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Short-term casting activity of earthworm *Pontoscolex corethrurus* (Oligochaeta: Glossoscolecidae) after biochar additions

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ABSTRACT

Conversion of forests to cultivated farms through slash-and-burn or chop-and-char practices often results in rapid loss of soil organic matter (SOM) or conversion of inherent SOM into pyrogenic organic matter (PyOM). However, there is little knowledge about the short-term changes in soil macrofauna that may occur when large amount of biochar are added to the soil. A thirty-day microcosm study was conducted to assess effects of biochar derived from two trees, *Croton megalocarpus* Hutch. and *Zanthoxylum gilletii* (De Wild.) P.G.Waterman, on the activity of a geophagous earthworm, *Pontoscolex corethrurus*. A portion of the biochar was leached with either acetone or 2 M HCl, to remove easily mineralizable organic matter and ash contents, respectively. Each of the biochar types was mixed with soil at a rate equivalent to 5, 10 and 25 Mg ha^{-1}. Casts were collected after 30 days and used as a measure of earthworms' activity. Casts dry weight was affected more by amount than the type of biochar. The highest cast weight (188.1 g and 176.5 g) was recorded in microcosm that received 5 Mg ha^{-1} of *C. megalocarpus* and *Z. gilletii* biochar, respectively. Notably, the weight decreased with increasing biochar additions. Cast weight decreased by 4% in microcosms that received 10 Mg of *C. megalocarpus* biochar ha^{-1} and by 15% in microcosms that received the same biochar type at a rate of 25 Mg ha^{-1}. Similarly, there was a 6% decline in cast weight in microcosms that received 10 Mg of *Z. gilletii* biochar ha^{-1} and an 8% decline in microcosms amended with 25 Mg ha^{-1} of the same biochar type. Easily mineralizable organic matter or nutrients were not responsible for the observed differences in cast production since leaching with acetone or HCl did not change the effects. The C and N content in casts and bulk soil were not significantly different, an indication that earthworms did not seek out biochar, but rather indiscriminately utilised the soil rich in biochar.

1. Introduction

Soil organic matter (SOM) is among the major soil components that are considered as key indicators of soil quality (Lal, 2004). However, continuous cultivation with minimal or no external organic and inorganic inputs, a common practice in most smallholder agroecosystems in Africa, leads to rapid loss of SOM (Mbau et al., 2015). SOM loss has been linked to decreasing crop productivity and increased soil degradation in tropical agroecosystems (Six et al., 2002). Thus, retention or addition of organic matter to soil is the most direct intervention of reversing such trends. Activities such as slash-and-burn and charcoal making during forest clearance are also common across sub-Saharan Africa, which leaves large amount of pyrogenic organic matter (PyOM) on site. This creates soil heterogeneity, with some areas rich in PyOM and others with unmodified SOM. Such areas may become 'hotspots' of favourable or unfavourable conditions, bringing about changes in the abundance, diversity and distribution of soil macrofauna (Kamau et al., 2017a). In other occasions, organic residues and by-products from diverse farming

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systems are deliberately converted into biochar for sequestering C in the soil (Lehmann et al., 2006). Addition of large amount of biochar or replacement of the native soil organic matter with biochar, could alter carbon substrates and nutrients available for soil macrofauna through a cascade of effects within the soil food web (Domene et al., 2014). Kamau et al. (2017a), for example, reported that the abundance of *Nematogenia lacuum*, an endogeic earthworm species, was negatively affected by high concentration of pyrogenic C (PyC) in charcoal-making spots. Thus, soil changes that may occur after forest clearance and addition of biochar may negatively influence forest-specific species and favour peregrine species that are more adapted to disturbed soils or can withstand effects arising from biochar additions. For instance, *Pontoscolex corethrurus*, a geophagous endogeic earthworm species, is said to be highly adapted to tropical cultivated soils over a wide range of soil conditions due to its capacity to consume low-quality organic matter (Topoliantz and Ponge, 2005; Ponge et al., 2006). This species has been found to have significant influence on soil structure due to its burrowing and casting activities and thus may have played a significant role in formation of *Terra Preta* soils of the Amazon through incorporation of charcoal particles throughout the soil profile (Ponge et al., 2006). Nonetheless, its casting activity has also been shown to compact soils when there is low or absence of a diverse soil macrofauna community capable of fragmenting the large coalescent casts produced by this species (Blanchart et al., 1997; Barrios et al., 2005). This demonstrates how dominance of a single macrofauna species, resulting from soil management decisions, could significantly affect soil functions.

Endogeic earthworms are known to play a critical role in changing soil physical properties through ingesting and excreting soil and making extensively branched, sub-horizontal networks of burrows in search of organic matter rich soil (Shipitalo and Le Bayon, 2004; Barrios et al., 2018). Lavelle (1988) reported that a single endogeic, geophageous earthworm can ingest up to 30 times its body weight in a single day. Estimates show that cast production can go as high as 1200 Mg ha^{-1} year^{-1} in tropical soils (Brown et al., 2000). However, the rate of burrowing and cast production can be affected by the type of substrate and earthworm species. Topoliantz and Ponge (2003), for instance, reported that earthworms (*P. corethrurus*) avoided ingestion of charcoal particles by pushing them aside, as the earthworms burrowed through soil amended with charcoal. Nonetheless, the authors suggested that earthworms may ingest charcoal particles for purposes other than nutrition, such as to benefit from detoxifying effects of the charcoal. A close examination of soils with dark-coloured organic matter in French Guiana confirmed the presence of casts from earthworms (*P. corethrurus*), which had numerous small charcoal fragments intimately mixed with the soil (Ponge et al., 2006). This implies that chemical properties of a soil amended with biochar may be greatly affected by the presence of earthworms and their activities.

