

Effect of sewage sludge and sugarcane bagasse biochar on soil properties and sugar beet production



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ABSTRACT

Recently, biochar has shown to be an alternative to waste disposal and a source of nutrients, acting as a soil amendment. The effects of two types of biochar on soil properties and sugar beet production as well as potential for carbon (C) sequestration were evaluated: biochar produced from sewage sludge (SB) and biochar produced from a 1:1 mixture of sewage sludge and sugarcane bagasse (MB). A greenhouse pot experiment was conducted using a sandy loam soil from the Brazilian savanna under treatments of MB applications at 2.5%, 5.0%, 7.5%, and 10.0%, SB application at 5.0%, and a conventional fertilization (CF) using lime and mineral fertilizers, with no fertilization as a control. After incubation for 45 d, seedlings were transplanted into each pot and cultivated for 55 d. Biochar characterization showed that pyrolysis reduced the biomass volume drastically, but concentrated the trace elements per unit of biochar weight. The MB treatments increased soil total C (by 27.8%) and pH (by 0.6), reduced the concentrations of nutrients, except for potassium (K), and chromium (Cr), and did not significantly alter lead (Pb) and cadmium (Cd) concentrations. Results of stable isotopes showed that all biochar treatments increased the total soil C stock and stability, suggesting a potential for application in C sequestration, and improved overall soil fertility. However, the biochar treatments also increased the concentrations of trace elements in the soil and plants. The sugar beet yields at 10.0% MB and 5.0% SB corresponded to 55% and 29% of the yield obtained in the CF treatment, respectively. These results may be due to biochar nutrients not being bioavailable when required by plants or to biochar nutrient adsorption.

Key Words: carbon sequestration, food safety, organic wastes, plant fertilizers, soil fertility, soil organic matter fractions, stable isotopes, waste management

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INTRODUCTION

With increasing demand for plant nutrients, concurrent with increasing waste generation and the growing need for waste management alternatives, the transformation of organic wastes into plant fertilizers has emerged as a low-cost solution to both sides of the problem (Robinson *et al.*, 2018). However, before their widespread use in agricultural production systems, it is important to assess their economic viability and the potential for adverse environmental outcomes (Cha *et al.*, 2016).

Sewage treatment produces a semi-solid waste known as sewage sludge, which is either disposed of in landfills or applied to land as a soil amendment for fertilizing crops, depending on its concentrations of pathogens and trace elements (Lu *et al.*, 2012; Paz-Ferreiro *et al.*, 2018). With 2.5 billion people expected to be living in urban areas by 2050 (UN DESA/P, 2014), a considerable increase in the production of sewage sludge is expected, necessitating

the development of adequate systems for its disposal in the environment (Mateo-Sagasta *et al.*, 2015). Pyrolysis of sludge for biochar production has the advantages of eliminating pathogens, reducing its volume to facilitate transportation, concentrating total carbon (C) and nutrients, and serving as a potentially beneficial soil amendment (Méndez *et al.*, 2012). Biochar refers to the solid, C-rich material produced *via* the thermochemical transformation of biomass in an oxygen-limited environment also known as pyrolysis (Lehmann and Joseph, 2015).

However, pyrolysis of sewage sludge also concentrates potentially toxic trace elements and the biochar produced is still low in C (ranging from 15% to 40%, depending on the pyrolysis temperature), when compared to other types of biochar (Agrafioti *et al.*, 2013; Waqas *et al.*, 2014; Zhao *et al.*, 2014; Pituello *et al.*, 2015). The quantity of C is the main property used to classify the quality of the biochar as per the International Biochar Initiative (IBI, 2015) and the European Biochar Certificate (EBC, 2012). Additionally, the

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legislation for the land application of biosolids has become more restricted (Clarke and Cummins, 2014); therefore, the combination of other material may generate a product of higher value. Sugarcane bagasse, a type of agricultural residues produced in large quantities in Brazil (about 170 Mt in 2016) (IEA, 2017), requires disposal solutions.

The use of biochar in agriculture is justified as both a means of recycling large quantities of an organic waste (Abdelhafez *et al.*, 2014) and a means of reducing the contamination associated with such waste (Ahmad *et al.*, 2014; Gwenzi *et al.*, 2015; Waqas *et al.*, 2015). Compared to natural organic matter, which tends to turn over rapidly under tropical conditions, biochar provides a form of C that is more stable against decomposition (Joško *et al.*, 2013; Gwenzi *et al.*, 2016) and thus has been suggested as a potential strategy for increasing soil C stocks (Racek *et al.*, 2019). Additionally, biochar can act as a soil conditioner, with some evidence showing the potential to increase soil water retention, pH, nutrient availability, and cation exchange capacity (CEC), while reducing soil bulk density and influencing plant-microbial interactions (Hammer *et al.*, 2014).

Concerns over the feasibility of using biochar and sewage sludge in agriculture (Gwenzi *et al.*, 2015; Maroušek *et al.*, 2017) require further investigation into plant-soil interactions following biochar application, as well as the overall influence on agricultural yield. In the present study, the addition of sugarcane bagasse to sewage sludge when making biochar was evaluated in an attempt to increase total C and reduce the concentrations of potentially toxic elements in biochar. We hypothesized that the pyrolysis process is a viable technology for final use/disposal of sewage sludge in the environment. More specifically, i) blending sugarcane bagasse will result in biochar with a higher and more stable C content and lower concentration of heavy metals, ii) at the same application rate, the mixed biochar will outperform the sewage sludge biochar, and iii) waste-derived biochar can be a source of nutrients, which may outperform mineral fertilizers. As such, the objectives of this study were to i) determine whether mixing sewage sludge with sugarcane bagasse, at a 1:1 ratio, can provide biochar of higher quality that complies with the environmental regulations for soil application; ii) determine an optimum application rate for the mixed biochar; iii) determine whether biochar serves as a source of nutrients and increases sugar beet yield compared with soil alone and soil plus similar additions of mineral fertilizers; and iv) evaluate whether biochar can be used for C sequestration purposes. To the best of our knowledge, this is the first study to examine the mixture of two waste products to manipulate biochar characteristics.

MATERIALS AND METHODS

Soil, sewage sludge, and sugarcane bagasse used

The surface layer (0–20 cm) of an Oxisol, developed from sandstone, was collected from an area of the Brazi-

lian savanna with natural vegetation (16°54'14.99" S, 43°57'41.28" W, 600 m above sea level) close to Montes Claros in the State of Minas Gerais (MG), Brazil. According to the Köppen's climate classification (Alvares *et al.*, 2013), the area has a tropical dry climate characterized by a dry winter, with an annual rainfall of 1 000–1 300 mm and an annual mean temperature ≥ 18 °C.

Sewage sludge was collected at the municipal wastewater treatment plant in Montes Claros, which receives and treats only domestic sewage. The treating system consists of removing the sludge from the water system and processing it first in an extended aerobic sewage activation system and then in a continuous flow system, followed by drying pools. The sewage was sampled and handled following Rule 503 defined by the US EPA (1994), as stipulated by the Resolution 375/2006 of the National Environment Council (CONAMA, 2006), which defines the criteria for the agricultural use of sewage sludge in Brazil. Sugarcane bagasse was obtained after the mechanical extraction of the juice for ethanol production in the Sada Bioenergia and Agropecuária industrial plants (Jaíba, MG, Brazil).

