Biochars from local agricultural waste residues contribute to soil quality and plant growth in a Cerrado region (Brazil) Arenosol

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Abstract

Arenosols (sandy soils) in the Cerrado region of Mato Grosso, Brazil, are increasingly used for maize production, the second most important crop in the region after soybean. Yet, these soils are typically nutrient poor with low soil water retention, requiring high fertilizer inputs that are often lost in surface runoff or leached. The addition of biochar, a more recalcitrant organic amendment, may therefore be beneficial in Cerrado Arenosols, contributing to sustainable crop production in the region. To examine biochar contribution to soil nutrient levels and maize growth in a Cerrado Arenosol, we conducted a greenhouse experiment using biochars made from local agricultural waste feedstocks. These were cotton husks, swine manure, eucalyptus sawmill residue, and sugarcane filtercake, pyrolyzed at 400 °C, and applied to soil at five rates: 0%, 1%, 2%, 3%, and 4% by weight. Maize plants were grown under unstressed conditions (e.g., no nutrient or water limitations) to highlight any possible negative effects of the biochars. After 42 days, soils were analyzed for nutrient levels, and plant physical and physiological measurements were taken. Filtercake biochar had the highest plant biomass and physiological properties (e.g., photosynthesis, respiration, nitrogen use efficiency), while cotton biochar had the lowest. Importantly, maize biomass decreased with increasing application rates of cotton and swine manure biochars, while biomass did not vary in response to biochar application rate for filtercake and eucalyptus biochars. In this study, we found that while each biochar exhibited potential for improving chemical and physical properties of Cerrado Arenosols, filtercake biochar stood out as most promising. Biochar application rate was identified as a key factor in ensuring crop productivity. Transforming these agricultural residues readily available in the region into more stable biochar can thus contribute to sustainable crop management and soil conservation, providing an alternative form of waste disposal for these residual materials.

Keywords: agricultural wastes, Arenosol, biochar, Brazil, Cerrado, plant physiology, soil nutrients, sustainable crop management

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Introduction

The state of Mato Grosso, Brazil, encompasses a large portion of the Brazilian savanna biome, known as the Cerrado. Characterized by grassland vegetation, the biome is increasingly being transformed for agricultural use. Arenosols are sandy soils that cover about 13% of the area of Mato Grosso and are particularly used for growing maize (SEPLAN, 2008). The importance of maize, especially the crop grown during the dry season, has risen significantly in the last 10 years, with total maize production in Mato Grosso exceeding 20 Mt year⁻¹ in recent years (IBGE, 2016). However, the region’s high precipitation rates (mostly during the rainy season) and base cation leaching lead to the development of acidic soils, which results in soils with low cation exchange capacity (CEC), low base saturation levels, and low water-holding capacity (Fageria, 2001; da Costa et al., 2013).

Biochar (charcoal derived from waste biomass by pyrolysis) has been observed to increase soil water and nutrient retention under some conditions (Lehmann, 2007; Spokas et al., 2012). Its amendment to sandy soils,
particularly tropical, could potentially be beneficial in this system (Jeffery et al., 2017). Furthermore, transforming crop residue from farms into biochar can provide an alternative way to reutilize these waste materials (Lehmann & Joseph, 2009), reducing carbon emissions and contributing significantly to climate change mitigation (Woolf et al., 2010). In this study, four feedstock materials were chosen to produce biochar based on their environmental impact and the agricultural aptitude of Mato Grosso: cotton husks, sugarcane filtercake (a residue left over from filtration of sugarcane juice after clarification), swine manure, and eucalyptus residue. Filtercake, cotton husks, and swine manure all currently pose disposal challenges and environmental contamination issues. While eucalyptus residues are currently used for bioenergy, this material was included to facilitate comparison to a woody feedstock, as woody feedstocks are commonly used in biochar studies. Using these organic wastes readily found in Mato Grosso as potential biochar feedstocks could substantially reduce their biomass volume and result in biochars comprised of more stable organic material that minimizes nutrient loss and, significant for the case of swine manure, is free of pathogens following pyrolysis (Lehmann & Joseph, 2009). In this way, these organic wastes become safe to apply to the soil, becoming a more environmentally beneficial form of waste disposal.

In addition, biochars with high pH can improve soil nutrient availability and reduce some limiting requirements in acidic soils (Biederman & Harpole, 2013). Depending on the feedstock, biochar can increase soil nutrient content and potentially boost plant growth (Spokas et al., 2012; Jeffery et al., 2015), in combination with other current management practices. Less understood, however, is the effect of biochar on plant physiological characteristics, such as photosynthesis rate, which is a key indicator of plant fitness (Xu et al., 2015). Recent studies have found biochar additions can reduce (Kammann et al., 2011) or not affect (Alburquerque et al., 2013) photosynthesis. To address these uncertainties and evaluate locally available wastes for biochar potential, our study had the following objectives: (1) to determine the effect of biochar type and application rate on maize biomass production and physiological characteristics, and (2) to evaluate the impact of biochar applications on resulting nutrient levels in the soil. As the intent of the study was to test the role of different biochars to observe if there would be negative interactions or positive synergies, plants were not stressed and were not exposed to limiting conditions in terms of water and nutrients. It was expected that swine manure biochar would increase soil nutrient levels and lead to greater maize biomass compared to the other biochar feedstocks as animal manures are often rich in nutrients (Chan et al., 2008).