With the increasing interest in utilisation of biochar as a soil amendment, there is also an increasing concern about possible accumulation of organic and inorganic contaminants which may be incorporated into the soil (Verheijen et al., 2010). Volatile organic compounds (VOCs) are common organic contaminants often formed during pyrolysis of organic materials. These compounds are probably formed either by breakdown and/or by rearrangement of the original structure of organic biomass (Spokas et al., 2011). For instance, complex organic compounds may be cracked into smaller unstable fragments which recombine with other free reactive molecules or radicals into more stable but potentially toxic compounds (Hale et al., 2012). Studies have confirmed presence of the most common group of toxic compounds, polycyclic aromatic hydrocarbons (PAHs), as well as traces of dioxins and furans in biochar (Verheijen et al., 2010; Domene et al., 2015). Other potential direct negative effects on soil macrofauna could be brought about by the presence of heavy metal contaminants or due to excessive salinization or liming effects after application of biochar (Domene et al., 2015). In spite of such pertinent issues being raised, there is little information on how farm management decisions such as application of biochar or addition of large amount of PyOM due to charcoal making can affect soil fauna.

Use of earthworms as a model bio-indicator organism is predicated on the ease of assessing their response to environmental perturbations in tests such as growth, mortality or activity rate as well as reproduction patterns, among others (Li et al., 2011; Bart et al., 2018). Therefore, we sought to study the short-term effects of biochar application on earthworms (*Pontoscolex corethrurus*) cast production, as a measure of earthworm activity. Our study includes three application rates of two biochar types derived from two dominant tree species; *Croton megalocarpus* Hutch. and *Zanthoxylum gilletii* (De Wild.) P.G.Waterman, commonly found along the Nandi-Kakamega forest complex and were subject of an earlier study by Kamau et al. (2017a). The two types of biochar were applied untreated or leached either with 2 M HCl or with acetone to remove ash and possible toxic substances that could affect earthworm casting activity. Since biochar adds more persistent organic matter which can have negative effects on earthworms (Kamau et al., 2017a), we hypothesised that (i) cast production would decrease with biochar addition, regardless of the source and application rate, and (ii) C and N content would increase with biochar addition.

2. Materials and methods

2.1. The experimental site

The study was conducted at the World Agroforestry Centre's (ICRAF) Soil Ecology Facility, located at Latitude 1° 14′ 08″ S and Longitude 36° 49′ 11″ E. At an altitude of 1700 m above sea level, mean daily temperature ranges between 11 °C and 25 °C. The soil used in the experiment was obtained from South Nandi (Latitude 0° 10′ N and Longitude 35° 0′ E), the same sites where Kamau et al. (2017a, 2017b) conducted their study. Soils are predominantly kaolinitic Acrisols (FAO/UNESCO classification) or Ultisols (USDA classification) showing deep reddish-brown colouration and humic topsoil with 45–49% clay, 15–25% silt and 26–40% sand. About 200 kg of the top soil was collected from each of the three study catchments which have been cultivated for 10, 16 and 62 years after conversion from the forest to cultivated lands, thus providing chronosequence sites where effects of land-use change could be systematically studied. The chronosequence sites were established after extensive data collection, which included farmer interviews regarding land use history and supported by local records, aerial photos, sampling over 150 farms and in-depth experimentation on 70 farms, as summarized by Kamau et al. (2020). These farms were similar in many aspects, including soil type, land use history and hydrology. Soil was taken from the upper 0.1 m layer; any organic material at the surface was removed prior to collection. The soil was air-dried, thoroughly mixed and passed through a 2 mm sieve. Finally, the soil from the three sites were composited to make a homogenous mass from where portions to be used in each microcosm were drawn. Chemical properties of the composited soil before the experiment were as follows: the soil was slightly acidic (pH of 5.6), low in available P (13.8 mg kg^{-1}) and exchangeable K (0.4 g kg^{-1}), but with relatively high total C (36.2 g kg^{-1}) and N (3.2 g kg^{-1}) (Namoi et al., 2019).

2.2. Biochar preparation and pre-treatment procedure

Biochar was prepared from *Croton megalocarpus* and *Zanthoxylum gilletii* tree branches which are commonly used in traditional charcoal making in South Nandi (Kamau et al., 2017a). The branches were separated from the leaves and the wood chopped into 2 m pieces. Two portions of land (about 3 m in diameter each) where the kilns were to be located were cleared of any farm residues, levelled and compacted. The chopped wood was placed upright, leaning towards a central pole. Leaves from the trimmed branches were spread over the stack and then covered with soil from around the kiln site. Wood from each tree was pyrolysed separately at temperatures of about 500 °C for four days. Once the process was completed, the biochar was ground to pass a 2 mm sieve and divided into three portions. One portion of the biochar was kept untreated, a second portion was leached with acetone to remove possible toxic volatile matter while the third portion was leached with 2 M HCl to reduce ash contents. Reduction of ash from biochar by acid treatment is achieved through demineralization of trapped inorganic constituents as suggested by Peiris et al. (2019). In each of the treated portions, the biochar was mixed with the leaching agent at a ratio of 1:10 (*w/v*), and the mixture shaken overnight using a reciprocating shaker as described by Güereña et al. (2015). The treated biochar were then filtered through Whatman number 42 filter paper. After filtration, the biochar that had been leached with acetone was dried overnight at 60 °C. The portion that was leached with 2 M HCl was further treated with 1 N NaOH in order to readjust the biochar material to its original pH and then filtered. The biochar material was further leached twice using deionised water at a ratio of 3:5 (*w/v*) in order to remove the excess Na^+ and Cl^-. The biochar was dried overnight at 60 °C.

