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Title: Contrasting responses of macro- and meso-fauna to biochar additions in a bioenergy cropping system

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Abstract

Combining bioenergy land use with biochar production could represent a win-win management strategy to increase energy production whilst reducing greenhouse gas emissions. However, a fuller understanding of the effects that these changes in land use and soil amendment could have on soil biodiversity and processes is needed. We performed a 2-year field experiment to determine the consequences of adding three different amounts of biochar (10 t ha^{-1}, 25 t ha^{-1} and 50 t ha^{-1}) to a commercial *Miscanthus* bioenergy plantation on soil invertebrate community structure and abundances of enchytraeids, collembolans, mites and earthworms. We also used stable isotope analyses to determine shifts in feeding preferences and to quantify C assimilation by those soil organisms most likely to be affected by soil amendments (i.e. soil ingesters: earthworms and enchytraeids). Results showed that biochar additions to the soil had a negative effect on larger-sized soil fauna (earthworms) significantly reducing their population sizes and species richness whereas, in contrast, mesofauna appeared to benefit from the input of the biochar. Although significant assimilation of new C by anecic earthworms was observed, it was clearly insufficient to support population growth and, more importantly, the dominant ecological group in these agricultural soils (endogeics) showed the lowest assimilation values. These results indicate that biochar additions might result in the loss of some of the ecosystem services provided by earthworms, an important concern in these intensively managed agricultural soils. Finally, our findings highlight the need for more field research at species level to fully elucidate the mechanisms driving the biological responses of these types of ecosystem management.

Keywords: *Miscanthus; pyrolised carbon; soil invertebrates; stable isotopes*

1. Introduction

Biochar application to soil and bioenergy crop production are two management options that have significant potential for attaining climate change mitigation whilst increasing soil carbon (C) stocks (Winsley, 2007). Biochar can be produced from bioenergy crop residues and applied to the fields to promote plant growth, with the combined use of these two strategies representing a good example of circular economy, aimed at a more sustainable use of limited land resources, whilst enhancing C sequestration and improving soil quality and water holding capcity (Laird, 2008; Gaunt and Lehmann, 2008; Sohi et al., 2009; Roberts et al., 2010; Hammond et al., 2011; Case et al., 2014). Since soil amendments and land use changes can have a strong influence on soil biota, interest has been raised on how these treatments could affect abiotic and biotic properties and in turn, ecosystem functioning as reviewed by McCormack et al. (2013).

The addition of biochar to soils has been shown to increase C retention (e.g. Schmidt et al., 2019), soil fertility (Ding et al., 2016; Glaser and Lehr, 2019), water-holding capacity (Omondi et al., 2016; Nagel et al., 2019) and plant productivity (Katterer et al., 2019), while reducing greenhouse gas emissions (e.g. Wang et al., 2011; Cayuela et al., 2014; Jeffery et al., 2016; Azeem et al., 2019). However, it remains unclear whether these benefits can be extrapolated across climates (e.g. Jeffery et al., 2017) and soil types (e.g. Noguera et al., 2010; Streubel et al., 2011; Zhang et al., 2019). Although research on the effects of biochar on soil biota has increased in recent years (see reviews by Lehmann et al., 2011; Ameloot et al., 2013; Domene, 2016), the available evidence indicates no consistent responses (i.e. either positive, negative or no effect), hampering the application of this technique to improve soil fertility and mitigate climate change. Part of the problem is that the majority of the studies focus on single individual groups of soil organisms, such as microorganisms (e.g. Anderson et al., 2011), collembolans (e.g. Amaro, 2013; Marks et al., 2014; Domene et al., 2015), but mostly earthworms (e.g. Noguera et al., 2010; Weyers and Spokas, 2011; Li et al., 2011; Tammeorg et al., 2014; Elmer et al., 2015), and they have often been performed under laboratory incubations (primarily toxicity assays using single species that can be easily reared under laboratory conditions).

The limited number of field observations show very contrasting results, with some studies reporting positive effects on certain soil faunal groups (Gruss et al., 2019; McCormak et al., 2019), others describing reductions in densities and diversity as well as avoidance behaviours (Fontodji et al., 2009; Marks et al., 2014; Godfrey et al., 2014). Others have shown no population changes (Zhang et al., 2013; Prober et al., 2014; Domene et al., 2014) and, interestingly, in some of these studies, the observations from laboratory trials were not confirmed in the field (Tammeorg et al., 2014; Gruss et al., 2019). This could be the consequence of having a greater number of interacting factors (both abiotic and biotic) and a more complex soil foodweb under more natural conditions. Additionally, a longer investigated period, compared to the laboratory trials, might have resulted in changes in physical and chemical characteristics of the biochar and/or the degradation of the potential contaminants released from biochar long after their application to the field, and therefore, its effects on soil biota.

Several factors have been proposed to explain the variety of observed biological responses of soil organisms to biochar: (i) the palatability and nutrition value of biochar is low (Salem et al., 2013), but it can be a source of energy (Ameloot et al., 2013); (ii) the porous nature of biochar can serve as a habitat for microorganisms, and in turn for microbial grazers, but the type of microorganisms that are enhanced (e.g. bacteria *versus* fungi) will dictate the microbivorous organisms that will benefit; (iii) the pollutant content of biochar (e.g. heavy metals and polycyclic aromatic hydrocarbons) could harm soil organisms (e.g. Elliston and Oliver, 2019), although concentrations do not usually reach the threshold levels indicated in relevant guidelines (Domene, 2016); (iv) biochar changes abiotic environmental conditions (pH, water availability), which could be beneficial for some organisms but not for others (McCormack et al., 2013).

Biochar palatability by decomposers is strongly dependent on its physical and chemical structure, which can vary depending on how the biochar is produced. Differing pyrolysis conditions and temperatures, even when used with the same starting biomass material, can result in a range of differing physico-chemical properties. For example, slow pyrolysis reduces the labile content of biochar and increases aromacity when compared to fast pyrolysis (Brewer et al., 2011). Similarly, higher pyrolysis temperatures (>350 °C) can also change the biochar elemental composition, with decreases in the O/C and H/C ratios, and aromaticity (Mimmo et al., 2014). From this, it may be expected that low-temperature and fast pyrolysis biochars would be preferred by soil organisms. However, Li et al. (2011) found that the earthworm *Eisenia fetida* avoided a slow pyrolysis wood biochar at ratios of 10% (w/w) and above, and Elmer et al. (2015) found that *Lumbricus terrestris* disliked fast-pyrolysis biochar made from hardwood sawdust, emphasising that the actual mechanisms of how these parameters influence soil biota responses remain unclear.

We performed a 2-year field study to investigate, under field conditions, the biological effects of adding biochar made from *Miscanthus* feedstock to a *Miscanthus* bioenergy crop. We assessed the effects of three different biochar addition rates (10 t ha^{-1}, 25 t ha^{-1} and 50 t ha^{-1}) on soil invertebrate community structure and abundances, including mesofauna (enchytraeids, collembolans, and mites) and macrofauna (earthworms). In addition, we used stable isotope analyses to reveal changes in feeding preferences and C assimilation by those soil faunal groups that directly ingest soil organic matter (e.g. earthworms and enchytraeids), as previous studies indicated that they are more likely to be affected by pyrolised C (McCormack et al., 2013). We applied combined ^{13}C and ^{15}N isotope analysis since it has been proved to be a powerful tool for tracing dietary changes ($\delta^{13}C$) and investigating ecological groupings ($\delta^{15}N$) in earthworm communities from agricultural soils (reviewed by Briones and Schmidt, 2004).

Previous studies at the same site have revealed that *Miscanthus* plantations provide a better habitat for bacterial grazers and a more functionally diverse earthworm community than other bioenergy crops such as Short Rotation Coppice (SRC) willow (Briones et al., 2019). We therefore hypothesised that biochar additions will promote enchytraeids and earthworm populations compared to those faunal groups that are fungal driven (e.g. collembolans). In addition, since earthworm ecological groupings are a direct reflection of their preferential diets, we also anticipated that endogeic worms feeding on more humified substrates would benefit from greater amounts of pyrolised carbon present in the soil.

2. Materials and methods

2.1. Site description

The field site used for this study was a commercial plantation of *Miscanthus giganteus* (11.56 ha) located in Lincolnshire, UK (53.318741, -0.590814). Prior to planting with *Miscanthus* in 2006, the field had followed a 1-year rotation of oilseed rape (*Brassica napus*) and 3 years winter wheat (*Triticum aestivum*). The soil was a fine loam over clay (59% sand, 36% silt and 15% clay; Robertson et al., 2017). The top 30 cm of soil had a mean total C and N concentration of 1.86% and 0.18%, respectively, with a soil pH ranging from 6.8 to 7.3. The soil bulk density before the experiment establishment was very high (1.67) because of compaction caused by long-term agricultural vehicle usage. The *Miscanthus* perennial crop was managed by spring harvest (March-April) and no fertilization. Meteorological data obtained from the nearest weather station (RAF Scampton, Lincoln; 53° 18′ 1″N, 0° 32′ 30″W) showed a mean annual minimum and maximum temperatures of 5.7 °C and 13 °C respectively and a mean annual rainfall of 613 mm (1981–2010). Further site details can be found in Robertson et al. (2017).

2.2. Biochar preparation and experimental set-up

Biochar was produced by slow pyrolysis from *Miscanthus* biomass by BTG Biomass Technology Group B.V. (Enschede, The Netherlands). The *Miscanthus* derived from a local farm in the Groeningen province, The Netherlands. Around 6 tons of chipped biomass (12.8% of moisture in average) was converted to around 2.3 tons of biochar in 17 runs, with an average yield of 35.6±7.5% (mean±SD, n=17) on dry weight (dw) basis. The pyrolysis unit used consisted of a screw reactor in which the biomass was subjected to a temperature of approximately 450 °C by means of hot combustion gases (~700 °C) mixed with air for an average time of 22.6±3 minutes. The resulting biochar had a total C content of 66.04±1.14% (n = 3), a total N content of 0.23±0.15% (n = 3), and an isotopic composition of $\delta^{13}C$ = -12.38±0.036‰ and $\delta^{15}N$ = 3.68±0.36‰. Organic elemental analysis of the biochar was performed by dynamic flash combustion (modified Dumas method) of the sample with a Flash 2000 analyzer (Thermo Fisher Scientific Inc.) set to a CNH configuration.