Biochar production

To produce mixed biochar (MB), a manually homogenized mixture of sewage sludge and sugarcane bagasse (1:1, volume/volume) was used. From this mixture, beads of approximately 3 cm in diameter were produced and, after drying at room temperature (25 ± 2 °C) for 48 h, were placed in a pyrolysis reactor. The temperature, controlled using a thermocouple inserted in the center of the carbonized mass, was elevated at a rate of approximately 5 °C min^{-1} until it reached 450 °C with a residence time of 30 min, followed by quenching in purified water at 20 °C. To produce sewage sludge biochar (SB), the same procedures were adopted as those described for MB, without the use of sugarcane bagasse. Both MB and SB were ground and passed through a 1-mm mesh sieve for subsequent analysis and application to the soil. Biochar yield was calculated as the ratio of the pyrolyzed biomass to the dry mass of the feedstock.

Soil and biochar characterization

Soil characterization included: i) textural class using the pipette method; ii) pH and electrical conductivity (EC) in water (1:2.5, volume/volume); 3) contents of available phosphorus (P) using colorimetry, exchangeable potassium (K) using photometric flame, and calcium (Ca) and magnesium (Mg) using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 8800 Triple Quadrupole, Agilent Technologies Inc., Palo Alto, USA) after extraction using the ionic exchange resin method; iv) sulfur (S) content using turbidimetry after extraction using calcium

phosphate ($\text{Ca}_3(\text{PO}_4)_2$) solution; v) aluminum (Al) content (exchangeable potential acidity) using titration with sodium hydroxide (NaOH) after extraction using potassium chloride (KCl) solution; iv) CEC determined as the sum of bases and potential acidity ($\text{Ca} + \text{Mg} + \text{K} + \text{hydrogen (H)} + \text{Al}$); vii) total nutritional and trace elements using ICP-MS (Agilent 8800 Triple Quadrupole) after microwave digestion (Microwave Digestion System MARS 6, CEM Corporation, Charlotte, USA) with concentrated nitric acid (HNO_3) using the US EPA (2007) method 3051 A; and viii) total C and N contents by the dry combustion method with an elemental analyzer (LECO CN-2000, LECO Corp., St. Joseph, USA).

Feedstocks and biochar were characterized by the same methods as soil; however, pH and EC in water (1:10, volume/volume) were determined following the methodology described by Rajkovich *et al.* (2012), and the ash content was measured according to the D1762-84 procedure (ASTM, 2013). Biochar morphology was determined with a scanning electron microscope (SEM, JEOL JSM -IT300LV, JEOL Ltd., Tokyo, Japan) at 20 kV. The samples were mounted on a conductive tape covered with a metal carrier (PELCO Tabs™, Ted Pella, Inc., Redding, USA) and coated in a 120-nm gold layer by an evaporator (Leica EM ACE 600, Leica Microsystems, Wetzlar, Germany). Biochar Fourier transform infrared (FTIR) spectroscopy analysis (Agilent Cary 640 FTIR, Agilent Technologies Inc., USA) was performed, at wavenumbers from 4 000 to 400 cm^{-1} , 64 scans per sample, and a 2- cm^{-1} resolution, with the potassium bromide (KBr)-pressed pellet technique by mixing 1 mg of dried biochar with 100 mg of pre-dried and pulverized spectroscopic-grade KBr (Merck and Co., Whitehouse Station, USA).

Greenhouse experiment

A greenhouse experiment was initially conducted in 2014 and repeated in 2016 at the Federal University of Minas Gerais at Montes Claros, MG, Brazil. The experiment was arranged in a completely randomized design with seven treatments in five replicates ($n = 35$). The treatments were: i) no-amendment control (CK); ii) five biochar additions, MB at 2.5%, 5.0%, 7.5%, and 10.0% (volume/volume) and SB at 5.0% (volume/volume); and iii) a conventional fertilization (CF) with lime (1 g dm^{-3} of calcium carbonate (CaCO_3) and 0.4 g dm^{-3} of magnesium carbonate (MgCO_3), ACS grade, to increase the soil pH to 6.5) and mineral fertilizers (monopotassium phosphate (KH_2PO_4), ammonium phosphate monobasic ($\text{NH}_4\text{H}_2\text{PO}_4$), magnesium sulfate (MgSO_4), boric acid (H_3BO_3), zinc chloride (ZnCl_2), and copper chloride pentahydrate ($\text{CuCl}_2 \cdot 5\text{H}_2\text{O}$), ACS grade). The application rates of biochar were defined following plant biomass production vs. biochar additions data for highly weathered soils in the humid tropics as shown by Lehmann and Rondon (2006), who found high yields

under biochar addition of 40 t C ha^{-1} . In the CF treatment, the quantity of nutrients was determined after soil characterization to obtain the highest sugar beet yield on low-fertility soils of the Brazilian savanna.

Pots containing 4 dm^3 of soil (< 4 mm) and biochar were incubated for 45 d to allow soil-biochar reactivity and stabilization. Soil moisture was maintained close to field capacity by daily irrigation with purified water (by reverse osmosis). Following the incubation period, soil samples from each pot were obtained for chemical analyses, and sugar beet seedlings, 1 per pot, were transplanted. The seedlings were previously grown for 30 d in polystyrene trays containing vermiculite without fertilization. Throughout the experiment, there was no need for phytosanitary control, and soil moisture was maintained similar to that during the incubation period. At 55 d post transplanting, the plants were harvested, and soil samples were collected. The tuberous roots were washed with purified water and dried at 55 °C.

Sugar beet root dry matter production was measured, and soil samples, collected before and after sugar beet cultivation, were analyzed following the characterization methods mentioned above. Additionally, plant-available elements in the soil, iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), chromium (Cr), nickel (Ni), lead (Pb), and cadmium (Cd) were extracted using diethylenetriaminepentaacetic acid (DTPA) at pH 7.3 according to the methodology described by EMBRAPA-CNPS (1997) and determined using ICP-MS.

Carbon sequestration assessment

For C sequestration purposes, total C and nitrogen (N) stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) were determined from the soil before and after sugar beet cultivation. The isotopic ratios were determined with a mass spectrometer (Thermo Scientific Delta Plus, Thermo Scientific, Bremen, Germany), coupled to an elemental analyzer (Carlo Erba CHN-1110, Carlo Erba Instruments, Milano, Italy). The C and N isotopic compositions (δ , ‰) were calculated using the following equation:

$$\delta = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 \quad (1)$$

where R is the ratio of $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The standards were the Pee Dee Belemnite (PDB) standards ($\delta^{13}\text{C} = 0.01124\text{‰}$) for C and atmospheric N_2 for N ($\delta^{15}\text{N} = 0$).