2.3. Assessment of casting activity

Casting activity of earthworms (*Pontoscolex corethrurus*) was assessed using modified microcosms (0.15 m in diameter and 0.30 m in height, see Fig. 1). The earthworm species *P. corethrurus* is a dynamic earthworm that produces large coalescent aggregates that can easily be separated from the rest of the soil, therefore making it suitable for the study of soil biological activity (Topoliantz and Ponge, 2003; Pauli et al., 2010). Each of the biochar type (untreated, leached with acetone or with acid) was weighed and mixed with soil at 5, 10, and 25 g kg^{-1}, which correspond to rates of 5, 10 and 25 Mg ha^{-1} respectively at a bulk density of 1 Mg m^{-3} and a depth of 0.1 m. Application rates of between 5 Mg and 10 Mg ha^{-1} of biochar is within the application rates currently employed in most agroecosystems in Africa, while 25 Mg ha^{-1} is at the outer range of maximum plausible application levels (Kamau et al., 2019). A microcosm with soil only was included as a control. For each amendment, 1.5 kg of soil alone or soil + biochar mixtures were placed into the microcosm. It should be noted that all the microcosms carried a total of 1.5 kg of the contents (either soil or soil + biochar mixture) before initial wetting and introduction of the earthworms. Inert sand was used at the base of the microcosm (at a depth of 0.05 m) to allow for capillary wetting. There were five replications for each amendment. The soil and soil + biochar mixture were moistened with water to 65% field capacity through capillary wetting and allowed to stabilize for 24 h to ensure that the contents of the microcosms (soil or soil + biochar) were evenly wet. A pre-test with three microcosms for each representative treatments showed consistency in moisture content in the whole soil column. After wetting, two mature *P. corethrurus* (average weight of 1.3 ± 0.03 g) were introduced at the top of microcosms. Top edges of the microcosms were then covered with wet muslin cloth to avoid desiccation of earthworms while also preventing them from escaping. The experiment was conducted for a period of 30 days. Casts were initially collected from the soil surface in the first 2 days and thereafter no additional surface casting was observed and hence collected from inside the soil column at the end of 30 days. To separate casts from the bulk soil (Fig. S1), the contents of microcosms were first air-dried for 48 h, after which they were placed on a 2 mm sieve and shaken lightly. No other physical force was applied to avoid breaking the casts. The casts and bulk soil were further oven dried separately at 60 °C overnight and their final dry weight recorded. Subsamples of the casts and bulk soil were drawn and fine-ground separately for C and N analysis.

2.4. Biochar and soil chemical analyses

After drying and grinding biochar, samples were analysed for pH and major macro-elements C, N (total, NH_4–N and NO_3–N), P, K, Ca and Mg as well as Na, Exchangeable Sodium Percentage (ESP) and Effective Cation Exchange Capacity (ECEC). Polycyclic Aromatic Hydrocarbons (PAHs) and heavy metals were also analysed. The pH was determined using a pH meter with a biochar-water solution ratio of 1:5 as described by Anderson and Ingram (1993). Total C and N were determined by FLASH 2000 NC Analyser (ThermoFisher Scientific, Cambridge, UK), extractable NH_4–N and NO_3–N was extracted using 2 M potassium chloride and determined using steam distillation method (Bremner and Keeney, 1965), while K, Na, Ca, Mg and ECEC were determined using the compulsive exchange method (Gillman and Sumpter, 1986). Heavy metals in biochar were determined using inductively coupled plasma atomic emission spectroscopy (Isaac and Johnson, 1998) while PAHs were determined using EP132-ST ALS Super Ultra Trace PAH method (ALS Environmental, 2015). Soil and casts were analysed for total C, N and pH. Total C and N were determined using NC Analyser while pH was determined using a pH meter with a soil- or casts-water solution ratio of 1:2.5 (Anderson and Ingram, 1993).

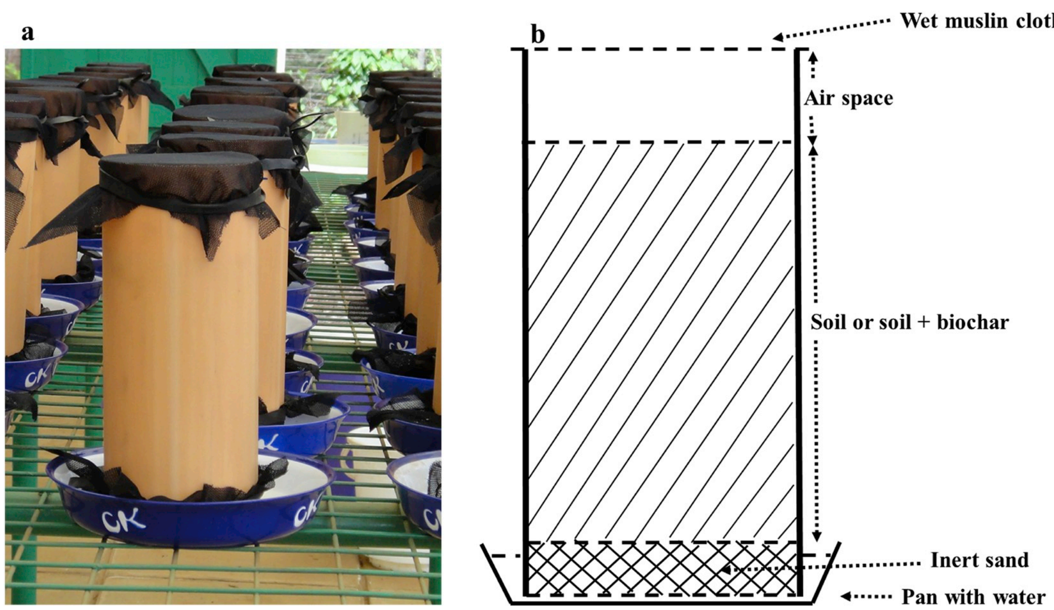

Fig. 1. Microcosms used in the study (**a**) and a schematic representation of the microcosm (**b**).