Four random sampling blocks were established within the *Miscanthus* field in May 2010, one month after harvest. In each block, four square plots of 2 m x 2 m, at least 5 m apart, were randomly assigned to one control treatment (i.e. no biochar added, CTRL), and to three amended treatments, where biochar was applied at a rate of either 10 t ha^{-1} (b10), 25 t ha^{-1} (b25) or 50 t ha^{-1} (b50) between the *Miscanthus* rows. Biochar was incorporated to an approximate depth of 10 cm with the help of a hoe and a tiller (Power digger, HSS Hire, UK) resulting in a mixing ratio of 0.6, 1.5 and 3% for b10, b25 and b50, respectively. This mixing ratio was calculated by weight, based on the application rate, the initial soil bulk density of 1.67 g/cm^3 and a 10 cm depth of application. Each plot (including the control plots) was ploughed twice, initially before and then after biochar application to the soil surface. The biochar was tilled into the soil, carefully avoiding the *Miscanthus* rhizomes and control plots without added biochar were tilled in the same way as for the biochar plots. *Miscanthus* straw and litter fall present on the ground were removed before tilling and repositioned after biochar incorporation into the soil.

2.3. Soil sampling and faunal extractions

Measurements were taken in September 2011 and October 2012 (i.e. 4 and 17 months after biochar was added to the soil). Litter and soil samples (taken to 10 cm depth) were analysed for isotopic composition (^{13}C and ^{15}N).

On both sampling occasions, soil macrofauna (earthworms) was collected by excavating one quadrat (50 cm x 50 cm x 10 cm deep) at three blocks, whilst two soil cores (PVC cylinders 10 cm Ø x 3 cm deep) at each of the treatments in the four blocks were sampled for extraction of soil mesofauna (one core for enchytraeids and one for microarthropods). Earthworms were hand-sorted in the field, whereas soil cores were taken to the laboratory to perform the faunal extractions: wet funnel extraction (O'Connor, 1955) in the case of enchytraeids, and the standard Tullgren method (Tullgren, 1918) in the case of microarthropods. Mites and collembolans were fixed in 70% ethanol and identified to order level, whereas oligochaetes were collected alive, washed with deionised water to remove any surface soil particles, and sorted by species (earthworms), and family (enchytraeids). After identification, earthworm species were assigned to ecological groupings (Bouché, 1977): i) epigeic worms living in the litter layers and feeding on fresh organic matter; ii) anecic worms building permanent or semi-permanent vertical burrows to feed on the organic layers at night; iii) endogeic earthworms inhabiting the mineral layers and feeding on more humified food sources.

Clean tissue samples of every earthworm species (after removing the gut by dissection) and all enchytraeids collected per replicate were then frozen at -20 °C for at least 24 h prior to freeze-drying and then weighed using a microbalance to determine their dry weight.

2.4. Isotopic analyses and calculations

Biomass C and N content as well as $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of soil and fauna samples were determined by continuous flow-combustion-isotope ratio mass spectrometry (CF-C-IRMS) using an elemental analyser (EA, Flash 2000, Thermo Scientific) coupled with a Continuous Flow-Isotope Ratio Mass Spectrometer (CF-IRMS, Delta V Advantage, Thermo Scientific) at the Stable Isotope Facility of the Free University of Bolzano (Italy). Analytical precision of < 0.2‰ $\delta^{13}C$ and 0.2‰ $\delta^{15}N$ was obtained.

The isotopic values are expressed as δ values:

$$\delta^{H}\mathrm{X} = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000$$

where R_{sample} is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in each sample. International Reference Standards were Vienna Pee Dee Belemnite (VPDB) for C ($R_{standard}$ = 0.011180) and Air (AIR) for N ($R_{standard}$ = 0.0036765).

The biochar derived from *Miscanthus* had a $\delta^{13}C$ value of -12.38‰, very different to that of the soil (-26.11‰), but not statistically distinguishable from the C isotopic signature of the *Miscanthus* litter (-12.04‰). Therefore, we used isotopic data derived from a previous study located adjacent to the current study site for reference soil, containing a Short Rotation Coppice (SRC) willow plantation, which had never grown *Miscanthu*s nor received biochar (Briones et al., 2019). From this, the fractional contribution (F) of plant/biochar C_4-derived C which had been incorporated into the worm tissues was estimated (after converting all $\delta^{13}C$ results to atom% values) using a two source mixing model (after Balesdent and Mariotti, 1996):

$$\mathrm{F\ (atom\%)} = ({}^{13}\mathrm{C}_{\mathrm{atom\%SAMPLE}} - {}^{13}\mathrm{C}_{\mathrm{atom\%SAMPLE\ SOIL\ REF}}) / ({}^{13}\mathrm{C}_{\mathrm{atom\%C4}} - {}^{13}\mathrm{C}_{\mathrm{atom\%SOIL\ REF}})$$

where ${}^{13}C$ atom%$_{SAMPLE}$ is the atom% value of the biological sample collected from the treatment plots, ${}^{13}C$ atom%$_{SAMPLE\ SOIL\ REF}$ is the atom% of the biological sample collected from the reference soil (see above), ${}^{13}C_{C4}$ is the atom% value of the C_4 food sources (*Miscanthus* litter), ${}^{13}C_{SOIL\ REF}$ is the atom% value of the reference soil (SRC willow soil; Briones et al., 2019). Because enchytraeids had not been measured in this previous study, and earlier work (Ostle et al., 2007) has shown that these oligochaetes show similar ${}^{13}C$ fractionation from basal food resources, we also used the earthworm values from the SRC willow plantation as our reference values for the enchytraeids.

Thereafter, the total new C assimilated into each group per square meter was calculated as following:

$$\mathrm{new\ C_4{-}derived\ assimilated\ C = F\ (atom\%)\ x\ biomass\ C\ (mg\ C\ per\ square\ meter)}$$

where biomass C was the dry weight of the animal tissue per area (mg m^{-2}) x % C in animal tissue.

2.5. Statistical analyses

Abundance data for soil invertebrates are expressed as numbers per square meter and have been log transformed (log10 (x+1)) to meet normality and homoscedasticity criteria for further statistical comparison. Isotopic data (delta values) were transformed to atom% values prior to statistical analyses.

Since all the measurements were taken on the same experimental plots over time, all data was analysed using linear mixed models (LMMs) with repeated effects (proc MIXED, SAS/STAT® Software, 2011). For an experiment with blocks, treatments and measurements over time, the repeated statement included the variable time (sampling year) and the experimental units which had been measured repeatedly (treatments or TREAT: biochar plots) and randomized within a block.

Significant effects of treatments, sampling years or the interaction between these two factors on faunal abundances/biomass of invertebrates gross groups, earthworm species and ecological groupings, as well as on atom% and new C assimilated values were further explored using LS-means within each level of each fixed factor.

3. Results

3.1. Effects of biochar amendments on soil fauna community composition and structure

Adding biochar to the soils significantly (TREAT: $p < 0.05$; see Table 1) altered the total abundances of invertebrates, but the responses differed between macro- and meso-fauna (Fig. 1). Thus, while earthworms were negatively affected by the presence of biochar in the soil (and proportionally to the application rate), enchytraeids and microarthropods appeared to benefit from the presence of the biochar. Indeed, both mesofauna groups increased their population numbers with higher concentrations of biochar, but in the case of microarthropods their populations peaked when the dosage application was 25 t ha^{-1}, whereas for enchytraeids the highest dose applied (50 t ha^{-1}) resulted in the highest increase in animal numbers. The sampling year also had a significant effect on total earthworm and microarthropod numbers (Table 1), with earthworm populations doubling those found in the previous year and microarthropods drastically reducing their densities by a factor of four when compared to the densities recorded in 2011 (Figs. 2 and 3). Despite this temporal effect, the responses to the treatments were consistent over time, since no significant effect of the interaction between treatment and year on animal abundances was observed.

Not only did the relative abundance of the smaller-sized organisms increase in the soil invertebrate communities with increasing biochar additions, but the community structure of both microarthropods and earthworms also exhibited significant changes. In the case of microarthropods, the statistical analyses of the most abundant groups (i.e. Collembola, Diptera, Mesostigmata, Oribatida and

Prostigmata) resulted in both treatment and sampling year having a significant effect as well as the interaction between these two factors (Table 2). Although biochar additions increased the average abundances of each of the investigated groups on both sampled years, the treatment effect only became evident in the second year, when populations of collembolans and oribatid mites became significantly greater in the b25 treatment than in the control (Fig. 2). Furthermore, on this sampling occasion, the population densities of oribatid mites reached their maximum values in the b50 treatment (Fig. 2).

In the case of the earthworms, no significant biochar effect was observed on their functional groupings structure (Table 2) and their communities were dominated by endogeic species (representing up to 77% of the total abundance) in all treatments across both sampling years. Increasing biochar application rates had negative effects on all three ecological groupings, but epigeics and anecics were the least affected (Fig. 3a). Similarly, biochar treatment did not appear to have a significant influence on earthworm species composition (Table 2); however, species richness tended to decrease with increasing biochar application rate ($p = 0.0321$). Up to 6 species were identified in control and b10 plots in both sampled years (in the case of the b25 only in 2012), whereas 4 and 5 species were recorded in the b50 plot in 2011 and 2012, respectively (Fig. 3b). Interestingly, *L. terrestris* was absent from the control plots and *L. rubellus* from those with the highest addition of biochar (b50).

3.2. Contribution of dietary C_4 sources in oligochaetes

No significant treatment effect on the proportion of new C (C_4 sources) uptake nor in the total amounts being assimilated by enchytraeids were observed (Table 3). However, all enchytraeids samples were isotopically enriched compared to the background soil, indicating that they were preferentially assimilating litter-derived C or root exudates from *Miscanthus*, rather than previous older C sources (Fig. 4a). In addition, there was a significant year effect (Table 3) and less new carbon was assimilated by these small oligochaetes in 2011 than in 2012, especially in the control and the b50 treatments (Fig. 4b).

