qMIC and H index and negatively with CO_2 efflux (Table 3). The SBR was positively correlated with qCO_2 and negatively correlated with CO_2 efflux. The qCO_2 correlated positively with qMIC (Table 3).

Table 3 – Pearson correlation coefficient between the variables microbial biomass carbon (Cmic), soil basal respiration (SBR), total organic carbon (TOC), CO_2 efflux, metabolic quotient (qCO_2), microbial quotient (qMIC), and Shannon diversity index (H). Mean of the two years of evaluation, 2018 and 2019.

Variable	Cmic	SBR	TOC	CO_2 efflux	qCO_2	qMIC
SBR	0.85**	-	-	-	-	-
TOC	0.34^{NS}	0.27^{NS}	-	-	-	-
CO_2 efflux	–0.76**	–0.68*	–0.70*	-	-	-
qCO_2	0.61*	0.93**	0.21^{NS}	–0.51*	-	-
qMIC	0.63*	0.70**	-0.26^{NS}	-0.30^{NS}	0.60*	-
H	0.68*	0.61*	0.38^{NS}	-0.46^{NS}	0.47^{NS}	0.20^{NS}

**, * and NS, significant at 1 %, 5 % and not significant, respectively.

Discussion

The height growth of sugarcane plants with increasing doses of biochar (Figure 2A) can be attributed to the improvement of the chemical, physical and biological properties of the soil. Biochar is a source of nutrients (Table 1) which contributes to increasing the cation exchange capacity of soils (Qian et al., 2023; Xu et al., 2024). Due to the large porosity of the particles and specific surface, it favors the ability to retain water. It promotes an environment conducive to the development of microorganisms, such as mycorrhizal fungi (Zhao et al., 2023). In the second year of evaluation, the lower growth in height in the NFC treatment, mainly in the lower doses of biochar (Figure 2A), can be attributed to the nutrients exported by the sugarcane stalks in the first harvest. Consequently, there was a decrease in the capacity of the soil to supply these elements to the plants.

The TOC contents increased linearly with the amount of biochar incorporated into the soil (Figure 2B). In addition to being a carbon source (Table 1), the aromatic structure of biochar is unfavorable to its biodegradation, which contributes to the maintenance of soil carbon stock over time. Other authors have also found an increase in TOC contents by applying biochar to the soil (Sun et al., 2021; Li et al., 2024). Undoubtedly, keeping sugarcane straw (harvest residue) on the soil surface contributes to increased TOC contents (Cherubin et al., 2021). Other researchers have reported that higher TOC levels resulting from the application of biochar and fertilizers may be associated, in addition to biochar being a source of carbon, to greater plant growth (root and shoot), greater leaf area index for production of photosynthesis and rhizodeposition and respiration of the roots provided by the greater availability of nutrients (Zhang et al., 2021). This study found that plant height growth increased linearly with biochar doses, with the highest values obtained in treatments with biochar and mineral fertilizer (Figure 2A).

The stability of pyrogenic carbon has been attributed to the maintenance of TOC stocks over time in soils that received biochar application (Oni et al., 2019). According to these authors, carbon from wood-derived biochar is highly recalcitrant in soils, with residence times between 100 and 1000 years, about 10-1000 times longer than non-pyrolyzed organic matter. Thus, biochar can increase soil carbon stocks and is a promising technique for mitigating greenhouse gas emissions (Li et al., 2024).

The Cmic values, according to the adjusted equation model, increased up to doses of 20 to 25 Mg ha^{-1} (Table 2). Cmic represents the most active and dynamic reservoir of soil organic C and nutrients and corresponds, on average, to 2 to 5 % of the TOC. Thus, Cmic is directly related to the quantity and quality of SOM, being much more sensitive to soil management practices than TOC (Tao et al., 2023). In the present

study, the final pyrolysis temperature was 350 °C. At this temperature, organic material, such as lignin, has not been wholly pyrolyzed.

In addition to biochar, the different amounts of residues, mainly leaves, deposited on the soil surface during plant growth until harvest may have influenced the Cmic. Under treatments with higher doses of biochar, there was greater plant growth in height (Figure 2A) and, consequently, a greater number of leaves deposited on the soil surface. Furthermore, higher doses of biochar incorporated carbon into the soil in more recalcitrant forms. Although it is unclear which factors influenced the Cmic, it can be inferred that the amount of sugarcane and biochar residues altered the quality and the carbon/nutrient ratio of SOM.