Materials and methods

Soil collection and biochar production

Soils from the top 0–20 cm layer were collected from an agricultural field located within the farm Fazenda Água Azul (15°13′55.2″S, 54°57′43.4″W) managed by the agribusiness Grupo Bom Futuro, 178 km northwest of the state capital of Cuiabá in Mato Grosso, Brazil, an area within the Cerrado. The climate is described as tropical, hot semihumid with average monthly temperatures above 18 °C year-round. The dry season lasts 4–5 months, beginning around May/June to September/October (IBGE, 2014). Precipitation rates are between 1500 and 2250 mm year⁻¹ (Maia et al., 2009). The soil collected was classified as an Arenosol (FAO soil classification), with a sandy texture (91% sand, 4% silt, 5% clay). Carbon (C) and nitrogen (N) levels in the soil were 0.7% C and 0.08% N as determined by elemental analysis (628 Series CHN Analyzer, LECO Corp., St. Joseph, MI). The average pH in water (pHwater) was 5.8, and average CEC was 5.3 cmol, kg⁻¹, with a bulk density of 1.6 g cm⁻³. Over the last 10 years, the crops sown on the study site included soybean, sorghum, maize, and cotton, with the latter two crops grown in rotation with soy for the last 3 years (Afonso Campos da Silva, Grupo Bom Futuro, personal communication, 2014). Biochars derived from four feedstocks (cotton husks, eucalyptus residue, sugarcane filtercake, swine manure) were pyrolyzed at 400 °C (SPPT Ltda., Mogi Morim, São Paulo, Brazil), then crushed, and sieved to <2 mm in order to have similar biochar particle sizes between the feedstocks. Chemical properties of the soil, as well as the biochars used, were determined according to the methodology of Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA, 2009). Biochar and soil total C and N were analyzed on a CHN Analyzer (628 Series, LECO Corp., St. Joseph, MI).

Experimental setup

In a greenhouse located at the Federal University of Mato Grosso, Cuiabá campus, the biochars were added to 8 kg of an Arenosol soil at 0%, 1%, 2%, 3%, and 4% on a dry weight basis (equivalent to 0, 16, 32, 48, 64 t ha⁻¹) and mixed in 9L pots by hand. The treatments were thus four biochar types applied at 1%, 2%, 3%, and 4%, each replicated four times, plus four replicates of the control (i.e., no biochar added) for a total of 68 pots. Water was then added to 60% water-filled pore space (WFPS) equivalence, and the pots were divided into four blocks (one block per greenhouse bench) for a randomized complete block design. Temperature in the greenhouse was set to 28 ± 2 °C, near temperatures during which the dry season maize is grown from January to June (INPE, 2012).

As fertilizer applications are a standard management practice in the region, fertilizer was added to the pots corresponding to the amount each maize plant requires at the rate of 150 kg NPK+S, 150 kg KCl and 200 kg urea for 60 000 plants/hectare in the field (Afonso Campos da Silva, Grupo Bom
Futuro, personal communication, 2014). After one week, 2.5 g of crushed NPK+S (12-46-0 + 7) was mixed into each pot. Four maize seeds (hybrid seed, Dekalb) were planted in all pots and thinned down to one plant per pot after 10 days. After 20 days, 2.5 g of crushed KCl and 1.2 g of urea diluted in 50 mL of water were added to each pot. Seven days later, another 1.2 g of diluted urea was added, followed by a third application of 1.2 g another 7 days later, replicating nutrient management strategies typical in the region. Soil moisture was maintained at 60%WFPS by checking soil moisture three times per week using a CS3 sensor (Decagon Devices, Inc., Pullman, WA, USA) inserted into the top 10 cm of soil. Soil electrical conductivity (EC) was recorded at the same time from GS3 sensor output. All plants, with and without biochar, thus received adequate amounts of water, fertilizer, and growing conditions. Pots were rotated within blocks to decrease effect of any temperature and light differences within the greenhouse space.

**Plant physiological measurements**

Plant physiological characteristics were measured halfway through the experiment (day 20) and on the last day (day 42) to observe biochar effects on plant processes. Physiological measurements were made in the morning (between 8 am and 1 pm) for a subset of treatments, with the 1% biochar application rate chosen as plant sizes at this dose were most similar for all biochar types. Measurements were made on four replicates per feedstock (one replicate per block). The third, fully expanded leaf of each plant (counting from the base of the plant) was used for measurements, based on a preliminary test carried out to confirm there were no physiological variations due to leaf senescence ($P > 0.05$).

Gas exchange measurements were made with a portable photosynthesis system LI-6400XT (LI-COR Inc., Lincoln, NE, USA). An area located in the middle third, 2 cm from the leaf edge, of each third leaf was subjected to a photon flux of 1000 μmol m$^{-2}$ s$^{-1}$ to guarantee light saturation for photosynthesis. A block temperature of 28 °C, a reference CO$_2$ air concentration fixed at 400 μmol mol$^{-1}$, and a reference relative humidity of 60% were used to minimize stomatal heterogeneity. Net photosynthetic rate ($P_n$, μmolCO$_2$ m$^{-2}$ s$^{-1}$), transpiration rate ($E$, mmol(H$_2$O) m$^{-2}$ s$^{-1}$), and stomatal conductance ($g_s$, mmol(H$_2$O) m$^{-2}$ s$^{-1}$) were calculated by the LI-6400XT data analysis program. Intrinsic water use efficiency (WUE$_i$, μmol CO$_2$ mol$^{-1}$ (H$_2$O) was calculated as $P_n/g_s$ (Dalmagro et al., 2016) and water use efficiency of photosynthesis (WUE$_p$, μmol CO$_2$ mmol$^{-1}$ (H$_2$O)) as $P_n/E$ (Kammann et al., 2011). Dark respiration rate ($R_d$, μmol m$^{-2}$ s$^{-1}$) was measured after switching off the light for about 20 min.