The amount of new C present in the earthworm tissues showed significant variations among the three ecological groupings depending on how much biochar was added to the soil (Table 3; Fig. 5a). Interestingly, the lowest preference for C_4 sources was exhibited by the anecic worms collected from the b25 treatment (Fig. 5a), although with a wide variation in their C isotopic values. This was the result of *Lumbricus terrestris* exhibiting the lowest isotopic enrichment ($\delta^{13}C$ = -19.95‰), when compared to the other anecic species (*Aporrectodea longa,* $\delta^{13}C$ = -13.58; Supplementary Table 1). The mass balance calculations confirmed that anecic earthworms showed the greatest incorporation of the new C in the b50 treatment (≈ 0.97 mg C per m^{-2}; Fig. 5b), whereas endogeic worms showed the lowest assimilation values, but with the highest values also being measured in the b50 treatment (≈ 0.82 mg C per m^{-2}; Fig. 5b). In the case of epigeics, their low abundances at the study site did not allow for a robust statistical comparison, but the available data suggest that they were assimilating similar amounts of new C across all investigated treatments (ranging between 0.77 and 0.99 mg C per m^{-2}; Fig. 5b). Similar to enchytraeids, earthworm assimilation was also significantly affected by sampled year (Table 3) and less new C was assimilated by all three ecological groupings in 2011 than in 2012 (on average 0.72 mg C per m^{-2} *versus* 0.85 mg C per m^{-2}, respectively).

Furthermore, although $\delta^{15}N$ isotopic ratios clearly reflected the different feeding strategies (i.e. lowest values for the litter feeders, such as epigeic worms, and the highest values for those species feeding on more humified sources, such as the endogeic worms), they also showed a wide variation across treatments, spanning nearly 5 delta units (Fig. 5a). In particular, epigeic species ranged from low isotopic values of 2.2 measured in the b50 treatment (very close to those values measured in the *Miscanthus* biochar/litter), to 4.8 in the ones collected in the b25 treatment, being more similar to those of anecic worms (Fig. 5a; see also Supplementary Table 1).

Discussion

Collembolans and mites are the most abundant microarthropod groups in agricultural soils (Behan-Pelletier, 2003; Coleman and Wall, 2014) and, in this study, these two groups were less negatively affected by increased biochar additions than earthworms. Several studies have reported the consumption of biochar by collembolans in laboratory incubations (Hale et al., 2013; Domene et al., 2015), although the evidence provided suggest that this group mainly feed on the fungi colonising the biochar particles rather than on the biochar itself (Lehmann et al., 2011). This has led Domene et al. (2015) to conclude that microorganisms play an important role in biochar consumption by collembolans. In support of this, it has been shown that the presence of biochar increased microbial biomass and that the soil microbial community composition shifted to higher fungal-to-bacterial ratios (Bamminger et al., 2014; Gómez et al., 2014; Paz-Ferreiro et al., 2015; McCormack et al., 2019). This is the result of a preferential advantage for fungi in the degradation of lignin (Lehmann et al., 2011), a plant-derived polymer whose content increases during pyrolysis (Mimmo et al., 2014). The affinity of collembolans for porous structures of char-like materials is supported by the fact that they are usually cultured in a mixture of plaster of Paris and activated charcoal (OECD, 2009; ISO 11267:2014). The enhanced porosity of such carbonised materials retains water, removing staling products and providing the high humidity conditions, which are essential for Collembola growth. Furthermore, the high internal surface area of biochar and its ability to adsorb soluble organic matter, gases and inorganic nutrients could provide a suitable habitat for microbes to colonise (Thies et al., 2015). However, other studies have not found a positive link between biochar-induced microbial increases and collembolan numbers and, for this reason, other factors such as soil pH and gut symbionts have been suggested as potential explanations for the lack of negative effects of biochar on collembolans (Domene et al., 2015).

The effects of biochar additions on soil mites has been much less investigated, probably because they are not model organisms in standard toxicity tests, unlike nematodes, collembolans, and enchytraeids. However, laboratory studies suggest either avoidance (Godfrey et al., 2014), negative effects (Ohsowski et al., 2015), or no effect (McCormack et al., 2019) of biochar on this group. In this study, the positive effects of biochar on mite abundances were mainly associated to the increases of Oribatid mites, which coincides with other field studies performed by Gruss et al. (2019) who suggested that biochar addition improved soil physicochemical properties, by increasing soil C, soil pH, CEC and water content. This group of mites have been defined as "choosy generalists" (Schneider and Maraun, 2005) and exhibit a great variety of fungal-based diets (e.g. Hubert et al., 2001) and hence, like collembolans, they could also have benefited from the microbes inhabiting biochar porous structure.

Enchytraeids are particularly abundant in C-rich soils (such as organic grasslands and peatlands), but also in no-tilled agricultural soils where they can reach high numbers and become the most dominant group of soil fauna (Davidson et al., 2002). Laboratory experiments have shown neither avoidance nor preference to biochar (Marks et al., 2014; Domene et al., 2015), although the passage of biochar particles through their gut has been reported (Domene et al., 2015) and Topolianz et al. (2006) found enchytraeid fecal pellets containing charcoal in tropical soils. In contrast to these short-term bioassays experiments using one culturable species (*Enchytraeus crypticus*), our field study clearly showed a positive stimulation of the population numbers with increased additions of biochar. The longer-term study might have allowed for greater microbial degradation of the biochar, reducing its particle size, and facilitating ingestion by these small worms; indeed, biochar particle size has been recently postulated as the main driver for soil biota responses (Prodana et al., 2019). However, the fact that C_4–derived C assimilation was similar between the biochar plots and the control plots suggests that biochar C may not be the main energy source for the growing population. Therefore, indirect effects through changes in the soil structure and physicochemical conditions might have played a more key role governing their responses to biochar treatments. More research involving these much less studied taxa needs to be performed to disentangle the interactive effects between abiotic factors and biochar additions.

A very different response to biochar was observed in the case of the large soil ingesters (earthworms), and they were the only group of invertebrates showing a negative response to all three biochar treatments. This finding contradicts previous studies indicating a positive interaction between earthworm activity and biochar (e.g. Topoliantz and Ponge, 2005; Elmer et al., 2015), but agrees with others who have reported avoidance responses (Tammeorg et al., 2014; Sanchez-Hernandez et al., 2019a). Active ingestion of biochar by earthworms have been widely reported both in the field and under laboratory conditions (Topoliantz and Ponge, 2003, 2005; Ponge et al., 2006; Elmer et al., 2015), leaving the question open of whether this ingestion is accidental or intentional. The presence of small biochar particles in their egested casts has led to the suggestion that earthworms are capable of grinding biochar in their gizzards (Topolizantz, 2002), but also they may use the biochar to help with the grinding of their selected food sources (Lehmann et al., 2011). In addition, a recent study (Sanchez-Hernandez et al., 2019a) has shown that the incubation of earthworms in biochar-amended soils led to a significant increase of digestive enzyme activity. In agreement with these observations, our isotopic results showed that earthworms were assimilating C_4 sources (including biochar), despite the negative effects on the abundances and diversity of their populations. This could suggest that only some earthworm species were able to get enough nutrition from these C sources to support population growth. Indeed, our results showed that the response to the biochar treatments varied according to the ecological group or even the species included in a particular grouping. This is also a reflection of the different feeding strategies exhibited by different earthworm species, which is one of the main criteria for their functional classification. Thus, epigeic and anecic species, that feed on fresh and less mineralised substrates (in terms of C:N ratios) deposited at the surface, assimilated more C_4-derived sources than the endogeics (living in the mineral layers). Furthermore, the two species included in the anecic group showed distinct responses, with *A. longa* showing a greater incorporation of C from C_4 sources in their tissues than *L. terrestris*. Oxidative stress in *Lumbricus terrestris* individuals exposed to biochar has been observed previously (Sanchez-Hernandez et al., 2019a), and broader feeding strategies have also been observed in the case of *A. longa* (Briones et al., 2005), which might explain these opposite responses. In support of this, Topoliantz and Ponge (2005) reported the endogeic *Pontoscolex corethrurus* having a preference for charcoal–soil mixtures over soil due to increased pH, but Tammeorg et al. (2014) observed avoidance by another endogeic species (*Aporrectodea caliginosa*) to a spruce biochar due a slight decrease in water availability. These findings highlight the need for more research on biochar's direct and indirect effects on individual species rather than on earthworms as a whole (and possibly for the other invertebrate groups investigated here).

Conclusions

Our findings partly confirm previous findings that pyrolysis products seem to have a negative effect on the soil fauna which directly ingest soil organic matter (e.g. earthworms and enchytraeids), whereas microbial feeders may indirectly benefit from the input of this organic substrate (McCormack et al., 2013). However, at least in our investigated systems, unlike earthworms, enchytraeids seem to benefit from biochar additions, although the driving factors behind these responses are not fully understood. Since biochar is not a uniform material (i.e. the physical and chemical properties vary depending on the feedstock used and the pyrolysis procedures; Theis et al., 2015), the interaction of specific biochars with the soil environment may be very different, and even the direction of biological responses. In addition, soil type and nutrient content have also been proposed as important factors influencing biological responses (Noguera et al., 2010; Paz-Ferreiro et al., 2015). Despite these uncertainties, the results from this field study clearly indicate that smaller-sized organisms (in particular, enchytraeids, collembolans and oribatid mites) were able to endure and even capitalise on biochar-induced changes in the soil environment, whereas earthworms experienced severe reductions in their population numbers and species richness. Due to the importance of all these soil organisms in soil processes, more information about the mechanisms driving these contrasting responses is needed, if we aim at increasing the benefits from using bioenergy crops, biochar and soil biodiversity in different soils. Recently, it has been suggested to use earthworms to activate biochar via extracellular enzymes (Sanchez-Hernandez et al., 2019b), which could represent a viable strategy to increase biochar acceptance by decomposers and be extended to other soil invertebrates capable to stimulate microbial activities (e.g. through grazing). Finally, we show that species identity and feeding strategies may also play an important role in the observed responses and therefore, they need to be considered before applying biochar to soils as a routine practice. More specifically, caution should be given to those intensively managed agricultural soils where soil communities may have already become less functionally diverse due to land management (Tsiafouli et al., 2015; Briones and Schmidt, 2017) and hence, less resilient to environmental changes.