2.5. Statistical analyses

The statistical software R, version 3.6.1 (R Core Team, 2019) was used in statistical analyses. Due to the fact that surface casting was observed only during the first two days, and thereafter occurred internally within the soil column, these casts were combined with those collected at the end of the experiment to represent the total cast production. Therefore, all analyses on casts parameters (weight, C and N) are based on the total casts collected. The influence of biochar on cast production and C and N content, pH and C/N ratio in casts and bulk soil was tested using generalised linear models using lme4 package (Bates et al., 2015). A full model was first used to test all the factors: biochar source, biochar pre-treatment method and biochar application rate and all two-fold and three-fold interactions between these factors. Factors that showed no significant contribution were dropped and the data were reanalysed only taking into consideration the effects of factors that showed significance. Tukey's post-hoc tests were used to separate the means at $\alpha = 0.05$ when analysis of variance (ANOVA) showed significant main or interactive effects.

3. Results

3.1. Chemical characteristics of biochar

The most outstanding differences between biochar derived from *Z. gilletii* and *C. megalocarpus* were NH_4–N, ECEC, Na and ESP (Table 1). Biochar derived from *Z. gilletii* had high NH_4–N content (23.0 mg kg^{-1}), ECEC (970 mmol (+) kg^{-1}), Na (22.3 g kg^{-1}) and ESP (95%) compared to biochar derived from *C. megalocarpus* which had very low NH_4–N content (0.5 mg kg^{-1}), ECEC (130 mmol (+) kg^{-1}), Na (0.1 g kg^{-1}) and ESP (0.7%). Pre-treatment seems to have been effective only on *Z. gilletii* biochar. Hence, leaching this biochar with HCl decreased the concentrations of NH_4–N (0.9 mg kg^{-1}) and Na (0.1 g kg^{-1}) and levels of ECEC (170 mmol (+) kg^{-1}) and ESP (0.6%). In contrast, there was no significant change observed in chemical characteristics of *C. megalocarpus* biochar after the HCl treatment. Very low concentration of heavy metals (<5 mg kg^{-1}) and PAHs (<0.5 mg kg^{-1}) were recorded in both biochar types, whether treated or untreated and therefore were not included in Table 1 (but reported in supplementary data file).

3.2. Biochar effects on earthworm cast production

Among the three factors (biochar source, biochar pre-treatment method and biochar application rate), only the rate of biochar application showed significant influence on earthworm cast weight. Further, there were no significant interactions between the factors (Table 2). Cast weight significantly declined with increasing additions of biochar (Fig. 2). In microcosms with untreated *C. megalocarpus* biochar, cast weight decreased significantly from 188.1 g in microcosms with 5 Mg ha^{-1} of biochar to 180.9 g and 160.2 g in microcosms with an equivalent of 10 and 25 Mg ha^{-1} of the biochar, respectively. This represented about 4% and 15% decline in cast weight, respectively. Similarly, cast weight decreased from 176.5 g in microcosms with 5 Mg ha^{-1} of untreated *Z. gilletii* biochar to 165.7 g and 163.5 g in microcosms which received the same biochar at an equivalent rate of 10 Mg ha^{-1} and 25 Mg ha^{-1}, respectively. This was about 8% decrease in the highest application rate (25 Mg ha^{-1}) and 6% in microcosms with an equivalent rate of 10 Mg ha^{-1} *Z. gilletii* biochar. Biochar pre-treatment method did not significantly influence earthworm cast production. Thus, cast weight in microcosms which received acetone-leached and acid-leached biochar, regardless of the feedstock source, showed similar differences to untreated biochar. Similarly, cast weight in microcosms with soil alone (0 Mg biochar ha^{-1}) was not significantly different from that in microcosms with lowest biochar application rates (5 Mg ha^{-1}), regardless of the source of biochar and biochar pre-treatment method.

3.3. Biochar effects on C and N content of casts and bulk soil

Similar to the cast weight, C and N content in casts was affected by amount, rather than the source of biochar or biochar pre-treatment method (Fig. 2). The C content in casts increased with increasing amount of biochar applied. Notably, however, only casts recovered from microcosms with the highest (25 Mg ha^{-1}) and the lowest (5 Mg ha^{-1}) biochar application rates showed significant difference in C content. In all cases, there were no differences either between 5 Mg and 10 Mg biochar ha^{-1} or between 10 Mg and 25 Mg biochar ha^{-1}. In microcosms with untreated *Z. gilletii* biochar, for instance, casts C content increased from 43.5 mg g^{-1} in the lowest application rate, to 48.7 mg g^{-1} in the highest application rate, which represents a 12% increase. C content in all the casts produced in microcosms with untreated *C. megalocarpus* biochar were not significantly different. In microcosms where acetone-leached biochar was applied, only those which received *Z. gilletii* biochar showed significant differences. In this case, C content increased from 41.5 mg g^{-1}, in microcosms with the lowest application rate, to 46.3 mg g^{-1} in microcosms with the highest amount, which was a 12% difference. Differences in C content between the highest and lowest

Table 1
Chemical quality parameters (means ± se) of untreated biochar and biochar leached with either acetone or 2 M HCl.