The percentages of total C derived from the natural vegetation (savanna) and biochar (MB and SB) were estimated via isotopic dilution, using the following equation, adapted from Balesdent *et al.* (1987):

$$\begin{aligned} \%C_{\text{NV}} \times \delta^{13}\text{C}_{\text{NV}} &= \%C_{\text{NV+biochar}} \times \delta^{13}\text{C}_{\text{NV+Biochar}} \\ &\quad - \%C_{\text{Biochar}} \times \delta^{13}\text{C}_{\text{Biochar}} \end{aligned} \quad (2)$$

where $\%C_{NV}$, $\%C_{NV+biochar}$, and $\%C_{Biochar}$ are the percentages of total C derived from natural vegetation only, natural vegetation and biochar, and biochar only, respectively.

The ^{13}C abundance of the soil derived from the natural vegetation ($\delta^{13}C_{NV}$) was $23.40\text{‰} \pm 0.17\text{‰}$ (eight replicates), the ^{13}C abundance of MB ($\delta^{13}C_{MB}$) was $-18.18\text{‰} \pm 0.13\text{‰}$ (eight replicates), and the ^{13}C abundance of SB ($\delta^{13}C_{SB}$) was $-23.08\text{‰} \pm 0.14\text{‰}$ ($n = 8$). The percentages of total C derived from biochar ($\%C_{Biochar}$) were estimated using the following equation:

$$\%C_{Biochar} = 100 - \%C_{NV} \quad (3)$$

Soil organic matter fractionation

Physical fractionation of soil organic matter was performed based on the methodology described by Christensen (1992) and denominated as light fraction, sand fraction ($> 53 \mu\text{m}$), and silt + clay fraction ($< 53 \mu\text{m}$). The total C content of each fraction was determined using a LECO CN-2000.

Statistical analysis

Data were assessed for normality and heterogeneity of variance using R software version 3.3.0. Regression equations were calculated to evaluate the effect of the biochar (MB) application rate. For all treatments, the means and

95% confidence intervals were calculated, using Student's t test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Characteristics of the soil used

The soil particle size distribution was sand, silt, and clay of 78%, 10%, and 12%, respectively, representing a sandy loam soil (USDA, 2017). Other results from the soil analysis include: pH of 4.7; available P content of 3.41 mg dm^{-3} ; exchangeable K, Ca, and Mg contents of 0.51, 2.0, and $1.0 \text{ mmol}_c \text{ dm}^{-3}$, respectively; Al content of $2.4 \text{ mmol}_c \text{ dm}^{-3}$; CEC of $48.9 \text{ mmol}_c \text{ dm}^{-3}$; and total C content of 6.91 g kg^{-1} .

Yield and characterization of biochar

The MB treatment increased the biochar yield from 16% to 24% (Table I) and increased total C from 21.2% to 27.1% (+27.8%). Both types of biochar had a higher EC than their original feedstocks; however, SB EC was 80% higher than MB EC, probably due to minerals originating from soil contamination during sewage treatment. These results can be attributed to an increase in the long-chain C compounds (such as lignin and cellulose) provided by the sugarcane bagasse. Lee *et al.* (2013) verified correlations

TABLE I

Properties of the feedstocks (sewage sludge and sugarcane bagasse), biochar produced from sewage sludge (SB), and biochar produced from a 1:1 mixture of sewage sludge and sugarcane bagasse (MB) and the limits in the CONAMA Resolution 375 (CONAMA, 2006)

Property	Sewage sludge	SB	Sugarcane bagasse	MB	CONAMA Resolution 375 limit
Yield relative to the feedstock (%)		16.0 ^{a)} (2.3) ^{b)}		24.0 (2.4)	
Ash (g kg^{-1})		622 (75)		515 (36)	
Moisture (%)	1.30 (0.36)	0.01 (0.01)	3.40 (1.24)	0.60 (0.10)	
Pyrolysis temperature ($^{\circ}\text{C}$)		450		450	
pH	5.9 (0.4)	6.5 (0.3)	5.4 (0.2)	7.1 (0.3)	
Electrical conductivity (dS m^{-1})	1.9 (0.3)	3.4 (0.3)	1.4 (0.2)	1.9 (0.2)	
Cation exchange capacity ($\text{cmol}_c \text{ kg}^{-1}$)	31.6 (1.7)	18.9 (2.5)	1.9 (0.4)	12.4 (1.1)	
Density (kg m^{-3})		730 (12.6)		840 (14.2)	
Total C (g kg^{-1})	321 (21)	212 (10)	313 (12)	271 (9)	
Total N (g kg^{-1})	26.4 (3.5)	18.3 (1.5)	23.0 (1.9)	16.1 (2.1)	
C/N	12.2 (2.4)	11.6 (1.5)	13.6 (1.7)	16.8 (2.8)	
B (mg kg^{-1})	40 (3.6)	100 (4.5)	60 (3.6)	66 (3.7)	NA ^{c)}
Ca (g kg^{-1})	38.0 (1.8)	54.2 (3.6)	20.6 (2.5)	45.4 (3.7)	NA
Cd (mg kg^{-1})	8.2 (1.3)	13.4 (1.2)	6.7 (1.4)	14.2 (0.9)	39
Cr (mg kg^{-1})	417 (20)	586 (13)	245 (18)	321 (24)	1000
Cu (mg kg^{-1})	170 (15)	220 (15)	50 (14)	130 (13)	1500
Fe (g kg^{-1})	20.2 (1.4)	33.8 (2.6)	10.8 (0.6)	28.8 (3.5)	NA
K (g kg^{-1})	1.8 (0.4)	3.1 (0.8)	3.0 (0.6)	5.4 (0.9)	NA
Mg (g kg^{-1})	2.9 (0.5)	4.5 (0.3)	1.8 (0.7)	4.1 (0.4)	NA
Mn (mg kg^{-1})	380 (21)	420 (22)	220 (16)	290 (13)	NA
Ni (mg kg^{-1})	64.0 (3.6)	87.3 (6.8)	45.4 (2.6)	78.9 (5.7)	420
P (g kg^{-1})	20.1 (2.6)	42.0 (3.2)	11.5 (1.2)	33.8 (2.6)	NA
Pb (mg kg^{-1})	0.2 (0.0)	7.8 (2.5)	0.2 (0.1)	9.8 (2.3)	300
S (g kg^{-1})	14.9 (2.6)	10.2 (1.9)	10.9 (1.8)	8.4 (1.3)	NA
Zn (mg kg^{-1})	820 (13)	1 070 (33)	370 (30)	990 (33)	2 800

^{a)} Average of five replicates.

^{b)} Values in the parentheses are the 95% confidence intervals.

^{c)} Not applicable.

between the lignin content in feedstock and biochar yield and C. Therefore, mixing sewage sludge with C-rich residues provides C enrichment and a reduction of EC, which can be detrimental to crops when values are high.

Both types of biochar had a higher pH (SB, 6.5; MB, 7.1) (Table I) than that of the soil used (4.7) and thus may serve to reduce soil acidity and increase the availability of soil nutrients for plants. Reduced ash (−17.2%), N (−12%), B (−34%), Ca (−16.2%), Fe (−14.8%), Mg (−8.9%), Mn (−31%), P (−19.5%), and S (−17.6%) contents and CEC (−34%), but increased density (+15.1%), C/N (+44.8%), and K (+74.2%) were observed in MB when compared to SB. These effects can be related to the chemical composition of the original feedstock (Table I). Regarding potentially toxic elements, MB had reduced the concentration of Cr (−45%), Cu (−40.9%), and Ni (−9.6%), but increased Pb (+25.6%) and did not significantly change Cd (−6%) when compared to SB. However, the Pb concentrations in both types of biochar were relatively low when compared to the maximum value established in the CONAMA Resolution 375 (CONAMA, 2006) and those in other studies (Uchimiya *et al.*, 2011; Ahmad *et al.*, 2014).