Water use efficiency of productivity (WUE$_{prod}$ g L$^{-1}$) was calculated as total dry aboveground biomass at the end of the experiment per total water consumed per pot (Kammann et al., 2011). Total water consumed (L) was the sum (Σ) of water added to the pots beginning 2 days after water was first added to 60% WFPS until the end of the experiment. Leaf area (LA, mm$^2$) of the third, fully expanded leaf of each plant was measured immediately after harvest on day 42 using a leaf area meter (CI-202, CID, Inc., Camas, WA, USA). LA of the entire plant was also determined. The third leaf tissue was subsequently ground using a ball mill (Mini Mill Pulversette 23, Fritsch GmbH, Oberstein, Germany) and analyzed for leaf total C and N on a CHN analyzer (628 Series, LECO Corp., St. Joseph, MI). Specific leaf area (SLA) was calculated as the ratio between total LA and total dry leaf biomass. Leaf nitrogen use efficiency (NUE$_{prod}$) was calculated as the ratio between total dry aboveground biomass and mg of leaf N (g mg$^{-1}$ leaf N) (Kammann et al., 2011). Additional properties calculated based on readings at the end of the experiment and the total leaf area were as follows: plant photosynthesis ($P_{plant}$, μmol (CO$_2$) m$^{-2}$ s$^{-1}$ mm$^{-2}$), aboveground plant respiration ($R_{plant}$, μmol m$^{-2}$ s$^{-1}$ mm$^{-2}$), plant stomatal conductivity ($g_{plant}$, mmol(H$_2$O) m$^{-2}$ s$^{-1}$ mm$^{-2}$), and plant aboveground transpiration ($E_{plant}$, mmol(H$_2$O) m$^{-2}$ s$^{-1}$ mm$^{-2}$) (Kammann et al., 2011). Photosynthetic nitrogen use efficiency (PNUE$_{plant}$) was calculated as $P_{plant}$ per mg of leaf N (μmol CO$_2$ g N$^{-1}$ s$^{-1}$) (Xu et al., 2015).

**Plant and soil analysis**

On day 42, maize plants were cut at the base and the following plant physical characteristics were immediately quantified: number of leaves, stem diameter, plant height to the tip of the top leaf, and total fresh biomass. Plant leaves from the 1% biochar application rate were tested for physiological characteristics. Leaves were first cut from the stems to measure total LA and then weighed fresh. Plant biomass was subsequently dried for 48 h at 60 °C and the dry weight recorded. Dried weight of the leaves for the 1% dose plants was recorded separately before combining with the stem for total dry aboveground biomass. Pots were destructively sampled to collect all fresh root biomass and soil samples. Soil samples were analyzed for macronutrient (available P, K$^+$, Ca$^{2+}$, Mg$^{2+}$, and S) and micronutrient (Zn, Cu, Fe, Mn, B) availability, pH$_{water}$ (1 : 2.5), and CEC according to the standard soil methodologies used by the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA, 2009) as described in Eykelbosh et al. (2014). Soil total C and N were analyzed on a CHN Analyzer (628 Series, LECO Corp., St. Joseph, MI).

**Statistical analyses**

The effects of biochar feedstock and biochar application doses on maize biomass, soil properties, and macro- and micronutrients were determined by multivariate ANOVA (MANOVA) using IBM® SPSS® Statistics (version 23, SPSS Inc., Chicago, USA). Where treatments were significant, a post hoc Tukey test ($P < 0.05$) was used to compare means between feedstocks and doses when variances were equal, and a post hoc Games–Howell test when variances were not equal. Pearson correlation coefficients and linear regression were determined between aboveground and belowground biomass and soil properties and nutrients. For the physiological characteristics, a repeated-measures ANOVA was carried out for $P_n$, $g_s$, $R_d$, $E$, WUE$_i$, and WUE$_p$ measured on day 20 and day 42, with Treatment as the between-subject factor and Time as the within-subject factor. A MANOVA was carried out for plant physiological properties based on
total leaf area or total aboveground dry biomass: $P_{\text{plant}}$, $k_{\text{plant}}$, $g_{\text{plant}}$, $E_{\text{plant}}$, $\Sigma_{\text{water consumed}}$, WUE$_{\text{prod}}$, NUE$_{\text{prod}}$, and PNUE$_{\text{plant}}$. A MANOVA was also performed for plant physical properties determined at the end of the experiment: total LA, stem diameter, number of leaves, plant height, total fresh leaf biomass, total dry leaf biomass, and SLA, as well as leaf C/N ratio. Correlations and regressions were also performed between plant and soil variables. Principal component analysis (PCA) was carried out on soil and plant properties of the 1% dose biochar treatments and the control using the FactoMineR package (Le et al., 2008) in R (version 3.3.1). Values are presented in text and graphs as means ± standard error.

**Results**

**Biochar properties**

The biochars showed a range of chemical properties depending on the feedstock, with cotton and swine manure biochars having higher pH, CEC, and levels of certain nutrients such as P, K, S, Cu, and Zn than filtercake and eucalyptus biochars (Table 1). Physical properties (e.g., porosity, particle size) of the biochars are described in Speratti et al. (2017) and their aromaticity in Table S1.

**Plant biomass and soil properties**

Biochar amendments had a significant effect on dry aboveground and belowground maize biomass (Fig. 1), which were positively correlated ($R = 0.88$, $P < 0.01$). Between the feedstocks, maize biomass for soil amended with filtercake and eucalyptus biochars at all application rates had significantly greater mean dry aboveground biomass than maize from soils with cotton and swine manure biochars at all rates ($P < 0.05$, Fig. 1a). In addition, filtercake biochars had higher mean aboveground biomass at three application rates (1% dose: $18.1 ± 0.8$, 2% dose: $16.4 ± 1.3$, and 4% dose: $18.1 ± 5.6$) compared to the control ($15.1 ± 1.7$). For eucalyptus and filtercake biochars, there were no significant differences between the application rates, but for cotton biochar, biomass decreased significantly with increasing application rate, while for swine manure biochar, the difference was only significant between the 1% dose and the other three doses. Belowground biomass exhibited similar patterns as the aboveground biomass, with maize root biomass from soils with eucalyptus and filtercake biochars not significantly different from each other or the control, but greater than root biomass from cotton and swine manure biochars (Fig. 1b).

pH levels for cotton and swine manure biochars increased with increasing dose, while varying less for eucalyptus and filtercake biochars (Table 2). Pairing soil pH levels with dry aboveground biomass showed a significant negative correlation ($R = -0.70$, $P < 0.001$) (Fig. S1a), which was the same for belowground biomass ($R = -0.70$, $P < 0.001$). Soil CEC increased significantly ($P < 0.05$) only in soils with swine manure biochars compared to the control (6.1 ± 0.5 cmol kg$^{-1}$). Comparing the biochars, soils with swine manure biochar (6.5 ± 0.4 cmol kg$^{-1}$) had greater CEC levels than with eucalyptus biochar (6.8 ± 0.3 cmol kg$^{-1}$), but neither differed from soils with cotton (7.5 ± 0.4 cmol kg$^{-1}$) and filtercake biochars (7.1 ± 0.3 cmol kg$^{-1}$). CEC levels were poorly correlated with aboveground dry biomass ($R = -0.32$, $P < 0.01$) (Fig. S1b).