The reduction in the activity of microorganisms associated with Cmic at doses above 20 to 25 Mg kg^{-1} of biochar (Table 2) is reinforced by the SBR and qCO_2 values (Table 2). As observed for Cmic, the highest SBR and qCO_2 were obtained at intermediate doses of biochar (Table 2). The factors that influenced Cmic values discussed earlier may have affected SBR and qCO_2. Corroborating this hypothesis was the positive correlation between Cmic and TOC, SBR, and qCO_2 (Table 3), regardless of the year of assessment, biochar dose or NPK application. The SBR and qCO_2 are related to the efficiency of microbial biomass, as the reduction in SBR and qCO_2 values indicates that less carbon in the form of CO_2 is being lost through respiration and, consequently, more carbon is incorporated into microbial tissues (Pires et al., 2020). In general, incorporating biochar into the soil reduces CO_2 emissions by up to 21 % compared to incorporating non-pyrolyzed raw materials (Zhou et al., 2017). Thus, lower qCO_2 values observed at higher doses of biochar (Table 2) may indicate less environmental stress and favor the soil microbial population with better habitat and optimization of carbon use (Li et al., 2021).

The incorporation of biochar into the soil increased the qMIC up to doses of 17 and 19 Mg ha^{-1} (Table 2). The reduction in qMIC at higher doses of biochar may be associated with the fact that biochar is a carbon-rich material (Table 1) since the qMIC was estimated by the relationship between Cmic and TOC. Thus, higher doses of biochar increased the TOC content (Figure 2B) and, consequently, reduced the qMIC values (Table 2). The qMIC is used as an indicator of the mineralization potential of organic matter. The lower the values of qMIC in the soil, the lower the tendency for mineralization of organic matter (Liyanage et al., 2021) and, therefore, the lower the emission of CO_2 into the atmosphere.

For the CO_2 efflux from the soil determined "*in loco*", in the two years of evaluation, lower values were found with the increase in biochar doses, regardless of the application of NPK (Table 2). CO_2 efflux values were negatively correlated with Cmic, SBR, and TOC, indicating that, regardless of the environmental factors that affect carbon dioxide diffusivity in the soil, the higher the values of these variables, the lower the soil CO_2 efflux. The efflux of CO_2 from the soil or soil respiration is related to the carbon emitted by plant roots, the activity of microorganisms and the oxidation of organic matter, and the environmental factors such as temperature and humidity, soil management, which affect the diffusivity of CO_2 in the soil (Nissan et al., 2023; Vigricas et al., 2024).

As regards microbial diversity, the addition of biochar to the soil significantly increased the Shannon index (Table 2). The highest values for the Shannon Index are related to the greater diversity of the soil microbial community, which favors the survival of microorganisms in stressful situations, promoted, for example, by agricultural activities (Wang et al., 2020; Osburn et al., 2023). Other studies have also observed an increase in the Shannon index due to the addition of biochar to the soil (Gao et al., 2021; Yan et al., 2020; Zhou et al., 2019). According to these authors, biochar improvements in soil chemical and physical properties favor both soil microorganisms and plants, which contribute carbon to the soil via biomass and rhizodeposition. Furthermore, biochar particles have a high specific surface and porosity, which provide a favorable habitat for microorganisms. Thus, the complexity of the matrix and the composition of biochars, depending on the raw material and pyrolysis conditions, affect soil microorganisms differently from non-pyrolyzed organic residues.

Shannon index values were positively correlated with Cmic, since this index measures fungi, bacteria, actinomycetes, protozoa, algae, and soil microfauna. It indicated that over time, there was an increase in the diversity of the soil microbial community with biochar, especially when combined with NPK, which favored an increase in SBR (Table 2). Furthermore, biochar's chemical composition and physical structure alter soil properties and directly affect the microbial population (Liu et al., 2023; Xiao et al., 2024). Regardless of the biochar dose, in general, treatments fertilized with NPK mineral fertilizers showed higher Cmic, SBR, qCO_2, qMIC, and Shannon index values and lower CO_2 efflux values (Table 2) when compared to treatments without the addition of biochar. These results can be attributed to changes in carbon/nutrient ratios, which altered the activity of soil microorganisms (Shi and Liu, 2021; Nissan et al., 2023; Vigricas et al., 2024). Sugarcane waste is rich in recalcitrant compounds such as lignin, cellulose and hemicellulose, while biochar has more stable forms of carbon (Lopes et al., 2021). However, the lower carbon/nutrient ratios resulting from the addition of NPK may have favored faster oxidation, both of the sugarcane straw deposited on the soil surface and of the biochar.