Characteristic	Units	Biochar source/pre-treatment method						Summary of *p*-values	
		Croton megalocarpus			Zanthoxylum gilletii				
		Untreated biochar	Acetone-leached biochar	Acid-leached biochar	Untreated biochar	Acetone-leached biochar	Acid-leached biochar	Pre-treatment	Biochar source
$pH_{(water)}$	pH units	9.6 (0.2)	9.3 (0.9)	9.6 (0.8)	8.8 (0.5)	8.8 (0.7)	8.3 (0.4)	0.155	0.058
Total C	g kg^{-1}	840.0 (2.7)	840.0 (5.6)	810.0 (9.2)	780.0 (9.6)	770.0 (3.8)	810.0 (6.0)	0.954	0.013
Total N	g kg^{-1}	6.8 (0.3)	7.2 (1.6)	6.2 (1.5)	6.7 (0.4)	5.8 (1.4)	6.5 (1.6)	0.414	0.074
Extractable NH_4–N	mg kg^{-1}	0.5 (0.2)	0.6 (0.2)	0.3 (0.0)	23.0 (5.1)	5.6 (0.8)	0.9 (0.3)	0.013	0.006
Extractable NO_3–N	mg kg^{-1}	1.0 (0.2)	1.1 (0.1)	1.4 (0.3)	0.9 (0.4)	1.7 (0.4)	1.5 (0.1)	0.085	0.085
Water-soluble P	mg kg^{-1}	65.0 (6.0)	66.0 (0.9)	60.0 (5.3)	47.0 (7.0)	51.0 (7.2)	50.0 (1.7)	0.162	0.292
Exchangeable K	g kg^{-1}	2.0 (0.7)	2.7 (0.6)	3.1 (0.6)	0.4 (0.1)	0.4 (0.2)	0.3 (0.1)	0.138	0.057
Exchangeable Ca	g kg^{-1}	1.4 (0.3)	1.3 (0.2)	1.7 (0.6)	1.5 (0.2)	1.2 (0.2)	1.5 (0.5)	0.164	0.059
Exchangeable Mg	g kg^{-1}	0.1 (0.0)	0.2 (0.1)	0.2 (0.0)	0.1 (0.1)	0.1 (0.1)	0.2 (0.1)	0.154	0.274
Exchangeable Na	g kg^{-1}	0.1 (0.1)	0.1 (0.0)	0.1 (0.0)	22.3 (3.1)	27.6 (4.9)	0.1 (0.0)	0.051	0.003
Effective CEC	mmol (+) kg^{-1}	130.0 (5.2)	150.0 (9.3)	180.0 (7.8)	970.0 (8.1)	1030.0 (10.9)	170.0 (4.8)	0.050	0.004
ESP	% of CEC	0.7 (0.4)	2.9 (0.2)	0.3 (0.1)	95.0 (7.3)	94.0 (5.3)	0.6 (0.4)	0.051	0.003

Table 2
Summary of *p*-values generated from fitting earthworm cast weight and C and N content, C/N ratio and pH of the casts and bulk soil as a function of biochar source, biochar pre-treatment method and biochar application rate using generalised linear models (GLM) (n = 5).

Property	Summary of the *p*-values						
	Biochar source	Biochar pre-treatment method	Biochar application rate	Source × Pre-treatment	Source × Application rate	Pre-treatment × Application rate	Source × Pre-treatment × Application rate
Casts							
Weight	0.136	0.345	<0.001***	0.845	1.000	1.000	1.000
$pH_{(water)}$	0.163	0.062	0.050*	0.312	0.355	0.434	0.592
Total C	0.219	0.052	<0.001***	0.143	0.135	0.176	0.429
Total N	0.138	0.437	0.193	0.202	0.623	0.296	0.147
C/N ratio	0.485	0.085	<0.001***	0.568	0.257	0.481	0.784
Bulk soil							
$pH_{(water)}$	0.016*	0.069	0.003**	0.345	0.491	0.501	0.126
Total C	<0.001***	0.955	<0.001***	0.872	0.205	0.855	0.109
Total N	<0.001***	0.315	<0.001***	0.247	0.146	0.290	0.140
C/N ratio	0.012*	0.602	<0.001***	0.599	0.216	0.222	0.257