Mean soil EC was highest in soils with cotton and swine manure biochars compared to eucalyptus and filtercake biochars and the control. EC between the doses was different only for cotton and swine manure biochars.

**Table 1** Chemical properties of an Arenosol and biochars made from agricultural waste feedstocks (cotton husks, swine manure, eucalyptus sawdust, sugarcane filtercake) pyrolyzed at 400 °C. Values are means (n = 3) ± SE. CEC, cation exchange capacity

| Soil          | Cotton400 | Swine400 | Eucalyptus400 | Filtercake400 |
|---------------|-----------|----------|---------------|---------------|
| Total C (%)   | 0.7 ± 0.03| 54.8 ± 2.0| 25.4 ± 9.6    | 54.6 ± 7.3    | 28.1 ± 0.5    |
| Total N (%)   | 0.08 ± 0.02| 3.1 ± 0.1| 2.8 ± 0.8     | 1.6 ± 0.01    | 2.9 ± 0.01    |
| pH$_{\text{water}}$ | 5.8 ± 0.1 | 10 ± 0.6 | 9.2 ± 0.3     | 7.7 ± 0.03    | 8.6 ± 0.6     |
| CEC (cmol kg$^{-1}$) | 5.3 ± 0.3 | 49.1 ± 3.8| 28.7 ± 1.1    | 3.7 ± 0.2     | 3.9 ± 0.3     |
| P (mg kg$^{-1}$) | 82.4 ± 23.0| 3700 ± 800| 6500 ± 400    | 200 ± 40      | 1300 ± 100    |
| K (mg kg$^{-1}$) | 43.3 ± 4.1 | 17 300 ± 1200| 9100 ± 700   | 700 ± 70      | 600 ± 100     |
| Ca (cmol kg$^{-1}$) | 2.2 ± 0.2 | 2.0 ± 0.2 | 2.9 ± 0.1     | 1.5 ± 0.06    | 1.9 ± 0.1     |
| Mg (cmol kg$^{-1}$) | 0.7 ± 0.04| 2.7 ± 0.5 | 2.7 ± 0.6     | 0.3 ± 0.01    | 0.4 ± 0.02    |
| S (mg kg$^{-1}$) | 7.6 ± 0.6 | 120.1 ± 4.5| 50.3 ± 16.8   | 9.5 ± 1.0     | 10.4 ± 1.1    |
| Cu (mg kg$^{-1}$) | 0.97 ± 0.3| 5.0 ± 1.3 | 12.1 ± 4.5    | 1.0 ± 0.1     | 2.4 ± 0.4     |
| Fe (mg kg$^{-1}$) | 79.0 ± 4.5| 35.7 ± 0.2| 12.7 ± 1.8    | 170 ± 13.2    | 266 ± 6.0     |
| Mn (mg kg$^{-1}$) | 9.8 ± 1.7 | 27.4 ± 4.0| 41.9 ± 7.3    | 9.1 ± 1.6     | 84.9 ± 1.0    |
| B (mg kg$^{-1}$) | 0.5 ± 0.03| 5.4 ± 2.5 | 2.2 ± 0.7     | 0.6 ± 0.1     | 0.8 ± 0.1     |
| Zn (mg kg$^{-1}$) | 2.8 ± 0.9 | 31.0 ± 5.3| 21.57 ± 7.7   | 2.4 ± 1.0     | 17.4 ± 0.3    |

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biochars, where the EC was generally higher in the high doses compared to the lower doses (Fig. 2). EC was significantly \((P < 0.001)\) and negatively correlated with both dry aboveground biomass \((R = -0.81)\) and dry belowground biomass \((R = -0.81)\), while positively correlated with soil pH \((R = 0.81, P < 0.001)\) (Fig. S2).

Aboveground and belowground dry biomass had negative correlations with total C and several soil nutrients (Table 3, Fig. S3, S4, S5). Dose and feedstock were both significant factors for soil total C levels (Fig. 3a). Cotton \((1.5 \pm 0.06\% C)\) and eucalyptus \((1.4 \pm 0.09\% C)\) biochars increased soil total C more than filtercake biochar \((1.2 \pm 0.04\% C)\), but not more than swine manure biochar \((1.3 \pm 0.1\% C)\). All biochars significantly \((P < 0.05)\) increased soil total C more than the control \((0.8 \pm 0.03\% C)\), and doses showed a trend of increasing

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**Table 2** pH\(_{\text{water}}\) levels of soils with cotton, swine manure, eucalyptus, and filtercake biochars at 1–4% doses after 6 weeks. Values are means ± SE. Lowercase letters indicate significant differences between doses at \(P < 0.05\) (Tukey test). Capital letters next to feedstocks indicate significant differences \((P < 0.05)\) between the feedstocks, and asterisk (*) indicates biochars that significantly \((P < 0.01)\) increased soil pH compared to the control soil pH (4.9).

| Dose | Cotton A* | Swine manure A* | Eucalyptus C | Filtercake B* |
|------|-----------|-----------------|--------------|--------------|
| 1%   | 5.8 ± 0.1 c | 6.1 ± 0.1 d | 4.9 ± 0.1 b | 5.3 ± 0.1 b |
| 2%   | 6.6 ± 0.1 b | 6.6 ± 0.1 c | 5.2 ± 0.1 ab | 5.7 ± 0.1 b |
| 3%   | 7.1 ± 0.4 ab | 6.9 ± 0.1 b | 5.1 ± 0.1 ab | 5.8 ± 0.2 b |
| 4%   | 7.5 ± 0.3 a | 7.2 ± 0.1 a | 5.3 ± 0.1 a | 6.4 ± 0.1 a |

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C with increasing dose (Fig. 3a). Biochar feedstock and dose effects varied for soil macronutrients, including total N. For total N, soils with swine manure biochar had the greatest N levels (0.06 ± 0.01% N), significantly (P < 0.05) greater than the control (0.02 ± 0.00% N) and eucalyptus biochar (0.02 ± 0.00% N), but not different from cotton (0.04 ± 0.00% N) and filtercake (0.04 ± 0.01% N) biochars. Only swine manure biochar showed significant differences between the doses for soil N, with the smallest dose (1%) significantly (P < 0.05) lower than the largest dose (4%) (Fig. 3b). Maize biomass had a higher correlation with C levels than with N levels (Table 3, Fig. S3).

