Agriculture, Ecosystems and Environment 191 (2014) 150–157

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Effects of biochar on earthworms in arable soil: avoidance test and field trial in boreal loamy sand

Priit Tammeorg[a,*], Tuure Parviainen[a], Visa Nuutinen[b], Asko Simojoki[c], Elina Vaara[c,1], Juha Helenius[a]

[a] Department of Agricultural Sciences, P.O. Box 27, Latokartanonkaari 5, Plant Production Sciences, FIN-00014 University of Helsinki, Finland
[b] MTT Agrifood Research Finland, Plant Production, FIN-31600 Jokioinen, Finland
[c] Department of Food and Environmental Sciences, P.O. Box 27, Latokartanonkaari 11, Environmental Soil Science, FIN-00014 University of Helsinki, Finland

ARTICLE INFO

Article history:
Received 25 May 2013
Received in revised form 3 February 2014
Accepted 24 February 2014
Available online 25 March 2014

Keywords:
Aporrectodea caliginosa
Biochar
Earthworms
Field experiment
Lumbricus terrestris
Wheat

ABSTRACT

Biochar is widely studied as a combined soil conditioner in agriculture and a potential carbon sink. The knowledge of the effects of field application of biochar on soil fauna remains limited. Earthworms are a globally common and important faunal group in arable soils and the purpose of our study was to determine the effects of biochar on earthworms under both laboratory and field conditions in a boreal loamy sand. An avoidance test using the earthworm *Aporrectodea caliginosa* Sav. was conducted for periods of 2 and 14 days with 16 g kg^{-1} spruce chip biochar. The same biochar was mixed into the top 10 cm of soil at 0 or 30 t ha^{-1} and its effect on earthworm density and biomass was studied over four and half months in a field experiment where wheat was grown with or without inorganic fertilizer application. In the avoidance test, biochar application did not affect the habitat choice of earthworms in the first 2 d, but after 14 d, they tended to avoid it. The avoidance was possibly the avoidance was possibly caused by a slight decline in soil water potential. Under field conditions the highest earthworm densities and biomasses were measured in biochar amended soils. None of the differences among the treatments studied were, however, statistically significant ($p > 0.05$). The time scale of the study was sufficient for reliably demonstrating the lack of strong toxic effects and immediate avoidance reactions caused by biochar application

1. Introduction

Biochar use as a soil amendment has been proposed as a greenhouse gas mitigation strategy due to the high stability of carbon (C) in it (Cheng et al., 2008; Lehmann et al., 2008; Woolf et al., 2010). The need for evaluating the suitability of biochar technology has increasingly promoted research in this area. Furthermore, biochar may also benefit soil functions (Glaser et al., 2002; Lehmann et al., 2008; Major et al., 2010; Vaccari et al., 2011) and, depending on its properties and those of the soil, it may increase soil pH (Vaccari et al., 2011), improve plant nutrition through the nutrients it contains (Major et al., 2010; Xu et al., 2013) and reduce nutrient leaching (Brockhoff et al., 2010; Güereña et al., 2012; Major et al., 2012). These effects may contribute to enhanced abundance and activity of soil organisms (Liang et al., 2010; Lehmann et al., 2011; Güereña et al., 2012), increased yields of agricultural crops (Major et al., 2010; Vaccari et al., 2011; Zhang et al., 2012) and improved environmental quality (Lehmann et al., 2011; Major et al., 2012).

The effects of biochar on the soil biological community have received little attention, with the exception of the rather well established increase in microbial biomass under most conditions (Liang et al., 2010; Lehmann et al., 2011; Güereña et al., 2012). In particular, the effects of biochar on soil fauna have so far been only sporadically studied (Lehmann et al., 2011). This is a clear shortcoming considering the high potential of soil animals, particularly earthworms, for ingesting, modifying and transporting biochar in the pedosphere (Topoliantz and Ponge, 2003, 2005; Eckmeier et al., 2007) and subsequently influencing the microbial activity (Lehmann et al., 2011). Earthworms are globally common members of soil communities and have favorable influences on soil physical structure, litter decomposition (Lavelle, 1988; Blouin et al., 2013) and soil nutrient availability for plants (Lavelle et al., 1998; Chaoui

* Corresponding author. Tel.: +358 504 480 431; fax: +358 9 191 58582.
E-mail addresses: priit.tammeorg@helsinki.fi (P. Tammeorg), tuure.parviainen@helsinki.fi (T. Parviainen), visa.nuutinen@mtt.fi (V. Nuutinen), asko.simojoki@helsinki.fi (A. Simojoki), elina.m.vaara@gmail.com (E. Vaara), juha.helenius@helsinki.fi (J. Helenius).
[1] Present address: Palmenia, Saimaankatu 11, FIN-15140 Lahti, Finland

http://dx.doi.org/10.1016/j.agee.2014.02.023
0167-8809/

et al., 2003; Blouin et al., 2013). These functions make earthworms important organisms for assessing the effects of any substances added into soil, including biochar (Yeardley et al., 1996; Hund-Rinke and Wiechering, 2001; Busch et al., 2011; Li et al., 2011).