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References

Amaro, A., 2013. Optimised Tools for Toxicity Assessment of Biochar-Amended Soils (Master dissertation). Departamento de Biologia, Universidade de Aveiro, Portugal.

Ameloot, N., Graber, E.R., Verheijen, F.G.A., De Neve, S., 2013. Interactions between biochar stability and soil organisms: review and research needs. European Journal of Soil Science 64, 379–390. https://doi.org/10.1111/ejss.12064

Anderson, C.R., Condron, L.M., Clough, T.J., Fiers, M., Stewart, A., Hill, R.A., Sherlock, R.R. 2011. Biochar induced soil microbial community change: Implications for biogeochemical cycling of carbon, nitrogen and phosphorus. Pedobiologia 54, 309–320. https://doi.org/10.1016/j.pedobi.2011.07.005

Azeem, M, Hayat, R., Hussain, Q., Ahmed, M., Pan, G.X., Tahir, M.I., Imran, M., Irfan, M., Mehmood-ul-Hassan, 2019. Biochar improves soil quality and N_2-fixation and reduces net ecosystem CO_2 exchange in a dryland legume-cereal cropping system. Soil and Tillage Research 186, 172–182. https://doi.org/10.1016/j.still.2018.10.007

Balesdent, J., Mariotti, A., 1996. Measurement of soil organic matter turnover using δ^{13}C natural abundance. In: Boutton, T.W., Yamasaki, S. (Eds.), Mass Spectrometry of Soils. Marcel Dekker, New York, pp. 83–111.

Bamminger, C., Zaiser, N., Zinsser, P., Lamers, M., Kammann, C., Marhan, S., 2014. Effects of biochar, earthworms, and litter addition on soil microbial activity and abundance in a temperate agricultural soil. Biology and Fertility of Soils 50, 1189–1200. https://doi.org/10.1007/s0037

Behan-Pelletier, V.M., 2003. Acari and Collembola biodiversity in Canadian agricultural soils. Canadian Journal of Soil Science 83, 279–288.

Brewer, C.E., Unger, R., Schmidt-Rohr, K., Brown, R.C., 2011. Criteria to select biochars for field studies based on biochar chemical properties. Bioenergy Research 4, 312–323. https://doi.org/10.1007/s12155-011-9133-7

Bouché, M.B., 1977. Strategies lombriciennes. In: Lohm, U. and Persson, T. (Eds.), Soil Organisms as Components of Ecosystems. Stockholm: NFR, Swedish Natural Science Research Council, pp. 122–132.

Briones, M.J.I., Schmidt, O., 2004. Stable isotope techniques in studies of the ecological diversity and functions of earthworm communities in agricultural soils. In: Pandalai, S. (Ed.), Recent Research Developments in Crop Science, vol. 1, Research Signpost: Trivandrum, pp. 11–26.

Briones, M.J.I., Schmidt, O., 2017. Conventional tillage decreases the abundance and biomass of earthworms and alters their community structure in a global meta-analysis. Global Change Biology 23, 4396–4419. https://doi.org/10.1111/gcb.13744

Briones, M.J.I., Garnett, M.H., Piearce, T.G., 2005. Earthworm ecological groupings based on ^{14}C analysis. Soil Biology and Biochemistry 37, 2145–2149. https://doi.org/10.1016/j.soilbio.2005.03.001.

Briones, M.J.I., Elias, D.M.O., Grant, H.K., McNamara, N.P. 2019. Plant identity control on soil food web structure and C transfers under perennial bioenergy plantations. Soil Biology and Biochemistry 138, 107603. https://doi.org/10.1016/j.soilbio.2019.107603

Case, S.D.C., McNamara, N.P., Reay, D.S., Whitaker, J., 2014. Can biochar reduce soil greenhouse gas emissions from a *Miscanthus* bioenergy crop? Global Change Biology Bioenergy 6, 76–89. https://doi.org/10.1111/gcbb.12052

Cayuela, M.L., van Zwieten, L., Singh, B.P., Jeffery, S., Roig, A., Sánchez-Monedero, M.A., 2014. Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. Agriculture, Ecosystems and Environment 191, 5–16. https://doi.org/10.1016/j.agee.2013.10.009

Coleman, D.C., Wall, D.H., 2014. Soil fauna: occurrence, biodiversity, and roles in ecosystem function. In: Paul, E.A. (Ed.), Soil Microbiology, Ecology, and Biochemistry, fourth ed. Academic Press, London, pp. 111-149. https://doi.org/10.1016/B978-0-12-415955-6.00005-0

Davidson, D.A., Bruneau, P.M.C., Grieve, I.C., Young, I.M., 2002. Impacts of fauna on an upland grassland soil as determined by micromorphological analysis. Applied Soil Ecology 20, 133–143.

Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., Zeng, G., Zhou, L., Zheng, B., 2016. Biochar to improve soil fertility. A review. Agronomy for Sustainable Development 36, 36. https://doi.org/10.1007/s13593-016-0372-z

Domene, X., 2016. A critical analysis of meso- and macrofauna effects following biochar supplementation. In: Ralebitso-Senior, K.R., Orr, C.H., (Eds.), Biochar Application - Essential Soil Microbial Ecology. Elsevier Inc., Amsterdam, pp. 268–292. http://dx.doi.org/10.1016/B978-0-12-803433-0.00011-4

Domene, X., Mattana, S., Hanley, K., Enders, A., Lehmann, J., 2014. Medium-term effects of corn biochar addition on soil biota activities and functions in a temperate soil cropped to corn. Soil Biology and Biochemistry 72, 152–162. https://doi.org/10.1016/j.soilbio.2014.01.035

Domene, X., Hanley, K., Enders, A., Lehmann, J., 2015. Short-term mesofauna responses to soil additions of corn stover biochar and the role of microbial biomass. Applied Soil Ecology 89, 10–17. https://doi.org/10.1016/j.apsoil.2014.12.005

Elliston, T., Oliver, I.W., 2019. Ecotoxicological assessments of biochar additions to soil employing earthworm species *Eisenia fetida* and *Lumbricus terrestris*. Environmental Science and Pollution Research 1–9. https://doi.org/10.1007/s11356-019-04542-2.

Elmer, W.H., Lattao, C.V., Pignatello, J.J., 2015. Active removal of biochar by earthworms (*Lumbricus terrestris*). Pedobiologia 58, 1–6. https://doi.org/10.1016/j.pedobi.2014.11.001

Fontodji, J.K., Mawussi, G., Nuto, Y., Kokou, K., 2009. Effects of charcoal production on soil biodiversity and soil physical and chemical properties in Togo, West Africa. International Journal of Biological and Chemical Sciences 3, 870–879. http://dx.doi.org/10.4314/ijbcs.v3i5.51051

Gaunt, J.L., Lehmann, J., 2008. Energy balance and emissions associated with biochar sequestration and pyrolysis bioenergy production. Environmental Science and Technology 42, 4152–4158. https://doi.org/10.1021/es071361i

Glaser, G., Lehr, V.-I.. 2019. Biochar effects on phosphorus availability in agricultural soils: A meta-analysis. Scientific Reports 9, art. 9338. https://doi.org/10.1038/s41598-019-45693-z

Godfrey, R., Thomas, S., Gaynor, K., 2014. Biochar and Soil Mites: Behavioural Responses Vary with Dosage and Feedstock. Centre for Global Change Science (CGCS), University of Toronto.

http://www.cgcs.utoronto.ca/Assets/CGCS+Digital+Assets/Robert+Godfrey.pdf (accessed 16.10.19).

Gómez, J.D., Denef, K., Stewart, C.E., Zheng, J., Cotrufo, M.F., 2014. Biochar addition rate influences soil microbial abundance and activity in temperate soils. European Journal of Soil Science 65, 28–39. https://doi.org/10.1111/ejss.12097

Gruss, I., Twardowski, J.P., Latawiec, A., Medyńska-Juraszek, A., Królczyk, J., 2019. Risk assessment of low-temperature biochar used as soil amendment on soil mesofauna. Environmental Science and Pollution Research 26, 18230. https://doi.org/10.1007/s11356-019-05153-7

Hale, S.E., Jensen, J., Jakob, L., Oleszczuk, P., Hartnik, T., Henriksen, T., Okkenhaug, G., Martinsen, V., Cornelissen, G., 2013. Short-term effect of the soil amendments activated carbon, biochar, and ferric oxyhydroxide on bacteria and invertebrates. Environmental Science and Technology 47, 8674–8683. https://doi.org/10.1021/es400917g

Hammond, J., Shackley, S., Sohi, S., Brownsort, P., 2011. Prospective life cycle carbon abatement for pyrolysis biochar systems in the UK. Energy Policy 39, 2646–2655. https://doi.org/10.1016/j.enpol.2011.02.033

Hubert, J., Zilova, M., Pekar, S., 2001. Feeding preferences and gut contents of three panphytophagous oribatid mites (Acari: Oribatida) European Journal of Soil Biology 37, 197–208. https://doi.org/10.1016/s1164-5563(01)01083-4.