Concentrations of potentially toxic elements for both feedstocks and both types of biochar were lower than the maximum concentrations for agricultural applications established by legislation (CONAMA, 2006). Therefore, the sewage sludge utilized in the present study was of relatively high quality, in terms of toxic metal contents. It is important to always consider the chemical composition of the sewage sludge because concentrations of trace elements in sewage sludge are variable depending on sludge type, treatment, and wastewater source.

The SEM images showed morphological differences between SB and MB (Fig. S1, see Supplementary Material for Fig. S1). The surface particles of MB had an increased laminar pattern when compared to those of SB, likely due to the vegetable morphology and presence of siliceous bodies from the sugarcane bagasse. In contrast, SB showed an increased amorphous structure when compared to MB.

The FTIR spectra for SB and MB were similar (Fig. 1). Both SB and MB showed transmittance bands in the regions of 3 018–2 993 cm^{-1} (C–H in CH_2 and CH_3), 1 603 cm^{-1} (C=O), and 1 021 cm^{-1} (Si–O or C–O), based on the interpretation proposed by Parikh *et al.* (2014) and Silverstein *et al.* (2014). The SB spectra showed a much larger peak at the 1 021 cm^{-1} band, which can be attributed to the Si–O stretching vibration, probably due to soil contamination during the sludge collection and treatment process. In addition, SB showed a band at 3 701 cm^{-1} , which may be attributed to N–H or O–H as proposed by Hossain *et al.* (2011), and a band at 1 423 cm^{-1} (C=C), whereas MB showed a band around 3 480 cm^{-1} (O–H) and 1 372 cm^{-1} (aromatic C–O

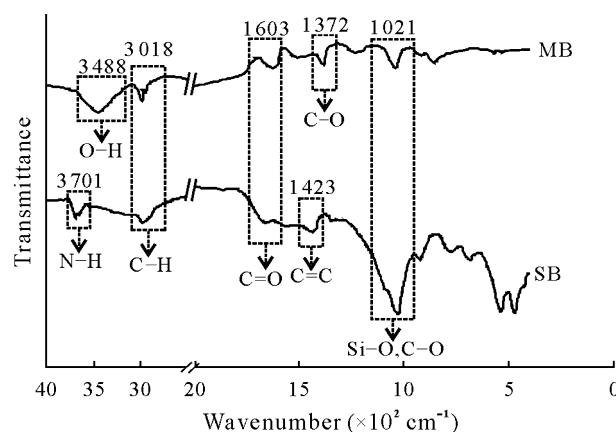


Fig. 1 Fourier transform infrared (FTIR) spectra, with functional groups indicated, of the biochar produced from sewage sludge (SB) and the biochar produced from a 1:1 mixture of sewage sludge and sugarcane bagasse (MB)

and C–H in CH_2 and CH_3). Overall, both biochar types contained numerous surface functional groups, which is expected for biochar made at a relatively low pyrolysis temperature (450 °C); for example, Koetlisi and Muchaonyerwa (2017) found that biochar produced from latrine waste had N–H functional groups at 350 °C (3 694.07 cm^{-1}) and 550 °C (3 691.69 cm^{-1}), but not at 650 °C. With N content of 18.3 g kg^{-1} (Table I), SB was rich in amino functional groups (N–H). The SEM image verified the amorphous structure and globular features, which may be oxides. Thus, the peaks in the 550–450 cm^{-1} area in the SB spectra can also be an indication of metal oxides.

In summary, MB was considered a type of biochar of higher quality (higher yield, C content, and pH and lower concentrations of potentially toxic elements) than SB and was thus selected for subsequent studies to determine the optimum application rates.

Biochar effects on the availability of soil nutrients

Greater amounts of N in the soil were found in the 5.0% SB and 5.0% MB treatments than in the CF treatment (Table II). Similar trends were found for P, K, Ca, Mg, S, boron (B), Zn, and Cu. Moreover, applications of both biochar types increased total C, Fe, and Mn. This corroborates the data shown in Table I, demonstrating that MB and SB were sources of these nutrients and their applications at 5.0% were capable of surpassing contributions from mineral fertilizers. Similar results were obtained by Glaser *et al.* (2002), Houben *et al.* (2013), and Griffin *et al.* (2017).

Biochar effects on soil properties

All the MB and SB treatments increased the soil pH to approximately 7 (Table III), which was likely due to the greater addition of ash. Corroborating results have been obtained by other researchers (*e.g.*, Albuquerque *et al.*,

TABLE II

Biochar amount and nutritional and trace elements added to the test soil (a sandy loam soil from the Brazilian savanna) under sugar beet cultivation in each 4-dm³ pot by different treatments, biochar produced from sewage sludge (SB) at 5.0% application rate, biochar produced from a 1:1 mixture of sewage sludge and sugarcane bagasse (MB) at 2.5%, 5.0%, 7.5%, and 10.0% application rates, and a conventional fertilization (CF) with lime and mineral fertilizers

Item	SB ^{a)}	MB ^{a)}				CF
		2.5%	5.0%	7.5%	10.0%	
Biochar amount in g dm ⁻³ /g pot ⁻¹	37/146 ^{b)}	21/84	42/168	63/252	84/336	
Total C in g dm ⁻³ /g pot ⁻¹	0.77/3.10	0.57/2.28	1.14/4.55	1.71/6.83	2.28/9.11	–
Total N in g dm ⁻³ /g pot ⁻¹	0.67/2.67	0.34/1.35	0.68/2.70	1.01/4.06	1.35/5.41	0.10/0.40
P in g dm ⁻³ /g pot ⁻¹	1.53/6.13	0.71/2.84	1.42/5.68	2.13/8.52	2.84/11.36	0.30/1.20
K in g dm ⁻³ /g pot ⁻¹	0.11/0.44	0.11/0.45	0.23/0.91	0.34/1.36	0.45/1.81	0.10/0.30
Ca in g dm ⁻³ /g pot ⁻¹	1.98/7.91	0.93/3.73	1.86/7.46	2.80/11.19	3.73/14.92	0.40/1.60
Mg in g dm ⁻³ /g pot ⁻¹	0.16/0.66	0.09/0.34	0.17/0.69	0.26/1.03	0.34/1.38	0.13/0.43
S in g dm ⁻³ /g pot ⁻¹	0.37/1.49	0.18/0.71	0.35/1.41	0.53/2.12	0.71/2.82	0.04/0.16
B in mg dm ⁻³ /mg pot ⁻¹	3.7/14.6	1.4/5.5	2.8/11.1	4.2/16.6	5.5/22.2	0.5/2.0
Fe in mg dm ⁻³ /mg pot ⁻¹	1 232/4 928	604/2 418	1 209/4 836	1 813/7 255	2 418/9 673	–
Zn in mg dm ⁻³ /mg pot ⁻¹	39.1/156.2	20.8/83.2	41.6/166.3	62.4/249.5	83.2/332.6	5.0/20.0
Mn in mg dm ⁻³ /mg pot ⁻¹	15.3/61.3	6.1/24.4	12.2/48.7	18.3/73.1	24.4/97.4	–
Cu in mg dm ⁻³ /mg pot ⁻¹	8.0/32.1	2.7/10.9	5.5/21.8	8.2/32.8	10.9/43.7	1.5/6.0
Cr in mg dm ⁻³ /mg pot ⁻¹	25.6/102.2	10.9/43.7	21.9/87.5	32.8/131.2	43.7/175.0	–
Ni in mg dm ⁻³ /mg pot ⁻¹	3.19/12.74	1.66/6.63	3.31/13.26	4.97/19.88	6.63/26.51	–
Pb in mg dm ⁻³ /mg pot ⁻¹	0.29/1.14	0.21/0.82	0.41/1.65	0.62/2.47	0.82/3.29	–
Cd in mg dm ⁻³ /mg pot ⁻¹	0.49/1.96	0.30/1.19	0.60/2.39	0.89/3.58	1.19/4.77	–