Favorable effects of biochar on earthworm behavior and activity have been attributed to decreased soil acidity (Topoliantz and Ponge, 2003, 2005; Van Zwieten et al., 2010; Busch et al., 2011) which could, when biochar particles are ingested and the pH in the gut of earthworms increased, assist earthworms in the assimilation of other resources (Weyers and Spokas, 2011). When earthworms ingest biochar particles containing high microbial biomass, it could contribute microbial enzymes to their digestive system (Topoliantz and Ponge, 2003). Negative responses to biochar by earthworms include avoidance and weight loss (Li et al., 2011) and decreased survival of *Eisenia fetida* Sav. (Liesch et al., 2010). These effects have been related to desiccation caused by high water retention of biochar (Li et al., 2011) or, in the case of poultry manure biochar, to the salinity, toxic effects of ammonia or to a rapid increase in soil pH (Liesch et al., 2010).

Previous experiments have not only given conflicting results on the effects of biochar on earthworms, but they have also been laboratory-based (Topoliantz and Ponge, 2003, 2005; Noguera et al., 2010; Van Zwieten et al., 2010; Busch et al., 2011; Li et al., 2011), and on earthworm species not common in agricultural soils (Van Zwieten et al., 2010; Busch et al., 2011; Gomez-Eyles et al., 2011; Li et al., 2011). Only two studies to our knowledge have been conducted under field conditions, neither of them comprised replicated treatments (Husk and Major, 2010; Weyers and Spokas, 2011).

Most field studies exploring the biochar-mediated changes in soil quality and plant growth have been conducted in (sub-) tropical (Major et al., 2010; Vaccari et al., 2011; Zhang et al., 2012) or temperate (Güereña et al., 2012; Jones et al., 2012) climates. Additional research is needed in colder climates, where soils are less affected by low contents of organic matter. For these reasons, this study explored the effects of spruce chip biochar on the earthworm species common in arable soils under both laboratory and boreal field conditions.

2. Materials and methods

2.1. Biochar

The biochar was produced by pyrolising chips of debarked spruce (*Picea abies* (L.) H. Karst.) in a continuously pressurized carboniser (Preseco Oy, Lempäälä, Finland) at 550–600 °C for 10–15 min. The biochar was cooled overnight in an airtight silo and then ground. The particle size distribution was determined by dry sieving. The ash content and the total elemental composition of the biochar were determined by dry ashing after Miller (1998). A 1.5 g sample was dry-ashed in a laboratory muffle furnace (Nabertherm Program Controller C19, Nabertherm, Lilienthal, Germany) by raising the temperature to 500 °C within 2 h and holding it there for 3 h. The ash was further transferred into an Erlenmeyer flask with 100 mL 0.2 M HCl, boiled for 30 min, transferred quantitatively into a 100-ml measurement flask, adjusted to the volume with deionized water, and finally filtered through a quantitative, ashless filter paper (Whatman, Grade 589/3, blue ribbon, pore size 2 μm, GE Healthcare, UK). Dilutions were carried out with 0.2 M HCl, if needed. The elemental concentrations of extracts were analyzed by an inductively coupled plasma optical emission spectroscopy (ICP-OES; Thermo-Fisher iCAP3600 MFC Duo, Thermo Fisher Scientific, Cambridge, UK). The total C and N contents of the biochar were determined by Dumas dry combustion with a VarioMax CN analyser (Elementar Analysensysteme GmbH, Hanau, Germany). The content of hydrogen (H) was determined by combustion with a CHN-1000 elemental analyser (LECO Corp. St. Joseph, MI, USA).

The volatile matter (VM) of the biochar was determined according to ASTM D3175-02 (2002), by heating the biochar in covered crucibles at 910 ± 30 °C for 7 min in the muffle furnace and determining the VM content as the weight lost from the sample. The pH of the biochar was measured in a 1:5 (w/w) suspension of biochar in deionized water with a combination pH electrode. The $CaCO_3$ liming equivalence of the biochar was determined by a method adapted after Rowell (1994). A 2.5 g sample of biochar was treated with 50 mL of 1 M HCl, for 2 h (including mild boiling for 45 min). Thereafter the solutions were cooled and titrated with 0.1 M NaOH.