Mean soil C/N ratios reflected the high total C levels in soils with eucalyptus and cotton biochars, with mean C/N ratios in eucalyptus biochar treatments (92.4 ± 15.3) not different from cotton biochar treatments (45.7 ± 5.7), but significantly greater than swine manure (27.1 ± 5.1) and filtercake (35.0 ± 6.7) biochar treatments and the control (36.6 ± 6.8). For phosphorus (P) levels, there were no significant differences between the feedstocks nor the doses (Fig. 4a). Potassium (K) levels in soils with cotton (1182 ± 153 mg K kg⁻¹) and swine manure (785 ± 127 mg K kg⁻¹) biochars were significantly higher than in soils with eucalyptus (251 ± 35 mg K kg⁻¹) and filtercake (227 ± 24 mg K kg⁻¹) biochars, and higher compared to the control (157 ± 11 mg K kg⁻¹) (Fig. 4b). Calcium (Ca) and magnesium (Mg) showed no significant differences between the doses, but had significant differences (P < 0.05) between the feedstocks (Fig. 4c, d). Soils amended with filtercake biochar (3.2 ± 0.1 cmol, Ca kg⁻¹) had significantly more Ca than cotton (1.7 ± 0.1 cmol, Ca kg⁻¹) and eucalyptus (2.6 ± 0.1 cmol, Ca kg⁻¹) biochars, but not more than swine manure biochar (2.8 ± 0.1 cmol, Ca kg⁻¹); filtercake biochar was the only feedstock to significantly (P < 0.05) increase Ca levels in the soil compared to the control (2.5 ± 0.3 cmol, Ca kg⁻¹). Like Ca, Mg levels also did not differ between doses and only soils with swine manure biochar (1.8 ± 0.4 cmol, Mg kg⁻¹) had significantly (P < 0.05) higher Mg levels than the other treatments which did not differ from each other. For sulfur (S), soils with cotton and swine manure biochar had significantly (P < 0.05) greater levels than eucalyptus and filtercake biochars and were significantly greater than the control. Only swine manure biochar had differences between the doses, where soil S levels increased with increasing dose (Fig. 5a).

Biochar feedstock type had a significant (P < 0.001) effect on all micronutrients except boron (B), while differences in doses were mostly observed for swine manure biochar on certain micronutrients (Figs 5, S6). For both zinc (Zn) and copper (Cu), soils with swine manure biochar had significantly (P < 0.05) higher levels than the other biochars which did not differ from each other nor from the control. The doses exhibited the same trend in swine manure biochar as seen for S (Fig. 5b, c). For iron (Fe), soils with cotton (54 ± 3.1 mg Fe kg⁻¹), swine manure (53 ± 3.7 mg Fe kg⁻¹), and eucalyptus (54 ± 3.4 mg Fe kg⁻¹) had significantly (P < 0.05) lower Fe levels than filtercake biochar (117 ± 6.5 mg Fe kg⁻¹), the latter also being higher than the control (47 ± 4.4 mg Fe kg⁻¹) (Fig. 5d). For manganese (Mn), soils with swine manure and filtercake biochars had significantly (P < 0.05) higher Mn

Fig. 2 Mean electrical conductivity (EC, dS m⁻¹) measured three times a week for 5 weeks (n = 4). Capital letters represent significant differences between the feedstocks, while lowercase letters represent significant differences between the doses (Tukey test; P < 0.05).

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levels than cotton and eucalyptus biochars and the control. Both swine manure and filtercake biochars had significant \( P < 0.05 \) differences between the lowest and the highest doses (Fig. 5e).

**Plant physiological properties**

Among measured physiological variables \( (P_{\text{av}}, \gamma_s, R_d, E, WUE_{\text{av}}, \text{and } WUE_d) \), the repeated-measures ANOVA showed a significant overall treatment effect only for \( R_d \) \( (P < 0.05) \). The time effect was also significant \( (P < 0.05) \) for most variables except for \( \gamma_s \) and \( R_d \). Differences between the time*treatment interaction (i.e., the treatment effects were different on day 20 compared to on day 42) were significant \( (P < 0.05) \) for \( P_{\text{av}}, \gamma_s, \text{and } WUE_t \).

Treatment differences were significant for several final plant physiological and physical characteristics (Table 4). Maize plants in soils with cotton and some swine manure biochars were consistently smaller than the other biochars and the control. Cotton biochar had the lowest WUEProd and filtercake biochar the highest, while the \( \Sigma \text{water consumed, NUE}_{\text{prod}}, \) and \( PNUE_{\text{plant}} \) were also lowest for cotton biochar.

\( P_{\text{plant}} \) was significantly correlated with total fresh leaf biomass \( (R = 0.54, P < 0.05) \) and with SLA \( (R = 0.58, P < 0.01) \), as well as with three soil properties: soil K \( (R = 0.73, P < 0.01) \), S \( (R = 0.57, P < 0.01) \), and pH \( (R = 0.54, P < 0.05) \) (Fig. S7). Biochar treatments with low fresh leaf biomass (e.g., cotton and swine manure biochars) had lower \( P_{\text{plant}} \) rates than other treatments. SLA, however, did not vary significantly between treatments, nor did leaf C/N ratio. There was a positive correlation between \( P_{\text{plant}} \) and the \( \Sigma \text{water consumed} \) \( (R = 0.75, P < 0.001) \) and \( NUE_{\text{prod}} \) \( (R = 0.82, P < 0.001) \), as well as between \( WUE_{\text{prod}} \) and \( NUE_{\text{prod}} \) \( (R = 0.75, P < 0.001) \).