^{a)} The densities of SB and MB are 730 and 840 g dm⁻³, respectively.

^{b)} Average of five replicates.

2014). This may have contributed to greater P availability, as acid soils have a high capacity for P fixation (Eduah *et al.*, 2019). In addition, the possible presence of soluble silica in the biochar ash could have contributed to soil P availability (Liu *et al.*, 2014). According to Sandim *et al.* (2014), silicate anions compete with phosphate anions for the same adsorption sites and can saturate sites that are otherwise available for P adsorption. Thus, biochar can contribute to soil fertility via P availability, which has strong interactions in highly weathered (rich in Al and Fe oxides) acid soils (Yuan and Xu, 2011; Alburquerque *et al.*, 2014). The high content of biochar ash, which is probably rich in oxides and carbonates of K, Ca, and Mg (Rehrah *et al.*, 2014), contributes to the reduction in soil acidity. Therefore, the biochar acted as a reducer of soil acidity and, consequently, may have decreased Al toxicity to the plants (Gwenzi *et al.*, 2016), which is common in highly weathered acid soils. To bring the pH to 7 in the CF treatment, it was necessary to add lime. Even after sugar beet cultivation, the pH of the soil with biochar remained high and comparable to the values obtained before cultivation.

In addition to increasing soil pH, the biochar applications increased the CEC and, thus, the availability of Ca, Mg, and K before and after sugar beet cultivation (Table III). The biochar applications also altered the levels of micronutrients in the soil, including Fe, Mn, Cu, Zn, B, and Ni, and potentially toxic trace elements, Cr, Pb, and Cd, because biochar is the source of these elements. Méndez *et al.* (2012), Houben *et al.* (2013), and Alburquerque *et al.* (2014) observed a reduction in the availability of cationic micronutrients and trace elements as a result of complexation with functional

groups in organic matter and/or precipitation because of the increase in soil pH with biochar application. In the present study, however, with an increase in the MB application rate, the availability of micronutrients and trace elements in the soil increased linearly (Table III), even with increases in soil C and pH.

Biochar effects on sugar beet production

Positive effects of MB and SB applications on the dry matter yield of tuberous roots of sugar beet plants were verified in comparison to CK; however, less positive effects were found when compared to the CF (Fig. 2). The dry matter yields for CK, the 2.5%, 5.0%, 7.5%, and 10.0% MB, 5.0% SB, and CF treatments were 0.42, 1.41, 2.36, 4.50, 5.81, 3.01, and 10.50 g plant⁻¹, respectively. Hence, the 10.0% MB treatment had the highest yield among the biochar treatments. Although the yields in the 10.0% MB and 5.0% SB treatments were 13.83 and 7.17 times higher than that of CK, they corresponded to only 55% and 29% of that obtained in the CF treatment, respectively. Although the present study did not have the objective to analyze the economic viability of the biochar applied, the application of 10.0% MB and 5.0% SB was estimated to correspond to an application of 100 and 50 L m⁻³ of biochar per hectare (0–20 cm depth), respectively. These applications rates would make biochar application expensive for commercial use.

The less positive effect of biochar application than CF was because biochar nutrients were not fully available during

TABLE III

Soil pH, cation exchange capacity (CEC), and nutritional and trace elements before and after sugar beet cultivation (SBC) on the test soil (a sandy loam soil from the Brazilian savanna) with different treatments, no-amendment control (CK), biochar produced from sewage sludge (SB) at 5.0% application rate, biochar produced from a 1:1 mixture of sewage sludge and sugarcane bagasse (MB) at 2.5%, 5.0%, 7.5%, and 10.0% application rates, and a conventional fertilization (CF) with lime and mineral fertilizers