The content of carbonate–C in the biochar was determined based on the gas chromatographic analysis of the carbon dioxide (CO_2) released by hydrochloric acid (HCl) as follows. First 1 g of biochar was accurately weighed and transferred into a 1205-mL glass bottle, and wetted with 5 mL of deionized water. Next, instantly after adding 20 mL of 1 M HCl into the bottle, the bottle was closed air-tight with a chlorobutyl septum. The bottles were kept for 3 d at 22 °C and gently shaken several times. A gas sample was taken from each bottle through the septum and the CO_2 evolved was determined by a gas chromatographic (HP 5890 Series II, Hewlett Packard, Palo Alto, CA, USA) method described in more detail by Penttilä et al. (2013). The amount of CO_2 evolved was calculated by multiplying the CO_2 concentration difference between samples and blanks with the volume of bottle, and converting it to the carbonate–C content assuming the validity of the ideal gas equation. The method was validated by measurements of known amounts of $CaCO_3$. Organic C (C_{org}) was calculated by subtracting carbonate–C from total C.

The concentration of total polycyclic aromatic hydrocarbons (PAH) was determined according to a protocol combining the methods of Hale et al. (2012) and Hilber et al. (2012). The Soxhlet extractions (0.5 g of biochar, 90 mL of toluene, 6 h, 160 °C) were spiked with 1,1-binaphthyl as an internal standard before extraction. The toluene was reduced to 1 mL and PAH content was analyzed by gas chromatography mass spectroscopy analysis.

The Brunauer–Emmett–Teller specific surface area (BET SSA) was determined by the N_2 adsorption technique with a single point (at 0.30 partial pressure) method using samples ground and sieved to pass 0.063 mm mesh and pre-heated at 300 °C for 30 min before analysis. The BET SSA was measured at −196.15 °C with a Micromeritics Flowsorb 2300 gas adsorption analyser (Micromeritics Co., Norcross, USA).

The scanning electron microscopy (SEM) images of 0.2 mm sieved biochar samples were obtained after scattering the samples onto double-sided tape fixed to an aluminum sample holder and sputter coating with 5 nm of platinum (Quorum Q150TS, Quorum Technologies Ltd., East Grinstead, UK). SEM images were taken with a FEI Quanta 250 Field Emission Gun Scanning Electron Microscope (FEI Co., Philips, Eindhoven, Netherlands) using primary electron beam energy of 10 keV.

2.2. Field experiment

2.2.1. Site and soil

A field experiment was started in May 2011 at the Viikki Research and Experimental Farm, University of Helsinki, Finland (60°13′42″ N 25°2′34″ E). The field had been cropped with wheat (*Triticum aestivum* L. emend Thell.) and barley (*Hordeum vulgare* L.) with conventional moldboard plowing for the preceding six years.

The experimental soil was an Endogleyic Umbrisol (WRB, 2007) with a loamy sand texture (Soil Survey Division Staff, 1993). Particle size analysis of samples taken from the uppermost 30 cm soil layer was conducted by the pipette method (Elonen, 1971). The soil comprised 83% sand, 15% silt and 2% clay. Soil chemical

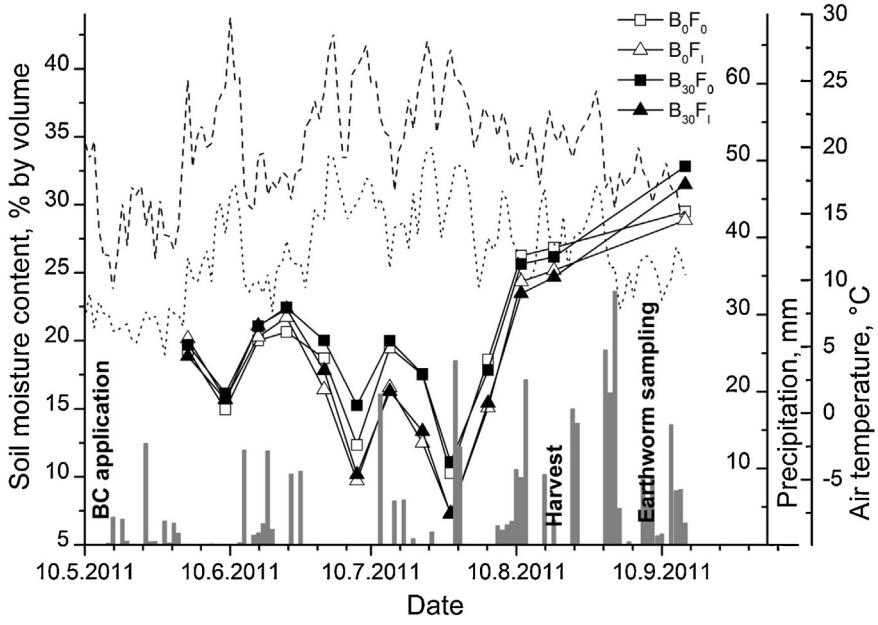

Fig. 1. Moisture content of topsoil (0–15 cm) as related to the precipitation (grey bars) and minimum and maximum air temperatures (dotted and dashed lines, respectively) during the growing season of 2011.