Dark respiration rate at the leaf scale varied significantly \( (P < 0.05) \) between treatments, but not over time, except for the cotton treatment (means compared by \( t \)-test, \( P < 0.001) \). Other biochars showed lower respiration at the end of the experiment compared to mid-experiment as well (Fig. 6). Cotton biochar reduced \( R_d \) compared to the control and eucalyptus and filtercake biochars. \( R_d \) was also significantly correlated with leaf C \( (R = 0.47, P < 0.05) \) and with total fresh leaf biomass \( (R = 0.69, P < 0.001) \). At the plant scale, plants in soils with cotton biochar had the lowest \( R_{\text{prod}} \gamma_{\text{prod}} \) and \( E_{\text{prod}} \) rates, while plants in soils with filtercake biochar had the highest, though not significantly different from the other two biochars or the control (Table 4). \( R_{\text{prod}} \gamma_{\text{prod}} \) and \( E_{\text{prod}} \) rates were significantly correlated with \( \Sigma \text{water consumed} \) \( (\gamma_{\text{prod}}: R = 0.79, P < 0.001; \gamma_{\text{prod}}: R = 0.63, P < 0.001; E_{\text{prod}}: R = 0.59, P < 0.001) \).
Principal component analysis

The PCA of soil and plant properties of biochar treatments applied at a 1% rate and the control found two groupings: \( R_{\text{plant}} \), S, EC, K, pH, Zn, and Cu (Group 1) and PNUE\(_{\text{plant}}\), \( P_{\text{plant}} \), \( E_{\text{plant}} \), \( g_{\text{plant}} \), total LA, dry aboveground biomass, plant height, \( \Sigma \) water consumed, and Ca/Mg ratio (Group 2). Cotton and swine manure biochars clustered by Group 1, while eucalyptus and filtercake biochars and the control clustered strongly by Group 2 (Fig. 7).

Discussion

As all soils were fertilized and adequately watered, including the control, this experiment examined which biochars and application rates might have a negative effect on plant growth when combined with standard fertilizing practices in a Cerrado Arenosol. Nevertheless, some biochars (i.e., filtercake biochar at 1%) increased soil nutrient contents and led to similar or even higher mean maize biomass compared to the control. The biochars’ effects on maize biomass, their contribution to high soil nutrient levels affecting maize biomass, and their effects on plant physiological properties are described in the next sections.

Biochar effects on maize biomass

Maize biomass decreased with increasing application rate in our study for cotton and swine manure biochars, while application rates did not have varying effects on maize biomass in soils with filtercake and eucalyptus biochars (Fig. 1a). The decrease in maize biomass was negatively correlated with increasing soil pH (Fig. S1a), suggesting the resulting higher soil pH levels may have affected nutrient availability for plant uptake in cotton and swine manure biochar treatments. For filtercake and eucalyptus biochars, however, maize biomass was likely less affected due to relatively
consistent pH levels at all rates. Soil acidity is one of the main limiting factors for crop production in tropical soils such as those of the Cerrado, which have an average pH of 5. Liming is often practiced in Cerrado soils to raise pH levels (Fageria, 2001) by increasing Ca$^{2+}$ and Mg$^{2+}$ content, thus reducing soil acidity (Frazão et al., 2008) and improving CEC (Silber et al., 2010). Prior studies have shown a similar liming effect of biochars in nutrient-poor soils due to the high pH of the biochars themselves which tend to be alkaline (Lehmann et al., 2003; Van Zwieten et al., 2010; Biederman & Harpole, 2013). Initial soil pH in our study was 5.8 (Table 1), suitable for plant growth, but by the end of the study decreased to 4.9 (Table 2). In contrast, in soils with biochar a liming effect was evident, where the high pH of three of the four biochars (Table 1) significantly increased the soil pH in biochar treatments compared to the control soil after 6 weeks (Table 2). The pH levels of biochars used in this study were comparable to those used by Rajkovich et al. (2012), where corn stover and animal manure (dairy and poultry) biochars had higher pH levels than wood biochars (oak and pine). The beneficial liming property of biochar can sometimes be deleterious, however, if biochar is over applied (Chan & Xu, 2009), as observed for cotton and swine manure biochars at increasing rates.

The high EC in soils with cotton and swine manure biochars may have also affected maize biomass, as suggested by the significant negative correlation (Fig. S2). EC can be used to indicate salinity and determine crop yield potential. High salinity can reduce plants’ ability to uptake water and disrupt their nutritional balance (Corwin & Lesch, 2005); thus, the former biochars may have led to excessive soil salinity which, combined with high pH and other factors, impacted plant growth. To prevent high salinity, Blok et al. (2017) suggest that feedstock salinity and alkalinity can be tested before biochar production, although a biochar’s high EC can still be lowered by prior washing with water or by combining with a lower EC biochar. Furthermore, differences in biochar contributions to soil nutrient levels and plant physiological variables may also have affected maize biomass, as discussed below.

**Biochar effects on soil nutrient levels**

Besides high pH and EC levels, high concentrations of soil nutrients may also have negatively affected maize biomass. Two in particular are soil K and S levels which had high, significant negative correlations with maize biomass (Table 3, Fig. S4), with both cotton and swine manure biochars contributing to high levels compared to the control and other biochars (Fig. 4).
to filtercake and eucalyptus biochars (Figs 4b, 5a). As biochar can alter nutrient availability in the soil, it can likely also affect photosynthesis and plant production (Xu et al., 2015). Cotton biochar had the lowest $P_{\text{plant}}$, although its total LA did not differ significantly from plants in soils with swine manure biochar (Table 4). $P_{\text{plant}}$ was significantly correlated with soil K and S, where biochars with high soil K and S (cotton and swine manure) had low $P_{\text{plant}}$ compared to the other two biochars. Potassium affects water relations in plants through its role in stomatal opening, and K deficiency can cause lower leaf $P_n$ and $g_s$ (Lu et al., 2016), reducing WUE (Kammann & Graber, 2015). Sulfur is essential for plant growth and metabolism, and S deficiency can lead to reduced photosynthesis rates (Resurreccion et al., 2001). In our study, as cotton and swine manure biochar treatments had the highest soil K and S levels, but the lowest $P_{\text{plant}}$ rates, the soil K and S were either not plant available and causing deficiency, or were too high causing toxicity and thus also impairing $P_{\text{plant}}$. As in our study, Chandra & Pandey (2016) observed that S toxicity (as well as S deficiency) in soybean plants caused reduced biomass with increasing S doses. For maize production in Brazil, soils with less than 10 mg S kg$^{-1}$ receive S fertilizers (typically as ammonium sulfate) (Coelho et al., 2011). Maize plants in our study grew well at levels around 45 mg S kg$^{-1}$, but were visibly smaller in soils with cotton and swine manure biochar (Fig. S8, S9), particularly in higher biochar doses where S levels almost doubled that of the control. Biochar has been known to decrease plant yield in certain instances, specifically in relation to high S levels and salinity, Al/Mn toxicity, and reduced nutrient availability (Jeffery et al., 2015).