Time	pH, CEC, or element	CK	CF	SB	MB			
					2.5%	5.0%	7.5%	10.0%
Before	pH	4.7 ^{a)} (0.3) ^{b)}	6.24 (0.4)	6.26 (0.4)	5.42 (0.3)	5.8 (0.4)	6.12 (0.3)	6.22 (0.4)
SBC	CEC (mmol _c kg ⁻¹)	3.04 (0.5)	4.02 (0.4)	6.34 (0.8)	3.46 (0.4)	4.52 (0.5)	4.89 (0.5)	6.23 (0.6)
	Total C (g kg ⁻¹)	6.92 (1.0)	6.93 (1.2)	13.45 (2.4)	8.82 (1.5)	11.34 (1.7)	12.98 (2.7)	14.33 (2.9)
	Total N (mg kg ⁻¹)	2.90 (0.6)	3.20 (0.9)	11.20 (1.5)	4.00 (1.0)	6.10 (1.0)	7.80 (1.3)	9.70 (1.7)
	B (mg kg ⁻¹)	8.89 (3.0)	10.91 (3.0)	11.99 (1.1)	9.99 (3.0)	10.51 (1.0)	10.84 (2.0)	10.59 (3.0)
	Ca (mmol _c kg ⁻¹)	2.00 (1.0)	10.01 (3.2)	26.60 (6.3)	9.40 (3.0)	14.62 (4.0)	16.00 (6.0)	25.00 (6.1)
	Cd (μg kg ⁻¹)	1.00 (0.5)	1.00 (0.4)	21.00 (4.0)	1.00 (0.5)	2.00 (1.0)	12.00 (2.0)	13.00 (3.0)
	Cr (μg kg ⁻¹)	150.00 (33.0)	260.00 (32.0)	490.00 (67.0)	180.00 (34.0)	390.00 (45.0)	350.00 (51.0)	400.00 (49.0)
	Cu (mg kg ⁻¹)	0.21 (0.1)	0.19 (0.1)	2.83 (0.6)	0.62 (0.2)	1.09 (0.4)	1.63 (0.4)	1.96 (0.5)
	Fe (g kg ⁻¹)	63.41 (23.1)	82.98 (20.9)	276.37 (62.9)	89.79 (24.0)	141.99 (42.9)	158.37 (43.0)	185.76 (53.9)
	K (g kg ⁻¹)	19.50 (4.1)	19.50 (6.9)	42.90 (8.0)	42.90 (6.0)	58.50 (7.0)	70.20 (8.1)	93.60 (9.2)
	Mg (mmol _c kg ⁻¹)	280.00 (0.3)	310.00 (1.0)	540.00 (1.1)	370.00 (0.8)	370.00 (1.0)	380.00 (1.1)	470.00 (1.1)
	Mn (mg kg ⁻¹)	2.83 (1.0)	7.07 (1.9)	9.60 (2.5)	4.55 (1.2)	5.65 (2.0)	6.42 (2.3)	7.21 (2.0)
	Ni (μg kg ⁻¹)	30.00 (14.0)	20.00 (7.0)	250.00 (46.0)	60.00 (21.0)	130.00 (36.0)	140.00 (42.0)	190.00 (44.0)
	P (mg kg ⁻¹)	3.75 (1.3)	5.20 (2.0)	67.95 (14.0)	21.92 (6.0)	40.06 (12.0)	56.45 (12.1)	73.87 (16.0)
	Pb (μg kg ⁻¹)	280.00 (67.0)	310.00 (54.0)	540.00 (97.0)	370.00 (65.0)	370.00 (69.0)	380.00 (75.0)	470.00 (89.0)
	S (mg kg ⁻¹)	6.54 (1.2)	9.54 (1.1)	10.23 (1.6)	8.43 (1.4)	10.34 (1.5)	12.34 (2.0)	14.54 (1.7)
	Zn (mg kg ⁻¹)	0.44 (0.2)	0.37 (0.1)	21.11 (4.0)	4.12 (2.0)	7.39 (3.0)	10.50 (3.0)	14.53 (3.0)
After	pH	5.44 (0.3)	6.26 (0.4)	6.24 (0.4)	5.57 (0.3)	5.98 (0.2)	6.4 (0.4)	6.38 (0.3)
	CEC (mmol _c kg ⁻¹)	3.07 (0.6)	4.52 (0.5)	7.00 (0.7)	4.58 (0.5)	4.88 (0.6)	4.92 (0.5)	5.41 (0.7)
	Total C (g kg ⁻¹)	6.85 (0.8)	7.16 (1.4)	13.24 (2.9)	8.90 (2.4)	11.28 (2.5)	12.84 (3.1)	14.68 (2.6)
	Total N (mg kg ⁻¹)	3.9 (0.7)	5.9 (0.7)	8.6 (1.6)	5.2 (0.7)	6.3 (1.0)	7.4 (1.4)	7.9 (1.8)
	B (mg kg ⁻¹)	10.31 (3.2)	12.86 (2.0)	13.07 (3.1)	12.55 (1.0)	11.95 (2.0)	9.59 (3.0)	11.74 (2.2)
	Ca (mmol _c kg ⁻¹)	9.0 (2.0)	14.8 (4.1)	29.6 (5.0)	12.9 (4.0)	18.2 (5.1)	18.7 (4.0)	22.30 (5.0)
	Cd (μg kg ⁻¹)	1 (0.5)	1 (0.5)	21 (3.0)	5 (1.0)	6 (2.0)	14 (2.0)	< 0.01
	Cr (μg kg ⁻¹)	180 (29)	220 (34)	440 (65)	240 (36)	290 (65)	360.00 (37)	400 (76)
	Cu (mg kg ⁻¹)	0.17 (0.1)	0.18 (0.1)	2.43 (0.5)	0.59 (0.2)	0.99 (0.3)	1.33 (0.4)	1.65 (0.4)
	Fe (g kg ⁻¹)	47.14 (21.0)	67.08 (31.0)	199.14 (58.9)	72.35 (23.1)	101.84 (41.0)	128.95 (53.0)	145.98 (24.1)
	K (g kg ⁻¹)	15.6 (3.0)	74.1 (8.1)	23.4 (5.0)	19.5 (5.0)	23.4 (6.0)	19.5 (6.0)	31.2 (7.1)
	Mg (mmol _c kg ⁻¹)	240 (0.4)	220 (1.3)	460 (1.2)	290 (0.7)	340 (1.3)	370 (1.4)	390 (1.2)
	Mn (mg kg ⁻¹)	2.74 (0.8)	5.41 (1.2)	9.18 (2.3)	4.19 (1.0)	5.31 (1.5)	6.55 (2.0)	7.33 (2.0)
	Ni (μg kg ⁻¹)	30 (10)	10 (3)	210 (41)	70 (19)	100 (21)	140 (340)	170 (39)
	P (mg kg ⁻¹)	4.73 (2.0)	53.57 (12.0)	71.80 (15.0)	39.45 (9.0)	51.30 (16.1)	66.35 (14.0)	77.58 (18.0)
	Pb (μg kg ⁻¹)	240 (54)	220 (32)	460 (87)	290 (56)	340 (65)	370 (76)	390 (76)
	S (mg kg ⁻¹)	8.54 (1.0)	10.31 (2.0)	12.32 (14.1)	10.67 (6.0)	11.32 (12.0)	14.56 (12.0)	16.88 (16.0)
	Zn (mg kg ⁻¹)	0.85 (0.3)	1.45 (0.3)	19.96 (4.0)	4.27 (3.0)	7.45 (3.0)	10.89 (3.0)	13.99 (4.0)

^{a)} Average of five replicates.

^{b)} Values in the parentheses are the 95% confidence intervals.

phases of greater demand by the plants, owing either to them not being released or to biochar nutrient adsorption. The adsorption capacity of biochar for cations is directly influenced by the surface chemistry and surface area of biochar (Ni *et al.*, 2019), both of which are influenced by the pyrolysis temperature and feedstock (Kwak *et al.*, 2019). In the present study, both biochar types were produced at a relatively low temperature (450 °C), and the maximum soil pH value did not exceed 6.38 (Table III). Another explanation is that the extractant DTPA, used in the present study, acted as a complexing agent and may have competed for metals with the biochar. Non-nutritional factors such as phytotoxicity were also possible reasons.