International Organization for Standardization (2014) Soil quality — Inhibition of reproduction of Collembola (*Folsomia candida*) by soil contaminants. ISO 11267:2014

Jeffery, S., Abalos, D., Prodana, M., Bastos, A.C., van Groenigen, J.W., Hungate, B.A., Verheijen, F., 2017. Biochar boosts tropical but not temperate crop yields. Environmental Research Letters 12, 053001. https://doi.org/10.1088/1748-9326/aa67bd

Jeffery, S., Verheijen, F.G.A., Kammann, C., Abalos, D., 2016. Biochar effects on methane emissions from soils: A meta-analysis. Soil Biology and Biochemistry 101, 251–258. https://doi.org/10.1016/j.soilbio.2016.07.021

Katterer, T., Roobroeck, D., Andren, O., Kimutai, G., Karltun, E., Kirchmann, H., Nyberg, G., Vanlauwe, B., de Nowina, K.R., 2019. Biochar addition persistently increased soil fertility and yields in maize-soybean rotations over 10 years in sub-humid regions of Kenya. Field Crops Research 235, 18–26. https://doi.org/10.1016/j.fcr.2019.02.015

Laird, D.A., 2008. The charcoal Vision: A Win–Win–Win Scenario for Simultaneously Producing Bioenergy, Permanently Sequestering Carbon, while Improving Soil and Water Quality. Agronomy Journal 100, 178–181.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota — A review. Soil Biology and Biochemistry 43, 1812–1836. https://doi.org/10.1016/j.soilbio.2011.04.022

Li, D., Hockaday, W.C., Masiello, C.A., Alvarez, P.J.J., 2011. Earthworm avoidance of biochar can be mitigated by wetting. Soil Biology and Biochemistry 43, 1732–1737. https://doi.org/10.1016/j.soilbio.2011.04.019

Marks, E.A.N., Mattana, S., Alcaniz, J.M., Domene, X., 2014. Biochars provoke diverse soil mesofauna reproductive responses in laboratory bioassays. European Journal of Soil Biology 60, 104–111. https://doi.org/10.1016/j.ejsobi.2013.12.002

McCormack, S., Ostle, N.J., Bardgett, R., Hopkins, D.W., Vanbergen, A.J., 2013. Biochar in bioenergy cropping systems: impacts on soil faunal communities and linked ecosystem processes. Global Change Biology Bioenergy 5, 81–95. https://doi.org/10.1111/gcbb.12046

McCormack, S.A., Ostle, N., Bardgett, R.D., Hopkins, D.W., Pereira, M.G., Vanbergen, A.J., 2019. Soil biota, carbon cycling and crop plant biomass responses to biochar in a temperate mesocosm experiment. Plant Soil 440, 341–346. https://doi.org/10.1007/s11104-019-04062-5

Mimmo, T., Panzacchi, P., Baratieri, M., Davies, C.A., Tonon, G., 2014. Effect of pyrolysis temperature on miscanthus (*Miscanthus x giganteus*) biochar physical, chemical and functional properties. Biomass and Bioenergy 62, 149–157. https://doi.org/10.1016/j.biombioe.2014.01.004

Nagel, K., Hoilett, N.O., Mottaleb, M.A., Meziani, M.J., Wistrom, J., Bellamy, M., 2019. Physicochemical characteristics of biochars derived from corn, hardwood, *Miscanthus*, and horse manure biomasses. Communications in Soil Science and Plant Analysis 50, 987–1002. https://doi.org/10.1080/00103624.2019.1594881

Noguera, D., Rondón, M., Laossi, K.-R., Hoyos, V., Lavelle, P., Crux de Carvalho, M.H., Barot, S., 2010. Contrasted effect of biochar and earthworms on rice growth and resource allocation in different soils. Soil Biology and Biochemistry 42, 1017–1027. https://doi.org/10.1016/j.soilbio.2010.03.001

O'Connor, F.B., 1955. Extraction of enchytraeid worms from a coniferous forest soil. Nature 175, 815–816. https://doi.org/10.1038/175815b0

OECD, 2009. Guideline for testing of chemicals 232: Collembolan Reproduction Test in Soil.

Ohsowski, B.M., 2015. Restoring grasslands in Southern Ontario sandpits: plant and soil food web responses to arbuscular mycorrhizal fungal inoculum, biochar, and municipal compost. PhD Thesis. University of British Collumbia.

Omondi, M.O., Xia, X., Nahayo, A., Liu, X., Korai, P.K., Pan, G., 2016. Quantification of biochar effects on soil hydrological properties using meta-analysis of literature data. Geoderma 274, 28–34. https://doi.org/10.1016/j.geoderma.2016.03.029

Ostle, N., Briones, M.J.I., Ineson, P., Cole, L., Staddon, P., Sleep, D., 2007. Isotopic detection of recent photosynthate carbon flow into grassland rhizosphere fauna. Soil Biology and Biochemistry 39, 768–777. https://doi.org/10.1016/j.soilbio.2006.09.025

Paz-Ferreiro, J., Liang, C., Fu, S., Mendez, A., Gasco, G., 2015. The effect of biochar and its interaction with the earthworm *Pontoscolex corethrurus* on soil microbial community structure in tropical soils. PLoS ONE 10(4), e0124891. https://doi.org/10.1371/journal.pone.0124891

Ponge, J.F., Ballot, S.D., Rossi, J.P., Lavelle, P., Betsch, J.M., Gaucher, P., 2006. Ingestion of charcoal by the Amazonian earthworm *Pontoscolex corethrurus*: a potential for tropical soil fertility. Soil Biology and Biochemistry 38, 2008–2009. https://doi.org/10.1016/j.soilbio.2005.12.024

Prober, S.M., Stol, J., Piper, M., Gupta, V.V.S.R., Cunningham, S.A., 2014. Enhancing soil biophysical condition for climate for climate-resilient restoration in mesic woodlands. Ecological Engineering 71, 246–255. https://doi.org/10.1016/j.ecoleng.2014.07.019

Prodana, M., Silva, C., Gravato, C., Verheijen, F. G. A., Keizer, J.J., Soares, A.M.V.M., Loureiro, S., Bastos, A.C., 2019. Influence of biochar particle size on biota responses. Ecotoxicology and Environmental Safety 174, 120–128. https://doi.org/10.1016/j.ecoenv.2019.02.044

Roberts, K.G., Gloy, B.A., Joseph, S., Scott, N.R., Lehmann, J., 2010. Life cycle assessment of biochar systems: estimating the energetic, economic and climate change potential. Environmental Science and Technology 44, 827–833. https://doi.org/10.1021/es902266r

Robertson, A.D., Whitaker, J., Morrison, R., Davies, C.A., Smith, P., McNamara, N.P., 2017. A *Miscanthus* plantation can be carbon neutral without increasing soil carbon stocks. Global Change Biology Bioenergy 9, 645–661. https://doi.org/10.1111/gcbb.12397

Salem, M., Kohler, J., Rillig, M.C., 2013. Palatability of carbonized materials to Collembola. Applied Soil Ecology 64, 63–69. https://doi.org/10.1016/j.apsoil.2012.10.009

Sanchez-Hernandez, J.C., Ríos, J.M., Attademo, A.M., Malcevschi, A., Andrade Cares, X., 2019a. Assessing biochar impact on earthworms: implications for soil quality promotion. Journal of Hazardous Materials 366, 582–591. https://doi.org/10.1016/j.jhazmat.2018.12.032

Sanchez-Hernandez, J.C., Ro, K.S., Díaz, F.J., 2019b. Biochar and earthworms working in tandem: Research opportunities for soil bioremediation. Science of the Total Environment 688, 574–583. https://doi.org/10.1016/j.scitotenv.2019.06.212

SAS Institute Inc. 2011. Base SAS® 9.3 Procedures Guide. Cary, NC: SAS Institute Inc.

Schmidt, H.P., Anca-Couce, A., Hagemann, N., Werner, C., Gerten, D., Lucht, W., Kammann, C., 2019. Pyrogenic carbon capture and storage. Global Change Biology Bioenergy 11, 573–591. https://doi.org/10.1111/gcbb.12553

Schneider, K., Maraun, M., 2005. Feeding preferences among dark pigmented fungal (“Dematiacea”) indicate limited trophic niche differentiation of oribatid mites (Oribatida, Acari) Pedobiologia 49, 61–67. https://doi.org/10.1016/j.pedobi.2004.07.010

Sohi, S., Lopez-Capel, E., Krull, E., Bol, R., 2009. Biochar, climate change and soil: A review to guide future research. CSIRO Land and Water Science Report 05/09, 1-64. https://doi.org/10.4225/08/58597219a199a.

Streubel, J.D., Collins, H.P., Garcia-Perez, M., Tarara, J., Granatstein, D., Kruger, C.E., 2011. Influence of contrasting biochar types on five soils at increasing rates of application. Soil Science Society of America Journal 75, 1402–1413. https://doi.org/10.2136/sssaj2010.0325

Tammeorg, P., Parviainen, T., Nuutinen, V., Simojoki, A., Vaara, E., Helenius, J., 2014. Effects of biochar on earthworms in arable soil: avoidance test and field trial in boreal loamy sand. Agriculture, Ecosystem and Environment 191, 150–157. https://doi.org/10.1016/j.agee.2014.02.023

Thies, J.E., Rillig, M.C., Graber, E.R., 2015. Biochar effects on the abundance, activity and diversity of the soil biota. In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management: Science, Technology and Implementation, second ed. Earthscan Publications Ltd., London, UK. https://doi.org/10.4324/9780203762264

Topoliantz, S., 2002. Réponse fonctionnelle de la pédofaune à la mise en culture itinerante et permanente des sols du sud-ouest de la Guyane française (PhD Thesis). Museum National d’Histoire Naturelle, Paris.

Topoliantz, S., Ponge, J.F., 2003. Burrowing activity the geophagous earthworm *Pontoscolex corethrurus* (Oligochaeta: Glossoscolecidae) in the presence of charcoal. Applied Soil Ecology 23, 267–271. https://doi.org/10.1016/s0929-1393(03)00063-5

Topoliantz, S., Ponge, J.F., 2005. Charcoal consumption and casting activity by *Pontoscolex corethrurus* (Glossoscolecidae). Applied Soil Ecology 28, 217–224. https://doi.org/10.1016/j.apsoil.2004.08.003

Topoliantz, S., Ponge, J.F., Lavelle, P., 2006. Humus components and biogenic structures under tropical slash-and-burn agriculture. European Journal of Soil Science 57, 269–278. https://doi.org/10.1111/j.1365-2389.2005.00736.x

Tsiafouli, M.A., Thébault, E., Sgardelis, S.P., Ruiter, P.C., Putten, W.H., Birkhofer, K., Hemerik, L., de Vries, F.T., Bardgett, R.D., Brady, M.V., Bjornlund, L., Jørgensen, H.B., Christensen, S., D' Hertefeldt, T., Hotes, S., Gera Hol, W.H., Frouz, J., Liiri, M., Mortimer, S.R., Setälä, H., Tzanopoulos, J., Uteseny, K., Pižl, V., Stary, J., Wolters, W., Hedlund, K., 2015. Intensive agriculture reduces soil biodiversity across Europe. Global Change Biology 21, 973–985. https://doi.org/10.1111/gcb.12752.