Micronutrient availability is particularly affected by pH levels, less micronutrient availability with increasing pH (Jensen, 2010). Excessive micronutrient levels, however, can cause toxicity and negative plant growth. The heavy metals, Cu and Zn, are essential micronutrients for plant growth, but if they are present at higher levels than the plant requires, they can cause toxicity (Nagajyoti et al., 2010). For Cerrado soils, average Cu levels are between 0.8 and 2.4 mg kg$^{-1}$ and between 1 and 3 mg kg$^{-1}$ for Zn (Coelho et al., 2011). Initial Cu levels in our study were within the average range (0.97 ± 0.3 mg kg$^{-1}$), but increased significantly under swine manure biochar (Fig. 5c) and its dry aboveground biomass was more closely correlated with Cu than that of the other biochars (Fig. S5c). Cu levels in soils with cotton biochar, in contrast, were not excessively high,
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suggesting Cu toxicity was not the cause of the decrease in maize biomass under that biochar. Zn levels in our initial soil were at the high end (2.8 ± 0.9 mg kg⁻¹) and were higher by the end of the experiment, again particularly under swine manure biochar (Fig. 5b) despite increasing pH. According to Fageria (2000), however, maize has a high tolerance to Zn toxicity compared to other crops such as rice, soybean, and wheat, withstanding a soil Zn toxic level up to 60 mg kg⁻¹. Zn levels in soils with swine manure biochar were high and may have contributed to lower dry aboveground biomass with increasing dose (Fig. S5a), although they did not reach the 60 mg kg⁻¹ toxic level.

Signs of Zn toxicity, like Cu, include low growth and development (Nagajyoti et al., 2010), but may have affected plants in swine manure biochar treatments more than in cotton biochar treatments (Fig. S5a). Increased Zn solubility could be beneficial in Cerrado soils as Zn is the main limiting micronutrient for maize production in Brazil, particularly in the Cerrado region (Coelho et al., 2011). Adding a low dose of swine manure biochar to the soil therefore could help raise Zn bioavailability.

Another heavy metal considered an important micronutrient in trace amounts is iron; it is essential in metabolic processes (Nagajyoti et al., 2010; Rout & Sahoo, 2015). Excess uptake, however, can cause toxicity in plants (Rout & Sahoo, 2015). Average Fe levels in Cerrado soils are 5 to 12 mg kg⁻¹, with levels above 12 mg kg⁻¹ considered high (Coelho et al., 2011). Soil Fe levels were highest in soils with filtercake biochar, including compared to the control (Fig. 5d). Despite the high levels, Fe did not seem to have a detrimental effect on plant growth (Fig. S5b) as maize plants in soils with filtercake biochar still had high above- and belowground biomass comparable to the control, and no signs of toxicity or deficiency compared to the cotton and swine manure biochar treatments (Fig. S10). Plants in filtercake biochar treatments also looked similar to those in eucalyptus biochar treatments (Fig. S11). Fe is not readily available in neutral to alkaline soils, as it is in insoluble oxidized forms, for example, Fe³⁺ (Rout & Sahoo, 2015). Although soils under filtercake biochar treatments remained slightly acidic (Table 2), the high soil Fe was likely unavailable for plant uptake, yet it appeared to be available in sufficient amounts to prevent Fe deficiency.

PCA of biochar treatments at the 1% application rate suggests a combination of soil factors affected maize biomass, where pH, EC, S, K, Zn, and Cu were negatively correlated with dry aboveground biomass; both cotton and swine manure biochars clustered near this group (Group 1) (Fig. 7). The availability of these nutrients for plant absorption would also be related to water availability, as examined below.
Biochar effects on plant physiological properties

Another explanation for the low \( P_{\text{plant}} \) and low biomass of plants with cotton and swine manure biochars may be related to their lower \( \Sigma \) water consumed and in turn, \( WUE_{\text{Prod}} \). The fact that soils containing these biochars remained visibly saturated for longer periods between waterings than the other two biochars and the control, suggests that they improved soil water retention. However, based on the low biomass, LA, and \( WUE_{\text{Prod}} \), the water retention may have been too high (particularly in soils with cotton biochar which had a high C/N ratio), causing the plants to suffer. Biochar is considered a porous material, and its porosity is determined both by the feedstock (which contributes mostly to macropores) and by the temperature of pyrolysis (which contributes to nanopores) (Uzoma et al., 2011a; Brown et al., 2015). When added to the soil, biochar has the potential to increase pores in the 30 to 0.3 nm diameter range (Verheijen et al., 2009). Yet, for plant available water content (AWC, water content at field capacity minus water content at the permanent wilting point) (Rajkovich et al.,

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**Fig. 6** Mean dark respiration rate (\( R_d \), \( \mu \text{mol} \text{ m}^{-2} \text{s}^{-1} \)) for maize plants in soils with different biochar and control (no biochar) treatments measured halfway (day 20) and at the end (day 42) of the experiment. Capital letters represent significant differences (Tukey test; \( P < 0.05 \)) between treatments. Lowercase letters represent significant differences between days 20 and 42 for a treatment (t-test, \( P < 0.001 \)).