In general, the values of the variables Cmic, SBR, qCO_2, qMIC, and Shannon index, in the two years of evaluation, regardless of the application of NPK, increased up to the intermediate doses of biochar, stabilizing or presenting a slight reduction from the 20 to

30 Mg ha^{-1} doses (Table 2). On the other hand, CO_2 efflux was more significant at lower doses of biochar, while TOC increased linearly (Table 2). A possible hypothesis to explain these results is the priming effect resulting from the addition of biochar to the soil. The priming effect is understood as intense short-term changes in the recycling of SOM caused, for example, by the addition of organic matter to the soil (Zhang et al., 2019). This effect refers to the acceleration of SOM mineralization when organic materials are added to the soil.

In contrast, the delay in SOM mineralization refers to the negative priming effect. In the present study, the acceleration of organic matter mineralization for intermediate doses can be understood as a positive priming effect since biochar adds soluble organic compounds to the soil and favors TOC mineralization. On the other hand, at higher doses of biochar, more stable amounts of carbon and higher carbon/nutrient ratios may have contributed to a lower TOC mineralization rate, that is to say, a negative priming effect. In addition to biochar, sugarcane straw may have contributed to the negative priming effect at higher doses of biochar over time.

It is important to point out that applying biochar to the soil can contribute to the maintenance of TOC, thereby reducing carbon losses in the form of CO_2 and altering soil microbial activity and diversity. Higher doses of biochar may have contributed to lower carbon emissions in the form of CO_2, as suggested by higher TOC contents (Figure 2B) and lower CO_2 efflux values (Table 2), which favor carbon sequestration in the ground.

Considering this hypothesis, it can be inferred that, soon after the incorporation of biochar into the soil, mineralization of more soluble organic compounds occurred, including the TOC native to the soil (positive priming effect). In the first evaluation, one year after the incorporation of biochar into the soil (first year), there may have been a predominance of more stable forms of pyrolyzed carbon. A similar trend was observed in the second year of evaluation. In addition, the highest values of Cmic (Table 2) indicate greater efficiency of carbon assimilation by microorganisms in higher doses of biochar since Cmic represents the most significant proportion of biomass formed per unit of carbon and the smallest amount of mineralized carbon of CO_2 (Vance et al., 1987; Sparling, 1992; Tao et al., 2023).

Another hypothesis is related to the complexity of the biochar matrix, which has a high porosity and specific surface and contributes to the growth and diversity of the population of microorganisms (Chen et al., 2018). It protects the organic matter from microorganisms and extracellular enzymes. According to this hypothesis, organic complexes would be formed, for example, by binding organic compounds to the surfaces of biochar particles by cationic bridges, protecting these compounds from heterotrophic microorganisms in the soil. By the same mechanism, extracellular enzymes can also be adsorbed to biochar particles and thus inhibit TOC mineralization. These reactions can also physically affect the formation of aggregates and protect the TOC from the action of microorganisms and extracellular enzymes. Other authors have proposed similar mechanisms when studying the effect of applying doses of retorted oil shale on the evolution of CO_2, Cmic and soil enzymatic activity (Doumer et al., 2011). Corroborating the results of the present research, in a study carried out in the same experimental area, an increase in the activity of the enzymes β-glucosidase, acid phosphatase, and urease was observed up to doses of 27, 24 and 34 Mg ha^{-1} of biochar incorporated into the soil, respectively (Lopes et al., 2021). With these doses, there was stabilization or reduction in the activity of these enzymes, which aligns with the hypotheses proposed to explain the CO_2 efflux results.

Regardless of the NPK fertilizer, during the two years of evaluation, the height growth of sugarcane plants and TOC increased linearly with the biochar doses. For Cmic, SBR, qCO_2, qMIC and H, the highest values were obtained under WFC treatments, regardless of the year of evaluation, in biochar doses between 20 and 30 Mg ha^{-1}. On the other hand, the highest CO_2 efflux values were obtained with zero doses of biochar, regardless of the NPK fertilizer, over the two years of evaluation.

We, as well as other authors (Zhang et al., 2020; Ghodszad et al., 2021; Novair et al., 2023), recommend the continuous evaluation of the effects of biochars on soil biological processes and the monitoring of risks to the environment, since biochars age over time after application to the soil and, consequently, their characteristics change.

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