Tullgren, A., 1918. Ein seh einfacher Ausleseapparat für terricole Tier-formen. Zeitschrift fur Angewandte Entomologie-Journal of Applied Entomology 4, 149–150.

Wang, J., Zhang, M., Xiong, Z., Liu, P., Pan, G., 2011. Effects of biochar addition on N_2O and CO_2 emissions from two paddy soils. Biology and Fertility of Soils 47, 887–896. https://doi.org/10.1007/s00374-011-0595-8

Weyers, S.L., Spokas, K.A., 2011. Impact of biochar on earthworm populations: a review. Applied and Environmental Soil Science 2011, art. 541592. http://dx.doi.org/10.1155/2011/541592

Winsley, P., 2007. Biochar and bioenergy production for climate change mitigation. New Zealand Science Review 64, 5–10.

Zhang, M., Riaz, M., Zhang, L., El-desouk, Z., Jiang, C., 2019. Biochar induces changes to basic soil properties and bacterial communities of different soils to varying degrees at 25 mm rainfall: more effective on acidic soils. Frontiers in Microbiology 10, 1321. https://doi.org/10.3389/fmicb.2019.01321

Zhang, X.-K., Li, Q., Liang, W.-J., Zhang, M., Bao, X.-L., Xie, Z.-B., 2013. Soil nematode response to biochar addition in a Chinese wheat field. Pedosphere 23, 98–103. https://doi.org/10.1016/S1002-0160(12)60084-8

Table 1 Results from Repeated Measures of ANOVA on the effects of the four treatments (TREAT): no biochar added (CTRL) and biochar applied at a rate of either 10 t ha^{-1} (b10), 25 t ha^{-1} (b25) or 50 t ha^{-1} (b50)) and sampling year (YEAR) on soil invertebrate total abundances

Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Earthworms				
TREAT	3	14	4.62	0.0190
YEAR	1	14	31.34	<0.0001
TREAT*YEAR	3	14	0.81	0.5089
Enchytraeids				
TREAT	3	21	3.33	0.0392
YEAR	1	21	0.00	0.0947
TREAT*YEAR	3	21	0.67	0.5812
Microarthropods				
TREAT	3	21	3.28	0.0409
YEAR	1	21	40.63	<0.0001
TREAT*YEAR	3	21	0.70	0.5653

Table 2 Results from Repeated Measures of ANOVA on the effects of the four treatments (TREAT): no biochar added (CTRL) and biochar applied at a rate of either 10 t ha^{-1} (b10), 25 t ha^{-1} (b25) or 50 t ha^{-1} (b50)) and sampling year (YEAR) on soil invertebrate community structure (microarthropod orders-ORDER, earthworm ecological groupings-ECOL and individual species-SPECIES)

Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Microarthropods dominant groups				
TREAT	3	100	4.22	0.0075
ORDER	4	100	22.73	<.0001
YEAR	1	100	45.08	<.0001
TREAT*ORDER	12	100	0.55	0.8745
TREAT*YEAR	3	100	0.97	0.4118
TREAT*ORDER*YEAR	16	100	2.11	0.0132
Earthworm ecological groups				
TREAT	3	46	2.03	0.1224
ECOL	2	46	68.41	<.0001
YEAR	1	46	11.82	0.0013
TREAT*ECOL	6	46	1.46	0.2127
TREAT*YEAR	3	46	0.42	0.7374
TREAT*ECOL*YEAR	8	46	0.96	0.4753
Earthworm species				
TREAT	3	57	1.96	0.1310
SPECIES	7	57	7.29	<.0001
YEAR	1	57	15.82	0.0002
TREAT*SPECIES	19	57	0.49	0.9575
TREAT*YEAR	3	57	0.40	0.7519
TREAT*SPECIES*YEAR	18	57	0.97	0.5015

Table 3 Results of linear mixed effects models showing the effects of the four treatments (TREAT): no biochar added (CTRL) and biochar applied at a rate of either 10 t ha^{-1} (b10), 25 t ha^{-1} (b25) or 50 t ha^{-1} (b50)) and sampling year (YEAR) on the proportion of new C uptake [F (%atom)] and the total amount of new C assimilated (as mg C m^{-2}) by enchytraeids, earthworm species and ecological groupings

	new C uptake [F (%atom)]				**new C_4–derived C assimilated (mg C m^{-2})**			
	Num DF	**Den DF**	**F Value**	**Pr > F**	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Enchytraeids								
TREAT	3	9	1.15	0.3818	3	4	0.42	0.7501
YEAR	1	9	37.71	0.0002	1	4	80.33	0.0009
TREAT*YEAR	2	9	5.81	0.0240	2	4	45.78	0.0018
Earthworm ecological groupings								
TREAT	3	69	6.31	0.0008	3	69	10.06	<0.0001
ECOL	2	69	0.96	0.3888	2	69	0.94	0.3945
YEAR	1	69	5.81	0.0186	1	69	16.22	0.0001
TREAT*ECOL	6	69	5.27	0.0002	6	69	3.78	0.0026
TREAT*YEAR	3	69	2.19	0.0970	3	69	3.06	0.0339
TREAT*ECOL*YEAR	6	69	1.55	0.1751	6	69	1.22	0.3052
Earthworm species								
TREAT	3	47	3.23	0.0308	3	47	6.51	0.0009
SPECIES	7	47	3.9	0.0020	7	47	1.70	0.1318
YEAR	1	47	6.46	0.0144	1	47	13.79	0.0005
TREAT*SPECIES	18	47	2.8	0.0024	18	47	1.58	0.1042
TREAT*YEAR	3	47	1.59	0.2049	3	47	1.82	0.1566
TREAT*SPECIES*YEAR	16	47	1.54	0.1252	16	47	0.71	0.7697

Figure legends

Fig. 1. Earthworm (a), enchytraeid (b) and microarthropod (c) densities in soils (0–10 cm) under the control (CTRL) and the three biochar treatments: 10 t ha^{-1} (b10), 25 t ha^{-1} (b25) or 50 t ha^{-1} (b50). Values are means + standard errors (S.E.) and different letters indicate significant differences between treatments.

Fig. 2. Average compositional differences between microarthropod communities in the control (CTRL) and the three biochar treatments: 10 t ha^{-1} (b10), 25 t ha^{-1} (b25) or 50 t ha^{-1} (b50) at each investigated year. Taxonomic groups that are significantly different from the control are denoted with asterisks.

Fig. 3. Average compositional differences between earthworm communities (as abundances of each ecological grouping (a) and species, including total species richness (identified species) in brackets (b)) recorded in the control (CTRL) and the three biochar treatments: 10 t ha^{-1} (b10), 25 t ha^{-1} (b25) or 50 t ha^{-1} (b50) at each investigated year. Species abbreviations: *Allolobophora chlorotica* (Ah), *Aporrectodea caliginosa* (Ac), *Aporrectodea rosea* (Ar), *Aporrectodea longa* (Al), *Lumbricus terrestris* (Lt), *Lumbricus rubellus* (Lr), *Lumbricus castaneus* (Lc), *Lumbricus* sp. (Lsp).

Fig. 4. Dietary preferences of enchytraeids collected in soils (0–10 cm) under the control (CTRL) and the three biochar treatments (10 t ha^{-1} (b10), 25 t ha^{-1} (b25) or 50 t ha^{-1} (b50)): (a) natural abundance isotopic signatures (^{13}C and ^{15}N) of enchytraeids together with the potential food sources (*Miscanthus* soil (Msoil) and C$_4$ sources (biochar/*Miscanthus* litter); (b) total amount of C$_4$–derived C incorporated into their tissues, with different letters indicating significant differences between sampling years per each treatment (NE = LS-means could not be estimated due to missing data in one of the cells of the interaction). Values are means ± standard errors (S.E.).

Fig. 5. Dietary preferences of earthworm ecological groupings (epigeics – EPI, anecics – ANE, endogeics – END) collected in soils (0–10 cm) under the control (CTRL) and the three biochar treatments (10 t ha^{-1} (b10), 25 t ha^{-1} (b25) or 50 t ha^{-1} (b50)): (a) natural abundance isotopic signatures (^{13}C and ^{15}N) of

earthworm ecological groupings together with the potential food sources (*Miscanthus* soil (Msoil) and C_4 sources (biochar/*Miscanthus* litter); (b) the total amount of C_4–derived C incorporated into the earthworm tissues of each ecological grouping, with different letters indicating significant differences between ecological grouping per each treatment (NE = LS-means could not be estimated due to missing data in one of the cells of the interaction). Values are means ± standard errors (S.E.).