**Fig. 7** Principal component analysis (PCA) of soil and plant properties of 400 °C biochar treatments at 1% application rate and the control (\( n = 4 \)).
the pore size range is 0.2 to 30 μm diameter; most plants are not able to take up water from pores smaller than 0.2 μm (Hardie et al., 2014). Thus, although biochar can improve the soil porosity, depending on the biochar, the effective plant AWC may not vary in response to biochar additions (Verheijen et al., 2009). Further, depending on the biochar, water can bind to its particle surface, increasing water storage, but keeping the water immobile and unavailable for plants or solute transport (Masiello et al., 2015).

Studies have shown that biochar improves WUE (Kammann et al., 2011; Uzoma et al., 2011b; Laghari et al., 2015). In our study, however, none of the biochars significantly improved WUEg, WUEv, or WUEp compared to the control. In a corn growth experiment with various biochars made of different feedstocks and temperatures of pyrolysis, Rajkovich et al. (2012) observed that AWC was neither correlated with plant growth nor that AWC in biochar–soil mixtures increased compared to the control. The authors suggested this was due to the fact that soil water was kept at optimum levels for all treatments to prevent plant stress, as was the case in our study. AWC in their case, however, did vary between biochars. In our study, AWC did not vary significantly between the treatments, including the control (Fig. S12), although soils with cotton and swine manure biochar had slightly higher mean AWC values. In most laboratory or greenhouse studies, biochar amendments ≥4% w/w are needed to see a significant increase in AWC (Kammann & Graber, 2015). Though not significant at the 1% rate, slightly higher mean AWC values in these biochar treatments may have caused short-term anoxic conditions that impacted plant growth, especially at higher doses. In fact, in a study including the same biochars applied at 5% w/w, cotton and swine manure biochars significantly (P < 0.05) increased AWC compared to the control (no biochar) (Speratti et al., 2017). Plants in soils with cotton and swine manure biochars in the present study had lower mean dry belowground biomass than the other biochars and the control. Wet or oxygen-poor soils can impede root growth, reducing plant water supply (Kammann & Graber, 2015). As nutrient concentrations were similar or even higher (e.g., K and S) in soils with cotton and swine manure biochars compared to soils with eucalyptus and filtercake biochars, it is possible that soil water retention also played a role in plant productivity, along with pH and nutrient availability.

Like Pplant, Rplant, Splant, and Eplant were lowest in soils with cotton biochar and highest with filtercake biochar (Table 4). These properties are also affected by soil water level and leaf N content (Kammann et al., 2011). In our study, Rplant, Splant, and Eplant were not correlated with leaf N, but they were significantly correlated with Σwater consumed, especially Rplant. All three properties increased with increasing Σwater consumed, in agreement with Kammann et al. (2011) who observed reduced stomatal conductance, transpiration, and Rleaf in low soil water availability (20% WHC) where Σwater consumed was lowest.

While in our study, there were neither significant differences between leaf N under the different treatments, nor a significant correlation between Pplant and leaf N, there were differences observed between biochars for NUEprod and PNUEplant. Cotton biochar had the lowest NUEprod and PNUEplant, and filtercake had the highest. Although available N was not measured in our study, when taking soil total N content into account, swine manure biochar had the highest soil N compared to the other biochars, and its NUEprod was not significantly different from filtercake biochar. As both biochars had lower C/N ratios than the other biochars (Table 1), they could potentially increase net mineralization and nitrification in the soil, increasing available N (Yoo & Kang, 2012). Eucalyptus biochar was not different from filtercake biochar, but it contributed to the lowest soil total N. Brantley et al. (2015) suggested that combining biochar with N fertilizer could improve maize production based on increased NUE and yield compared to no biochar. Increased soil N retention through biochar could be due to increased N immobilization, reduced N losses from gases and surface erosion, and higher organic N retention on biochar surfaces. In addition, biochar may increase N availability by providing substrate and habitat for microorganisms (Brantley et al., 2015).

Overall, the results of this study show that biochar combined with inorganic fertilizers can increase soil nutrient levels, but depending on the feedstock and application rate, it may lead to high pH, salinity, and possible toxicity and excessive water retention that can negatively impact plant growth. This was the case for cotton and swine manure biochars, while eucalyptus and filtercake biochars did not have a negative effect on maize biomass. PCA showed that increased PNUEplant, Pplant, Fplant, Splant, Σwater consumed, and Ca/Mg ratio (which can indicate improved soil structure and water infiltration (Hartz, 2007)) correlated with increased dry aboveground biomass, plant height, and total LA, particularly for eucalyptus and filtercake biochars, while Rplant and several soil properties were negatively correlated with these plant physical properties especially for cotton and swine manure biochars (Fig. 7). Mixing biochars derived from different feedstocks (e.g., swine manure with filtercake) or mixing biochars with raw feedstocks might enhance beneficial traits and reduce negative effects present in each feedstock individually (Lehmann & Joseph, 2015). Filtercake biochar showed the greatest potential to increase maize biomass in a
Cerrado Arenosol, although swine manure biochar still increased certain soil nutrients as hypothesized. If applied at proper rates (e.g., 1% or less for cotton and swine manure biochars), transforming these feedstocks into biochar offers a safe and beneficial alternative to disposing of farm waste readily available in the region.

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**Supporting Information**

Additional Supporting Information may be found online in the supporting information tab for this article:

**Table S1.** Properties of four biochars

**Figure S1.** Correlations between biomass and pH and CEC

**Figure S2.** Correlations between EC and biomass and pH

**Figure S3.** Correlations between biomass and total soil carbon and total soil nitrogen

**Figure S4.** Correlations between biomass and soil potassium, sulfur, and calcium

**Figure S5.** Correlations between biomass and soil zinc, copper, iron, and boron

**Figure S6.** Mean concentration of manganese and boron in soils with different biochar feedstocks and doses after 6 weeks

**Figure S7.** Correlations between plant photosynthesis and soil potassium, sulfur, and pH

**Figure S8.** Photo of maize plants in cotton biochar treatments

**Figure S9.** Photo of maize plants in swine manure biochar treatments

**Figure S10.** Photo of maize plants in filtercake biochar treatments

**Figure S11.** Photo of maize plants in eucalyptus biochar treatments

**Figure S12.** Plant available water content in 1% biochar treatments and the control