Regarding the amount of nutrients found in the roots, plants in the SB and MB treatments absorbed more P, S, Fe, and Zn, but less N and Mn when compared to the CF

treatment (Table IV). The higher N uptake by plants likely explained the higher yield in the CF treatment. In general, the concentrations of macronutrients in the roots of the sugar beet plants increased linearly with the MB application rates except for Fe, which reduced linearly, and for B, which was found to have no significant differences between treatments. The concentrations of the potentially toxic elements were not significantly different in the roots except for Cu, which was lower in the 2.5% MB treatment than in the CF and other biochar treatments. The potentially toxic elements were within the limits set in the International Standards for Heavy Metals for Plants proposed by the Codex Alimentarius Commission (FAO/WHO, 2011). Similar results were reported in the study by Wuana and Okieimen (2011), where the concentrations of these elements were below regulatory limits. Among the biochar treatments, the 10.0% MB treatment

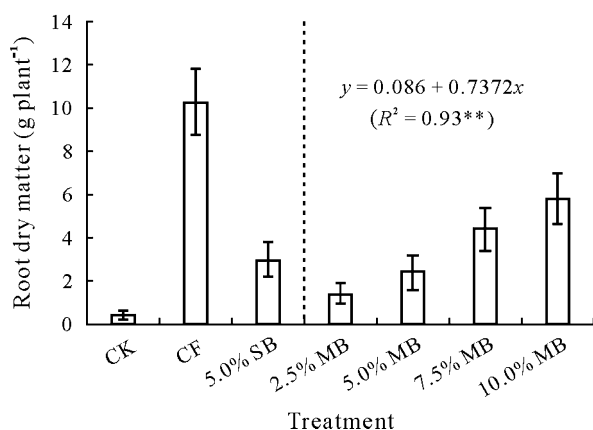


Fig. 2 Dry matter yields of tuberous roots of sugar beet plants (y) on the test soil (a sandy loam soil from the Brazilian savanna) with different treatments, no-amendment control (CK), biochar produced from sewage sludge (SB) at 5.0% application rate, biochar produced from a 1:1 mixture of sewage sludge and sugarcane bagasse (MB) at 2.5%, 5.0%, 7.5%, and 10.0% application rates (x), and a conventional fertilization (CF) with lime and mineral fertilizers. The asterisks ** indicate significance at $P < 0.01$ (Student's t test).

showed the lowest plant uptake of potentially toxic elements except for Cu, a micronutrient, and the highest yield. When compared to the 5% MB treatment, the 5.0% SB treatment presented a slightly higher yield and similar uptake of potentially toxic elements (except for Cu), demonstrating that the mixture of plant residues to sewage sludge when making biochar was not very advantageous for these parameters.

Two theories have been proposed to evaluate the potential concentrations of trace elements added to soil *via* biosolid: the plateau theory and time bomb theory (Silveira *et al.*, 2003). The plateau theory was used for the development of

the EPA Rule 503 (US EPA, 1994) and considered the effects of organic matter present in biosolid when restricting plant uptake of metals added by biosolid. According to this theory, the application of biosolid leads to linear increases in plant uptake and soil adsorption of metals. As the highest amount of biosolid was applied to soil, the strong binding sites of the biosolid matrix became dominant over the binding sites in soil, without altering the concentrations of metals in the plant tissues, thus reaching a plateau (Chang *et al.*, 1997). We emphasized that the present study was not designed with an objective to validate or disprove the plateau theory.

Extrapolating the plateau theory to biochar, the concentrations of micronutrients and trace elements in the sugar beet roots, except for B, tended to reach a plateau with an increasing MB application rate. In general, from the application rate of 7% upwards, the concentrations of these elements in the roots reached a plateau (Table V).

According to the time bomb theory, the elements present in biosolid may be available over time by the continuous mineralization of the organic matter present in biosolid. Biochar, due to interactions with the soil over the years, may increase the adsorption of metals into the biochar matrix (Jorio *et al.*, 2012) and not make it available again to the environment.

Although the concentrations of trace elements in sugar beet plants were not high enough to be of concern, the safety limits of these elements in the soil and plants should not only consider the individual concentration of each element. Because biochar is often a source of various elements, one should consider the interactions between them, especially synergists, in which one element may increase the toxic potential of the other.

TABLE IV

Concentrations of nutritional and trace elements in tuberous roots of sugar beet plants on the test soil (a sandy loam soil from the Brazilian savanna) with different treatments, no-amendment control (CK), biochar produced from sewage sludge (SB) at 5.0% application rate, biochar produced from a 1:1 mixture of sewage sludge and sugarcane bagasse (MB) at 2.5%, 5.0%, 7.5%, and 10.0% application rates, and a conventional fertilization (CF) with lime and mineral fertilizers

Element	CK	CF	SB	MB			
				2.5%	5.0%	7.5%	10.0%
B (mg kg ⁻¹)	10.3 ^{a)} (1.78) ^{b)}	12.86 (0.98)	13.07 (0.95)	12.55 (1.45)	11.95 (1.39)	9.59 (1.05)	11.74 (0.85)
Ca (g kg ⁻¹)	1.62 (0.23)	6.41 (1.34)	6.56 (0.67)	2.93 (0.43)	3.27 (0.42)	4.89 (0.09)	7.85 (1.04)
Cd (mg kg ⁻¹)	0.39 (0.23)	0.43 (0.06)	0.45 (0.04)	0.43 (0.09)	0.35 (0.04)	0.28 (0.03)	0.26 (0.03)
Cr (mg kg ⁻¹)	0.44 (0.11)	0.45 (0.05)	0.51 (0.05)	0.53 (0.13)	0.54 (0.06)	0.33 (0.03)	0.26 (0.03)
Cu (mg kg ⁻¹)	6.25 (0.35)	19.62 (2.01)	19.75 (2.35)	6.35 (1.03)	10.31 (1.84)	12.33 (1.05)	18.12 (2.46)
Fe (mg kg ⁻¹)	314.56 (39.85)	120.60 (17.42)	240.67 (23.56)	318.18 (29.82)	251.91 (42.65)	184.42 (21.56)	178.57 (13.56)
K (g kg ⁻¹)	10.64 (2.87)	23.25 (1.53)	18.44 (1.34)	12.50 (2.45)	13.09 (1.04)	18.78 (1.83)	24.60 (1.32)
Mg (g kg ⁻¹)	1.61 (0.76)	2.26 (0.47)	2.56 (0.18)	1.99 (0.45)	2.46 (0.09)	2.61 (0.03)	2.66 (0.64)
Mn (mg kg ⁻¹)	47.62 (14.93)	46.99 (3.78)	25.93 (1.56)	38.67 (4.23)	33.29 (2.45)	30.87 (6.83)	25.26 (1.76)
N (g kg ⁻¹)	12.20 (2.94)	28.10 (1.34)	26.30 (1.34)	22.14 (3.78)	24.24 (1.21)	25.32 (1.95)	27.80 (1.93)
Ni (mg kg ⁻¹)	0.37 (0.09)	0.43 (0.03)	0.45 (0.04)	0.46 (0.09)	0.55 (0.04)	0.62 (0.03)	0.35 (0.02)
P (g kg ⁻¹)	2.94 (0.84)	3.71 (0.23)	7.31 (1.23)	4.56 (0.7)	7.78 (0.09)	8.43 (0.74)	9.37 (0.75)
Pb (mg kg ⁻¹)	0.45 (0.12)	0.42 (0.03)	0.43 (0.02)	0.68 (0.12)	0.53 (0.08)	0.42 (0.03)	0.36 (0.03)
S (g kg ⁻¹)	0.90 (0.12)	1.90 (0.56)	4.71 (0.59)	2.98 (0.28)	3.99 (0.56)	4.05 (0.58)	4.75 (0.34)
Zn (mg kg ⁻¹)	102.46 (28.56)	91.01 (10.35)	118.21 (11.85)	121.72 (19.22)	129.69 (29.49)	112.38 (10.53)	109.54 (9.54)

^{a)} Average of five replicates.

^{b)} Values in the parentheses are the 95% confidence intervals.