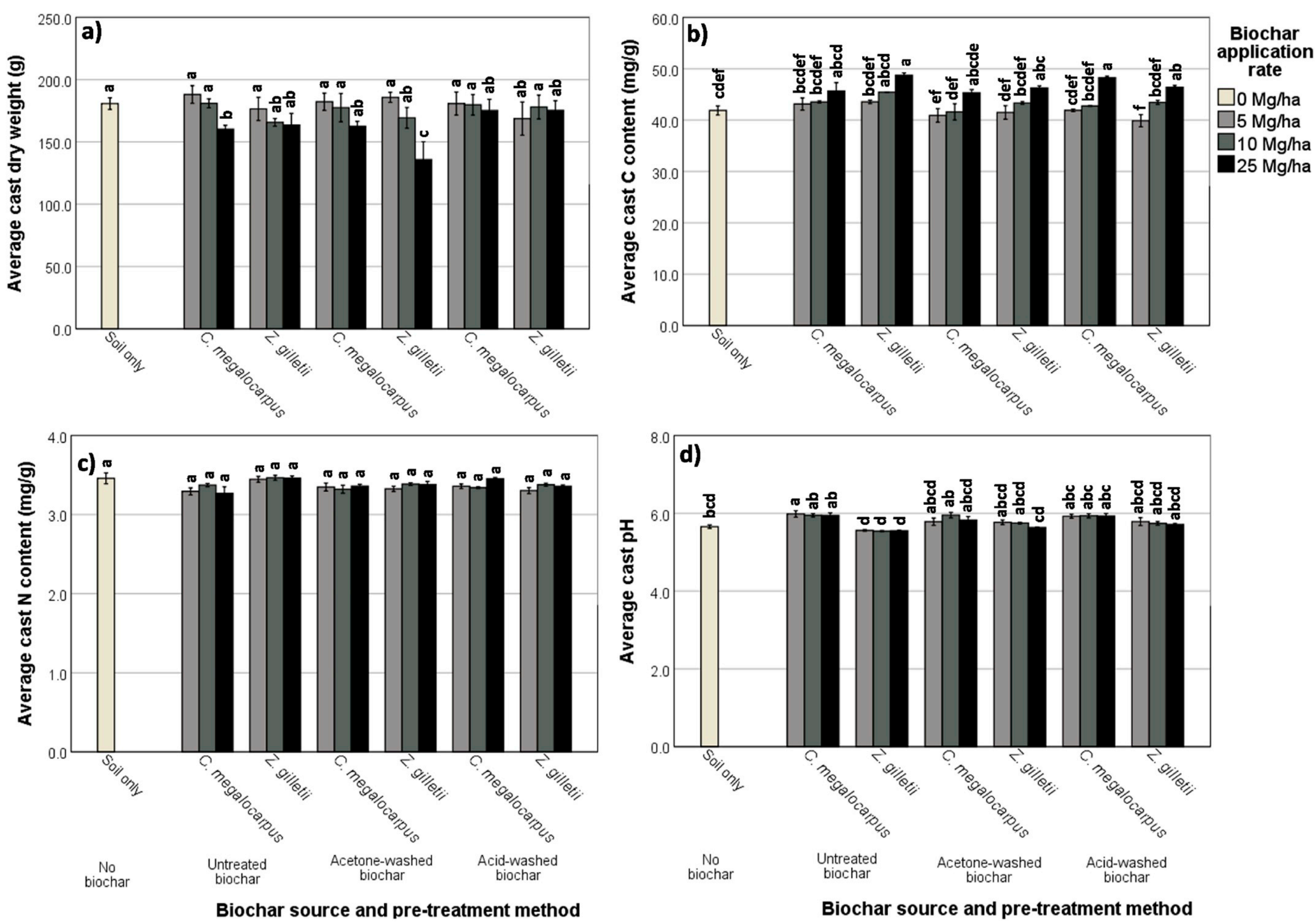

Fig. 2. Dry weight, total C and N content and pH of earthworm casts as affected by biochar amount, source and pre-treatment method. Bars with different lowercase letters indicate significant differences between the treatments at $p < 0.05$.

application rate in microcosms with acid-leached *C. megalocarpus* biochar was about 15% whereas those with acid-leached *Z. gilletii* biochar was about 17%. Contrary to casts, biochar source showed significant influence on bulk soil chemical properties (Table 2). Generally, bulk soil C was higher (46.0 mg g^{-1}) in microcosms that received biochar from *Z. gilletii* than those that received *C. megalocarpus* biochar (44.2 mg g^{-1}), regardless of pre-treatment method (Fig. 3). Bulk soil N showed similar differences as bulk soil C, with higher values in microcosms that received *Z. gilletii* biochar (3.4 mg g^{-1}) than those that received *C. megalocarpus* biochar (3.1 mg g^{-1}). On the other hand, bulk soil pH was higher in microcosms that received *C. megalocarpus* biochar (5.8) than those that received *Z. gilletii* biochar (5.5). Based on biochar application rate, the bulk soil C and N content and pH showed similar differences to those of casts (Fig. 4). However, there was no significant influence of biochar pre-treatment method or any of the two- and three-way interactions between the factors on bulk soil chemical properties.

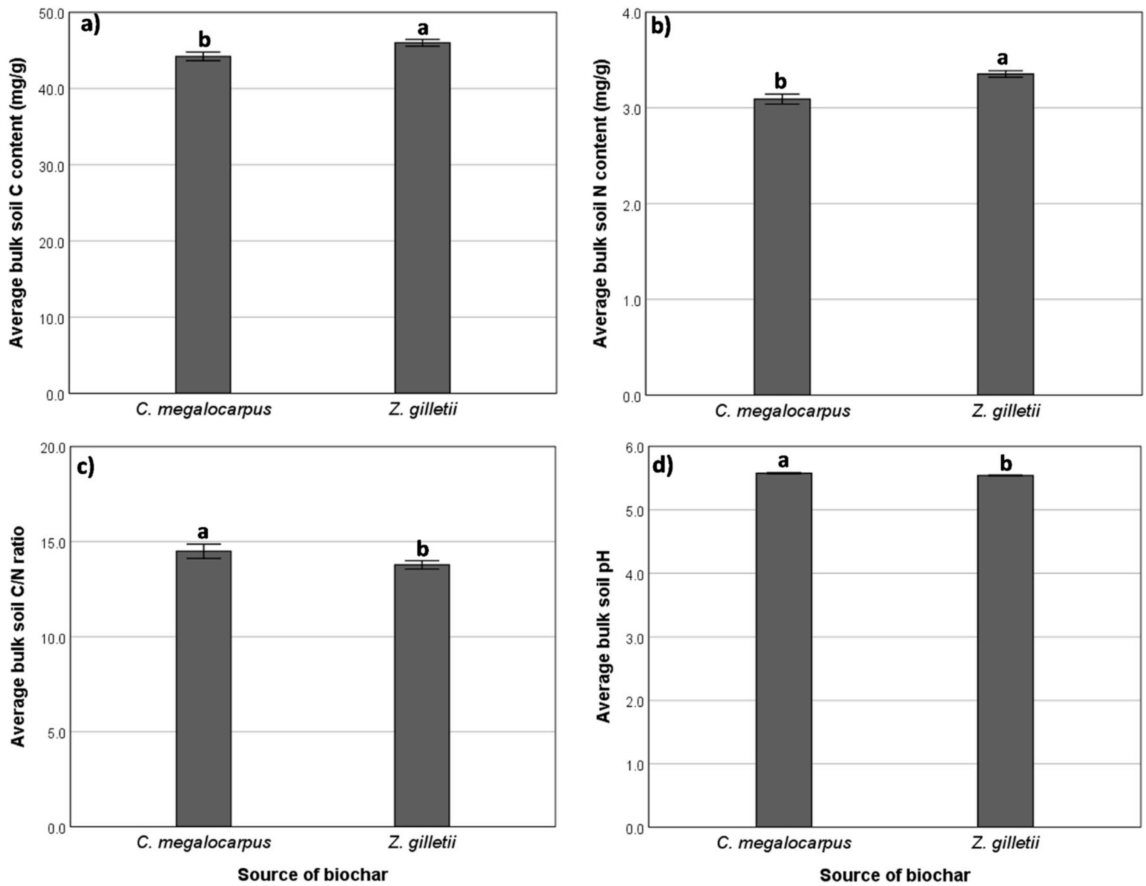

Fig. 3. Total C and N content, C/N ratio and pH of bulk soil as affected by biochar source, regardless of the amount and pre-treatment method. Bars with different lowercase letters indicate significant differences between the two sources at $p < 0.05$.