properties were analyzed twice during the field experiment, in May 2011 prior to biochar application and in August 2011, two weeks before earthworm sampling. Ten sub-samples were taken from 0 to 20 cm depth and mixed to form the composite sample from each plot (experimental design described in Section 2.2.3). The samples were extracted with acid ammonium acetate (Vuorinen and Mäkitie, 1955), followed by determination of elemental composition of the extracts by ICP-OES (Thermo-Fisher iCAP6500, Thermo Fisher Scientific, Cambridge, UK). Soil P content was determined by colorimetry with a molybdenum blue method (Lachat QuikChem 8000, Lachat Instruments, Milwaukee, USA). The electrical conductivity and pH of soil were measured from a 1:2.5 (w/w) soil-to-water mixture (Vuorinen and Mäkitie, 1955). The mineral nitrogen (NH_4–N and NO_3–N) was extracted from soil samples with 2 M KCl solution (1:5 v/v) for 1 h. The ammonium and nitrate concentrations of the extracts were determined by spectrophotometry with FIAstar 5000 Flow Injection Analyzer (Foss Tecator AB, Höganäs, Sweden). Total C and N in soil were quantified by VarioMax CN analyzer. All C was assumed to be of organic nature since the carbonate content in this soil was known to be negligible. The original content of soil organic matter (SOM) was 63.4 g kg^{-1}, assuming a 50% C content for the SOM (Pribyl, 2010).

2.2.2. Weather conditions

Weather conditions during the experiment are summarized in Fig. 1. Mean air temperatures of July and September 2011 were 3.4 and 2.7 °C higher than the long-term (1971–2000) average in Helsinki (FMI, 2012). The precipitation in early summer was similar to the long-term average, but in August–September it was 81% or 117 mm higher. Soil moisture contents at the depths of 0–15 and 0–28 cm were measured weekly at one point of the experimental plots with time domain reflectrometry (TDR; MiniTrase 6050X3, Soilmoisture Equipment, Santa Barbara, USA), and the moisture content at 15–28 cm layer was calculated. Weekly minimum and maximum temperatures together with the precipitation data were attained from the Vaisala WXT520 automatic weather transmitter (Vaisala Oy, Vantaa, Finland) located 1.6 km from the experimental site.

2.2.3. Experimental design

The experiment was a randomized complete block design with four replicates. It was a sub-set of a larger field experiment with additional factors and plots (Tammeorg et al., 2014). The biochar application was a factor with two levels (0 and 30 t dry matter ha^{-1}) and the inorganic fertilization was the other factor with two levels (0 and 361 kg fertilizer ha^{-1}). The biochar with a 25% (w/w) moisture content was applied with a sand spreader and mixed into the uppermost 10 cm soil layer by a rotary power harrow. The day after biochar application, a combine seeder was used to sow wheat cv 'Amaretto' at 650 viable seeds m^{-2} and to apply the inorganic fertilizer, a mixture of Agro 28-3-5 (with elemental contents of 28% N, 3% P and 5% K; Cemagro Oy, Lohja, Finland) and K_2SO_4 (K content 41.5%, Yara Suomi Oy, Siilinjärvi, Finland) providing 100 kg ha^{-1} of N, 10.8 kg ha^{-1} of P, and 19.5 kg ha^{-1} of K in easily soluble form. Fertilizer N was in the form of both NH_4^+ and NO_3^-.

Treatments were coded as B_XF_Y, where $_X$ refers to the biochar application rate (t ha^{-1}) and $_Y$ to the fertilizer treatment (0 and *I* corresponding to unfertilized control and inorganic fertilizer, respectively). The plot size was 2.2 × 10 m, and buffer plots of the same width were used between the experimental plots to minimize cross-contamination by wind-blown biochar. Integrated practices were used for crop management, including the use of chemical herbicides, fungicides and pesticides when necessary. Effects of biochar on the efficacy of the herbicides, fungicides and pesticides were not detected during the experiment.