Carbon sequestration

As shown in Fig. 3a, the $\delta^{13}\text{C}$ values obtained for CK and the 5.0% MB, and 5.0% SB treatments were -23.4‰ , -21.83‰ , and -23.08‰ before cultivation and -23.35‰ , -21.42‰ , and -23.1‰ after cultivation, respectively. The values obtained for CK (under natural vegetation) and the SB treatment correspond to those reported in the literature for C3 plants that discriminate ^{13}C with values ranging from -33‰ to -23‰ and the value obtained for the MB treatments approximates those obtained for C4 plants that discriminate ^{13}C ranging from -16‰ to -9‰ (Deines, 1980). The less negative result for the MB treatments can be explained by the incorporation of bagasse of sugarcane, a C4 plant, into the biochar.

As the MB application rates increased, the $\delta^{13}\text{C}$ values became less negative, ranging from -22.41‰ in the 2.5% MB treatment to -21.1‰ in the 10.0% MB treatment after sugar beet cultivation (Fig. 3a Balesdent *et al.* (1987) showed that the incorporation of C4 plants to the soil made the $\delta^{13}\text{C}$ values less negative, indicating an increase in organic C turnover. Thus, these results indicate a possible contribution of MB to a positive priming effect on the soil organic matter. Indeed, the 5.0% SB treatment increased soil C by 49.2% and 49.07% before and after cultivation, respectively, whereas the 5.0% MB treatment increased soil C by 39.8% and 39.4% before and after cultivation, respectively (Fig. 3a). These results suggest that even with SB having less total C than MB, SB may be more advantageous in terms of stability than MB.

Yousaf *et al.* (2017) compared soil total organic C content and $\delta^{13}\text{C}$ values under the treatment with biochar made from wood sawdust at 450 °C with those under the treatments with various biowastes (pressmud, farm manure, compost, sewage sludge, and poultry manure) and unamended control after incubating for 120 d. All amendments were applied at

the same rate, *i.e.*, 2% oxidizable C (OC) basis. Their results indicated that biochar increased total organic C (TOC), oxidizable C, and recalcitrant C (RC) ($P \leq 0.05$) compared with the biowaste amendments and control after 120 d. They also found significant decreases in the $\delta^{13}\text{C}$ value of the soil with the addition of biochar (becoming more negative), indicating a negative priming effect. Therefore, their biochar reduced the C mineralization *via* soil C stabilization because of higher RC content and probably because of sorption of labile organic C onto biochar particles, which resulted in less decomposition and higher potential for C sequestration.

Additionally, the results for the soil organic matter physical fractionation showed that with an increase in MB application rate, there was an increase in the light fraction and in the silt + clay fraction ($< 53\text{ }\mu\text{m}$) and thus a decrease in the bigger particles (sand fraction, $> 53\text{ }\mu\text{m}$) (Fig. S2, see Supplementary Material for Fig. S2). The light fraction of soil organic matter may be indicative of soil quality, as it is highly subject to management practices, whereas the silt + clay fraction represents more stable organic matter (Christensen, 1992; Lisboa *et al.*, 2009). However, as fractionation was based on particle density, the methodology used may not be the most appropriate for soils that have received biochar application. According to Schmidt and Noack (2000), the presence of charcoal in soil resulting from the burning of vegetation can complicate the interpretation of soil organic matter parameters, such as C concentration, physical and chemical properties, mineralization rates, and age.

According to Hgberg (1997), the determination of the $\delta^{15}\text{N}$ signal allows information to be obtained about processes related to N dynamics in soil because mineralization favors greater decomposition of the lighter isotope of N (^{14}N), making the remaining organic matter enriched in ^{15}N . In the present study, values of $\delta^{15}\text{N}$ in soil (Fig. 3b) were not significantly different among treatments, averaging -6.28‰

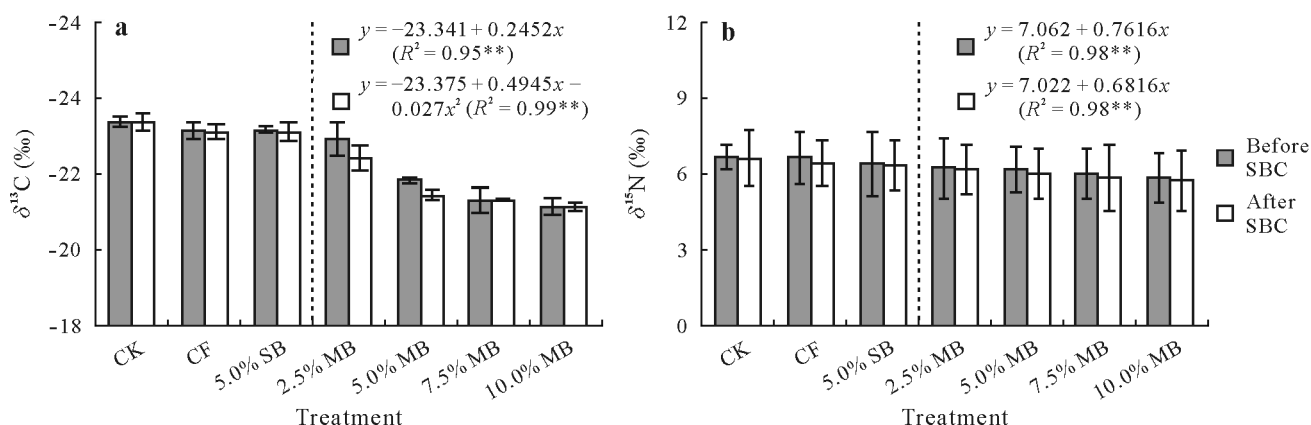


Fig. 3 Total C and N stable isotopes, $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b), respectively, in the test soil (a sandy loam soil from the Brazilian savanna) (*y*) before and after sugar beet cultivation (SBC) with different treatments, no-amendment control (CK), biochar produced from sewage sludge (SB) at 5.0% application rate, biochar produced from a 1:1 mixture of sewage sludge and sugarcane bagasse (MB) at 2.5%, 5.0%, 7.5%, and 10.0% application rates (*x*), and a conventional fertilization (CF) with lime and mineral fertilizers. The asterisks ** indicate significance at $P < 0.01$ (Student's *t* test).

and -6.17‰ before and after cultivation, respectively, were slightly more negative in the 5.0% SB treatment than in the 5.0% MB treatment, and slightly decreased with increasing MB application rates.

CONCLUSIONS

The pyrolysis process significantly reduced the volume of residue biomass to be disposed of in the environment, but increased the concentrations of trace elements per unit of biochar weight. The addition of sugarcane bagasse to the sewage sludge altered the morphology and types of functional groups of the biochar produced, increased the total C, and reduced the concentrations of nutritional elements and Cr, but did not significantly alter the concentrations of Pb and Cd. Both types of biochar, MB and SB, increased the total soil C concentration, which is an indication of the biochar C sequestration potential, and improved soil properties related to fertility. However, biochar increased the concentrations of trace elements in soil and plant, and in the biochar treatments even at higher application rates, sugar beet production was significantly lower than that in the CF treatment with limestone and mineral fertilizers, probably because biochar nutrients were not fully available when in greater demand by plants.

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