4. Discussion

4.1. Biochar effects on earthworm cast production

Studies investigating effects of biochar effects on earthworm abundance, diversity and activity have reported mixed results (Lehmann et al., 2011; Ameloot et al., 2013). Endogeic earthworms, those that mostly feed on and live in the soil, are presumably the most affected by biochar additions to soil (Kamau et al., 2017a). Given that our experimental species, *P. corethrurus* is an endogeic earthworm, addition of biochar, which is more persistent than non-biochar organic matter in SOM, could have made biochar a less desirable substrate. Presence of more persistent SOM may alter nutrient release patterns and carbon availability (Lehmann et al., 2011; Domene et al., 2014; Kamau et al., 2019). Decreasing available nutrients could trigger a change in abundance and diversity of soil microbiota through a cascade of effects in the food web and hence influence the earthworms' response to the newly added biochar (Domene et al., 2014; Kamau et al., 2017a). For instance, Kamau et al. (2017a) reported lower numbers of *Nematogenia lacuum*, also an endogeic earthworm species, with increasing concentration of PyOM. Though the authors were looking at abundance rather than the activity of earthworms, the decreasing abundance with increasing PyOM concentration could be an indication that the PyOM was exerting negative effects on the earthworms. In this case, earthworms were probably responding by moving away from the centre of charcoal-making spots where PyOM concentration was highest. Alternatively, increased nutrient utilisation efficiency after biochar application may reduce the need for higher substrate ingestion, and thus a reduction in cast production. Though this was not determined in our study, such a process may explain the observed differences in earthworm cast production. Besides nutrition, other direct and indirect biochar effects may play a significant role in shaping specific earthworm responses to biochar addition. For example, we cannot exclude the possibility that the earthworms were keeping away from soil rich in biochar to avoid desiccation as suggested by Li et al. (2011). Since we allowed the soil and soil + biochar mixture in the microcosms to wet and stabilize for 24 h, we cannot state with certainty that the biochar had reached its field capacity at this time when the earthworms were being introduced. Nonetheless, in their study, Li et al. (2011) observed a significant reduction in weight of the earthworms in biochar treated soils at the end of their 28-day study, which they suggested could have been due to the avoidance of the biochar-rich soil. In our study however, the earthworms did not show significant weight change. There was also no significant difference in weight across the treatments at the end of the experiment (Fig. S2).

The method of biochar pre-treatment in our study seems to have had little influence on the response of earthworms to biochar application. For instance, despite significant reduction in Na and ESP after leaching biochar derived from *Z. gilletii* tree with 2 M HCl, there were no significant differences in cast production in microcosms which received this biochar compared to microcosms which received untreated biochar from the same tree species. This suggests that the response of earthworms to biochar was not strongly affected by the mineral contents of biochar. We can also not relate the decreased cast production to presence of toxic compounds since the biochar used in this study had negligible amount of PAHs and heavy metals as reported in Table S1. It should also be noted that though the pre-treatment method did not seem to have significant influence on biochar quality, chemical and/or physical properties, other than what we analysed may be implicated in the observed differences in earthworm cast production.