Figure 1

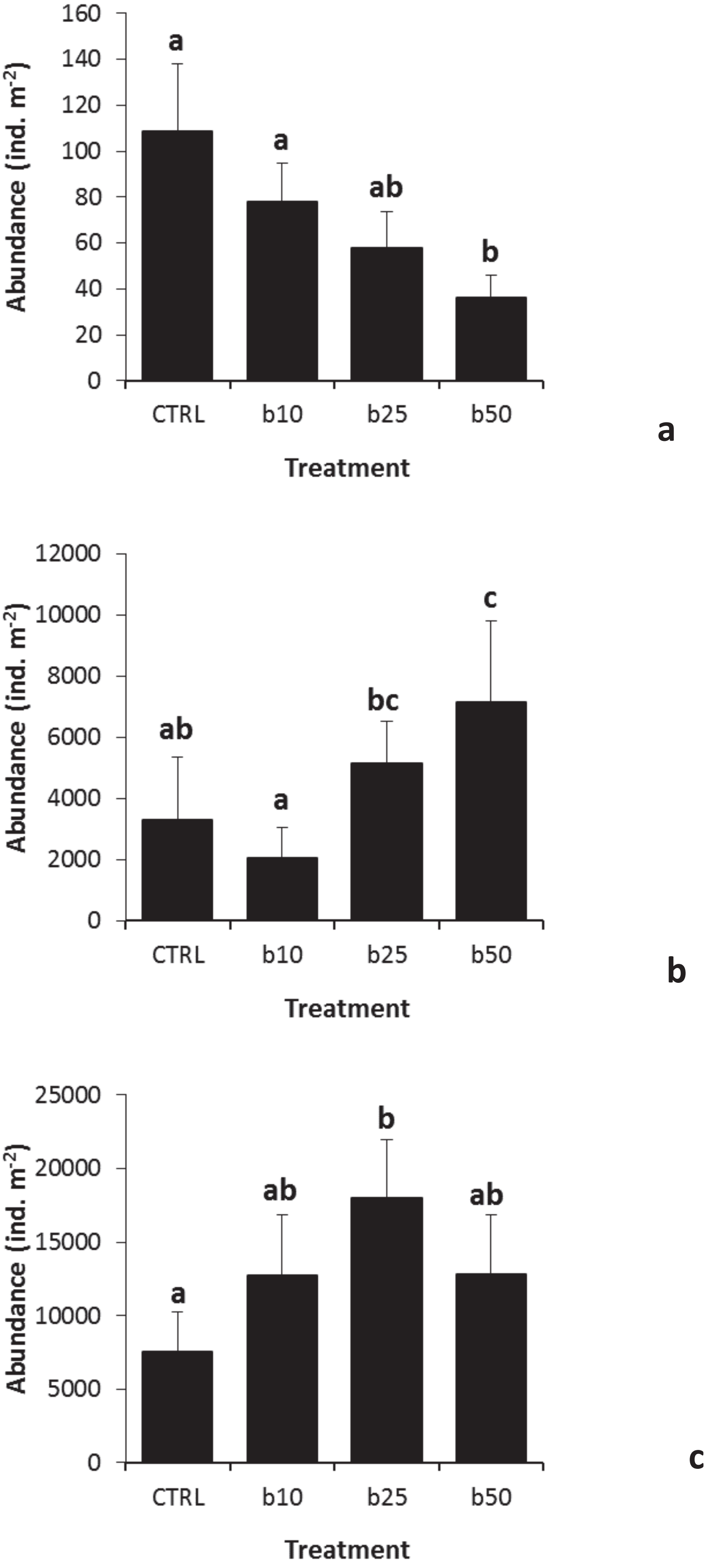

Abundance (ind. m^{-2})
160
140
120
100
80
60
40
20
0
a
a
ab
b
CTRL
b10
b25
b50
Treatment
a
Abundance (ind. m^{-2})
12000
10000
8000
6000
4000
2000
0
ab
a
bc
c
CTRL
b10
b25
b50
Treatment
b
Abundance (ind. m^{-2})
25000
20000
15000
10000
5000
0
a
ab
b
ab
CTRL
b10
b25
b50
Treatment
c

Figure 2

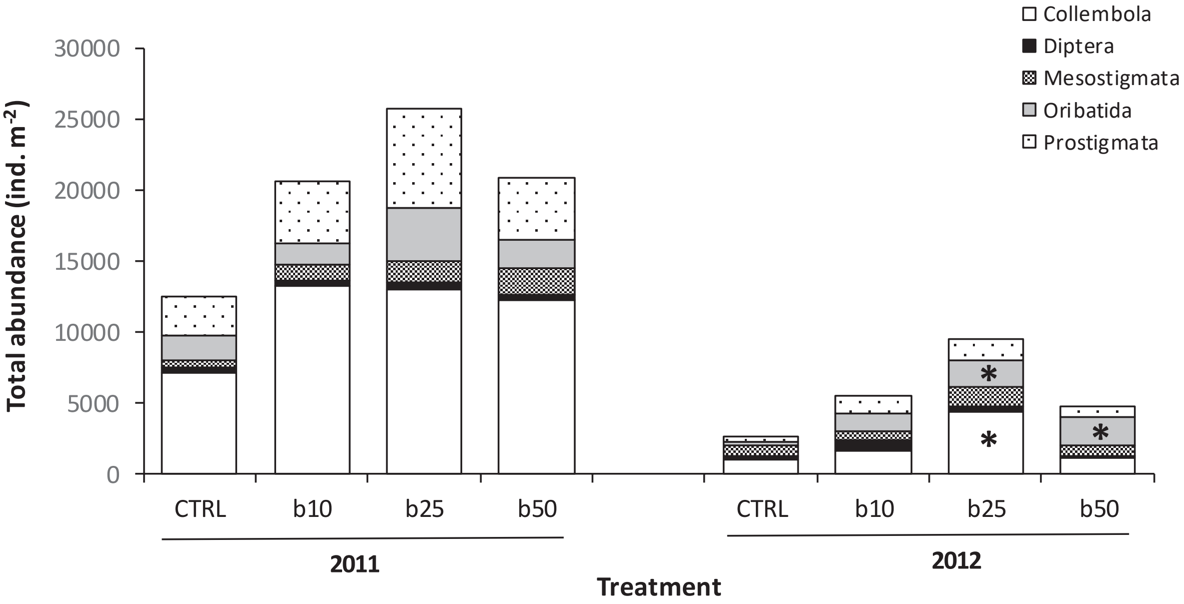
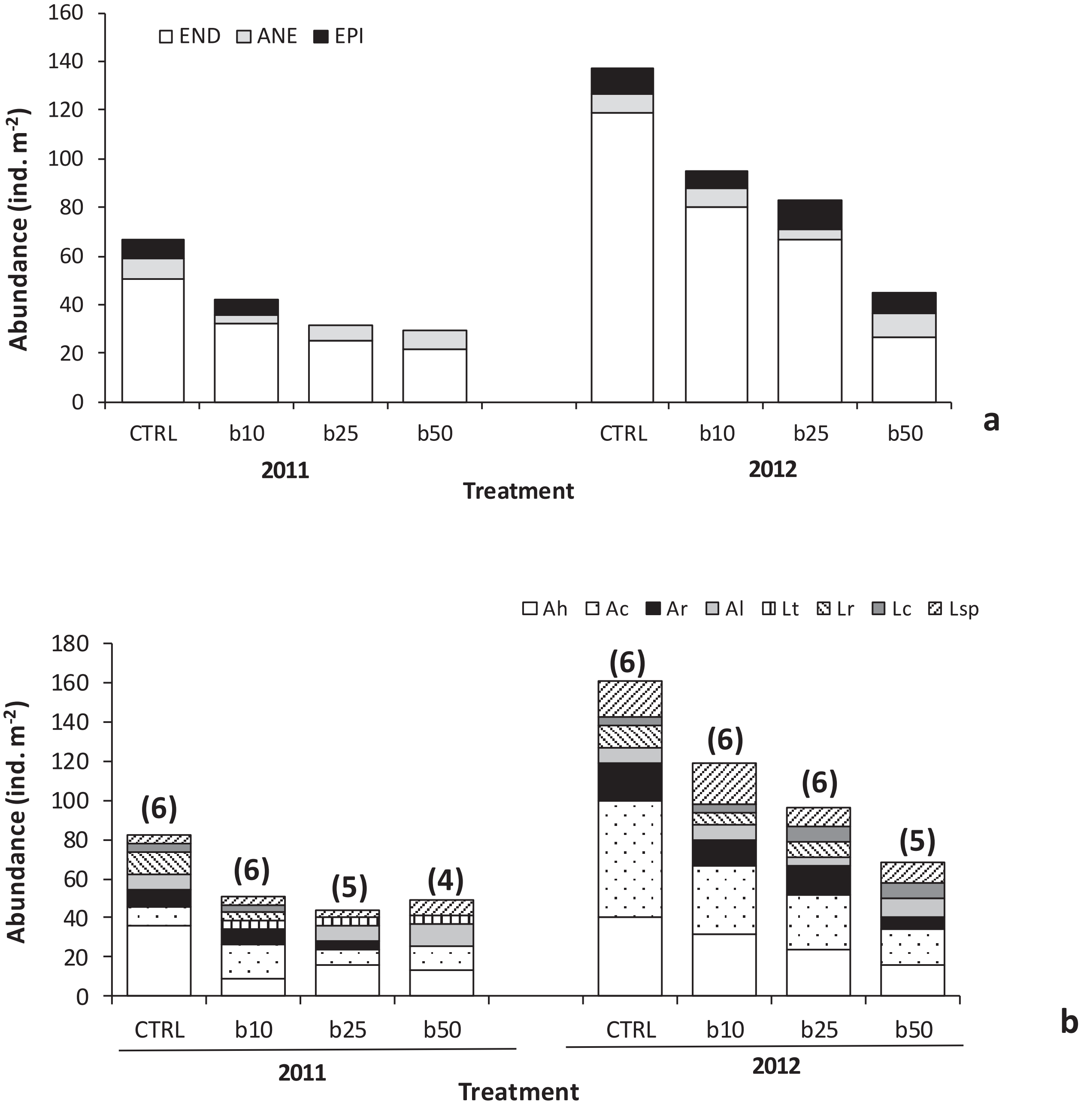
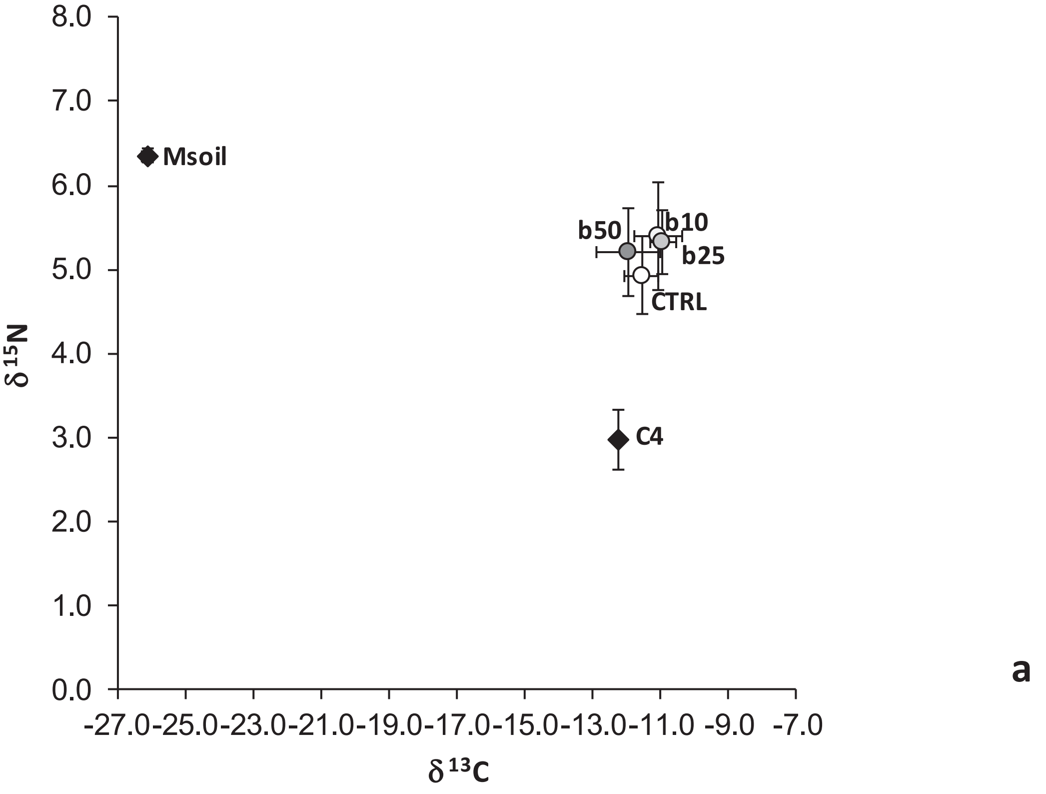

Collembola
Diptera
Mesostigmata
Oribatida
Prostigmata
Total abundance (ind. m-2)
30000
25000
20000
15000
10000
5000
0
*
*
*
CTRL
b10
b25
b50
CTRL
b10
b25
b50
2011
2012
Treatment

Figure 3



□ END ▨ ANE ■ EPI
Abundance (ind. m-2)
160
140
120
100
80
60
40
20
0
CTRL
b10
b25
b50
CTRL
b10
b25
b50
2011
2012
Treatment
a
□ Ah □ Ac ■ Ar ▨ Al ▥ Lt ▨ Lr ■ Lc ▨ Lsp
Abundance (ind. m-2)
180
160
140
120
100
80
60
40
20
0
(6)
(6)
(5)
(4)
(6)
(6)
(6)
(5)
CTRL
b10
b25
b50
CTRL
b10
b25
b50
2011
2012
Treatment
b

Figure 4


8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
δ 15N
Msoil
b50
b10
b25
CTRL
C4
-27.0
-25.0
-23.0
-21.0
-19.0
-17.0
-15.0
-13.0
-11.0
-9.0
-7.0
δ 13C
a

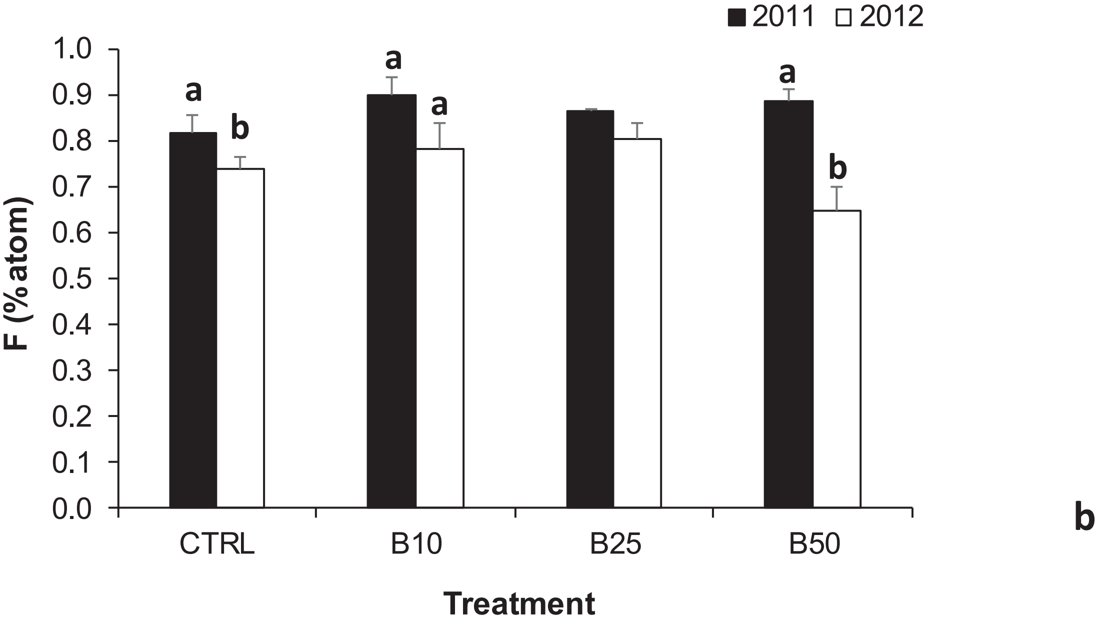
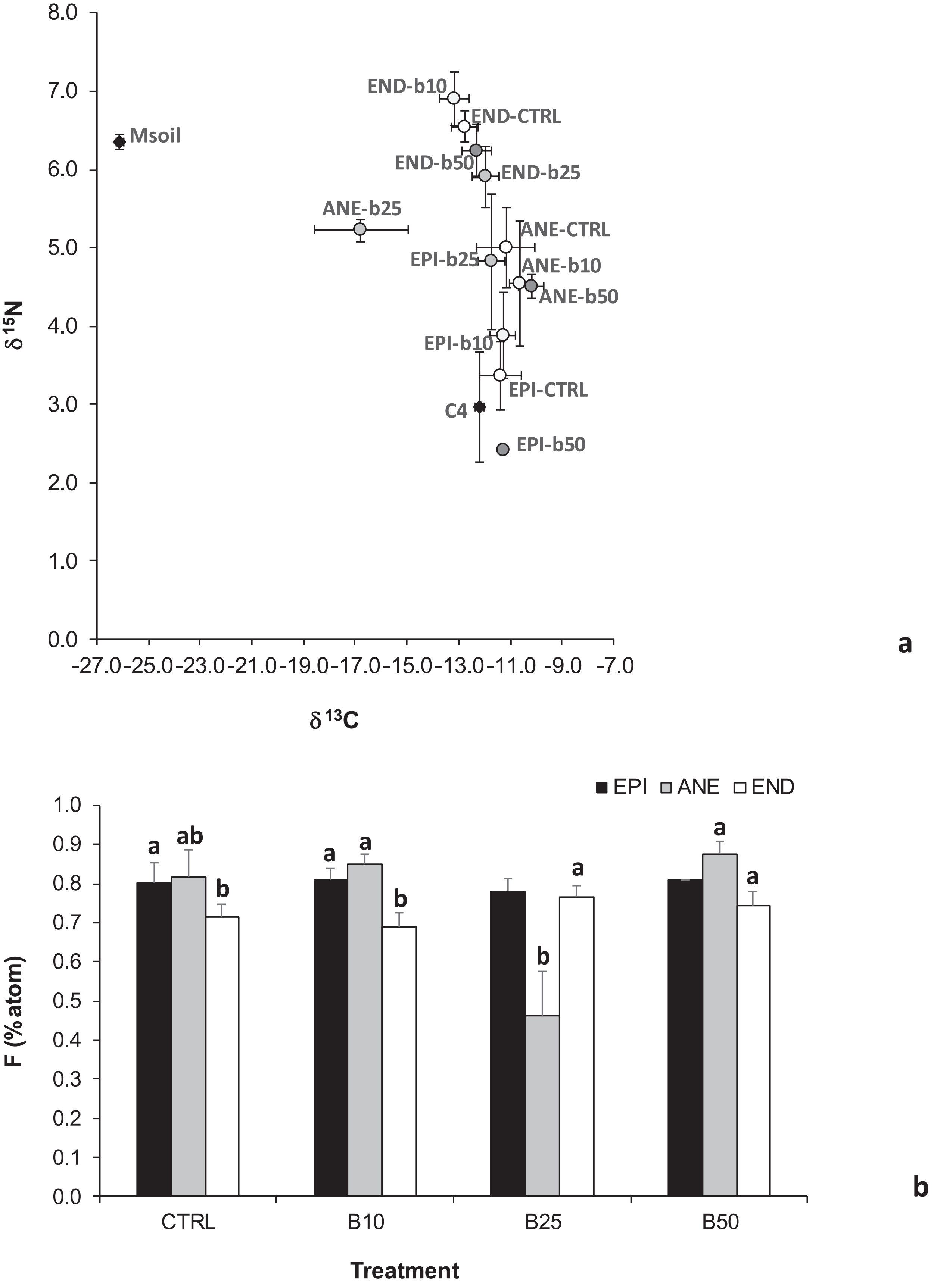

■ 2011 □ 2012
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
F (% atom)
a
b
a
a
a
b
CTRL
B10
B25
B50
Treatment
b

Figure 5


8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
δ 15N
-27.0
-25.0
-23.0
-21.0
-19.0
-17.0
-15.0
-13.0
-11.0
-9.0
-7.0
δ 13C
Msoil
END-b10
END-CTRL
END-b50
END-b25
ANE-b25
ANE-CTRL
EPI-b25
ANE-b10
ANE-b50
EPI-b10
EPI-CTRL
C4
EPI-b50
a
EPI
ANE
END
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
F (% atom)
a
ab
b
a
a
b
b
a
a
a
CTRL
B10
B25
B50
Treatment
b