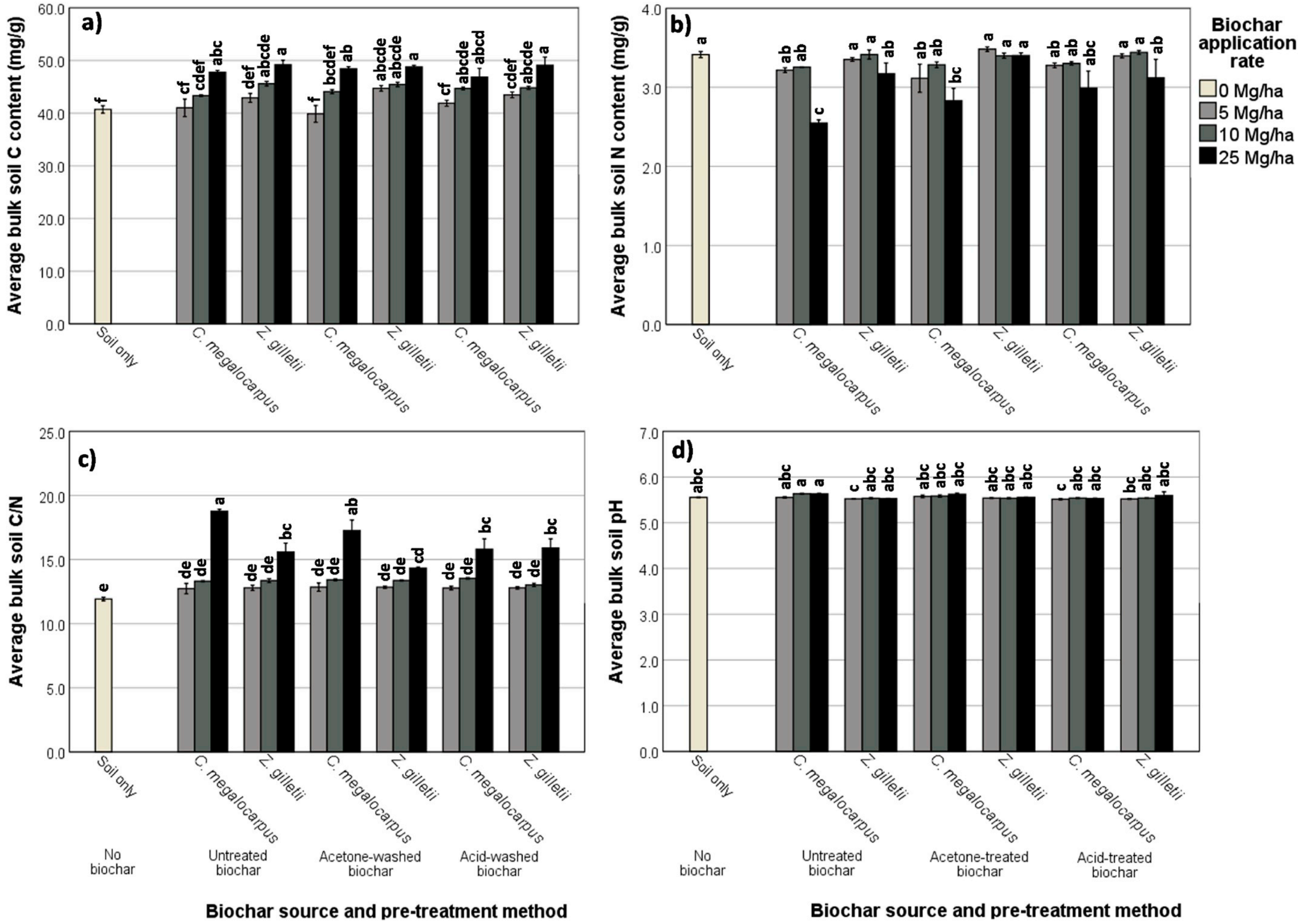

Fig. 4. Total C and N content, C/N ratio and pH of bulk soil as affected by biochar amount and pre-treatment method. Bars with different lowercase letters indicate significant differences between the treatments at $p < 0.05$.

4.2. Effects of biochar and earthworms interactions on C and N content of casts and bulk soil

Generally, it has been reported that the selective ingestion of mineral and organic particles by earthworms play a major role in determining C and N content of the casts or cast-derived micro-aggregates (Zhang and Schrader, 1993; Bossuyt et al., 2004; Fonte et al., 2007; Jouquet et al., 2008; Van Groenigen et al., 2019). Thus in most cases, studies investigating effects of earthworms' activities on soil properties have shown significantly higher C and N content in the casts than the bulk (surrounding uningested) soil. Two early studies showed clear differences between casts and bulk soil C and N. In a grass/legume management system, Guggenberger et al. (1996) reported that organic C in earthworm (*Martiodrilus* sp.) casts was more than double that of the bulk soil. Decaëns et al. (1999) also reported similar findings where anecic earthworms (*Martiodrilus carimaguensis*) casts had 1.5–1.9 times higher C and 1.4–1.6 times higher N than the adjacent bulk soil. In a recent meta-analysis of studies covering all continents (except Antarctica), Van Groenigen et al. (2019) also reported an average of 40–48% higher total organic C, N and P in earthworm casts relative to the bulk soil. However, though these studies show greater content of these elements in casts compared to the bulk soil, it should be noted that the earthworms' efficiency in assimilation of nutrients during digestion process affects the amount of C and N in the casts (Brown et al., 2000; Condron et al., 2010), which partly contributes to these differences. Assimilation efficiency may in turn vary depending on a number of factors including, but not limited to, earthworm species and their ecological grouping, the quality and quantity of organic substrates provided to the earthworms. Generally, C assimilation efficiency rates for endogeic earthworms have been reported to range between 8 and 19% (Brown et al., 2000), but can be as low as 1% in some earthworm species (e.g. *Aporrectodea rosea* – Condron et al., 2010). In this study, since we had a single earthworm species, differences in C and N can be attributed to the quality and quantity of the biochar used. Thus, the little difference in C and N content between casts and bulk soil could be an indication that earthworms did not seek out biochar, but rather indiscriminately utilised soil rich in biochar. This could possibly result from earthworms being in a restricted volume of soil, and lacked any other alternative organic substrate they could utilise. Several studies provide insights that we could use to explain the observed trends in our study. For instance, Pulleman et al. (2005) reported that limited availability of easily assimilable organic residues could have been the reason why cast-derived aggregates in soils from conventional agriculture were hardly enriched with C compared to those derived from permanent pasture and organic agriculture. Similarly, Decaëns et al. (1999) reported that casts produced in tropical pastures had significantly higher levels of total C and N due to availability of large quantities of easily assimilable legume litter, compared to those produced in the native savanna where plant debris had a lower palatability. Nonetheless, in our study, the observed differences may change over time with casts age. In addition, other direct and indirect effects of earthworms and/or biochar in the casts and bulk soil, and which were not measured, cannot be excluded. For example, physical aspects of the soil such as aeration and drainage affect microbial activity, mineralization rates and nutrient availability, which may contribute to these differences.

The increasing C content with increasing amount of biochar shows that the earthworms were able to successfully incorporate greater amount of C into these biogenic structures. This can be an important process given that mass application of biochar as a soil amendment hinges on anticipation that soil organisms will incorporate this amendment into the soil. Topoliantz and Ponge (2003, 2005) suggested that endogeic earthworm *P. corethrurus* is an important organism in incorporating charcoal to soil in slash-and-burn systems, and thus could have been responsible for the formation of the ancient nutrient-rich Amazonian Dark Earths. Further, since *P. corethrurus* produce large coalescent casts that protect soil organic matter from microbial degradation, this could be an important characteristic attribute contributing to enhanced physical and chemical soil properties over the long-term. Its extensive burrowing could also become an important factor in incorporation of biochar within the soil profile after application.

5. Conclusion

Endogeic earthworms are among the soil macrofauna that are most sensitive to biochar application because they feed on soil organic matter or to benefit from the microbes growing on this substrate or their metabolites. This study has shown that increasing the concentration of biochar in soil led to an initial short-term decrease in earthworm cast production, possibly an indication that earthworms may avoid consumption of soil with high amount of biochar. Alternatively, increased nutrient use efficiency after addition of biochar could reduce substrate intake and therefore the decreased earthworm casts production. Nonetheless, C and N content in the casts and bulk soil were not significantly different, thus supporting the notion that earthworms did not seek out biochar, but indiscriminately utilised soil rich in biochar. In general, the method of biochar pre-treatment seems to have had little effects on chemical properties, except in *Z. gilletii* biochar where Na and ESP decreased significantly. Nonetheless, these changes did not affect earthworms' response towards biochar application and thus contributed to the observed insignificant differences in cast production and C and N content. Future studies should consider looking at C and N dynamics of casts, taking into consideration the cast ageing process.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.107736.

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