2.2.4. Earthworm sampling

Earthworms were sampled in September 2011 after the crop was harvested and before the autumn tillage. From the four experimental treatments (B_0F_0, $B_{30}F_0$, B_0F_I and $B_{30}F_I$), three soil samples (with an area of 25 × 25 cm and a depth of 28 cm) were taken with a spade in regular intervals from each replicate plot and earthworms were hand-sorted from these in the field. Chemical extraction of the earthworms from deeper soil horizons was not deemed necessary, as the density of the deep burrowing *Lumbricus terrestris* L. was with high likelihood very low due to the frequent plowing of the field. Similarly to the TDR measurements, the earthworm

samples were taken in two layers (0–15 and 15–28 cm depths) and the sampling was conducted within a 10-day period (31 August–9 September 2011). During the sampling, the mean topsoil temperature at 15 cm soil depth was 15 ± 1.3 °C (measured with a handheld Pt 100 digital thermometer) and the mean volumetric soil moisture content was 28.1 ± 4.5% (measured with TRIME-FM time domain reflectrometry, IMKO, Ettlingen, Germany). The moisture content measurements were made in the 0–15 cm layer of the soil immediately before sampling and the temperature and moisture content of the soil were not significantly different between biochar and fertilizer treatments ($p > 0.05$; data not shown). According to our experience the prevailed conditions were favorable for efficient earthworm sampling. The number of earthworms collected from each sample was recorded in the field and the individuals were rinsed in water and stored in 3.7% formaldehyde solution for 1.5 months at 22 °C. Next, the individuals were transferred to 85% ethanol solution, weighed and their species identified according to Timm (1999). The mean values of the three replicate samples from the plot were converted to individuals m^{-2} and $g\,m^{-2}$ for the earthworm density and biomass, respectively.

2.3. Avoidance test

The earthworm avoidance test (Hund-Rinke and Wiechering, 2001) was conducted in the laboratory, using soil taken from 0–15 cm depth from the experimental field (above) in spring 2011. Prior to use, the soil was heated at 60 °C for 4 days to eradicate earthworms (Butt et al., 2005), passed through a 2 mm sieve, homogenized and moistened to 300 $g\,kg^{-1}$ DM moisture content. Sixteen cylindrical PVC vessels with the diameter of 15 cm and the height of 22 cm were used as experimental units. They were divided into two equal sections by a vertically introduced 3 mm wide wide polycarbonate divider (Makroclear®, Etra Oy, Helsinki, Finland). One half of each vessel was filled with control soil to the bulk density of 1.3 $g\,cm^{-3}$ (soil depth 15 cm), while the other half was filled with 16 $g\,kg^{-1}$ moist biochar–soil mixture (after the biochar had been sieved through a 2-mm mesh) to the same volume (of height 15 cm). Assuming a bulk density of 1.3 $g\,cm^{-3}$ and a furrow slice of 15 cm, the application rate corresponded to 30 $t\,ha^{-1}$ of dry biochar. Next, the divider was removed, and eight randomly chosen, mature *Aporrectodea caliginosa* Sav. individuals were placed on the separating line of each test vessel. This species was chosen as it is the most common earthworm species in Finnish agricultural soils (Nieminen et al., 2011). It is also subsurface feeding and geophagous, so it is suitable for testing the effects of materials mixed in the soil. The individuals used were collected from a location at the immediate vicinity of the field experiment one week prior to the experiment and stored in field-moist soil in a closed bucket at 15 °C. A perforated plastic wrap was installed over the experimental vessels in order to prevent escape.

During the test, the vessels were kept in a dark, temperature-controlled (15 °C) room where the positions and orientation of the vessels were completely randomized. For half of the vessels, the test lasted for 48 h, and for the other half, two weeks ($n = 8$ for both durations). In the two week test, the soil moisture content was checked by weighing the vessel after one week and replenishing the weight loss with deionized water. Additionally, the pH was measured periodically in the both parts of the vessel directly from the soil with a portable pH meter (Model 150, IQ Scientific Instruments, Carlsbad, USA).

The experiment was ended by inserting the divider swiftly into its original position and emptying out the contents of different sides of the vessel separately. The number of earthworms found in each side was recorded. Individuals damaged by the insertion of the divider were not considered in the statistical analysis. The percentage of preference/avoidance was calculated according to the equation proposed by Busch et al. (2011):

$$X_{\text{avoid}} = \frac{-100(n_c - n_t)}{N} \quad (1)$$

where X_{avoid} is the avoidance in percent, n_c is the number of worms in the control soil (mean of all eight replicates), n_t is the number of worms in the test soil (as above), and N is the total number of earthworms.

The effect of biochar on the soil water potential was followed over 11 days in a separate arrangement with two vessels equipped with tensiometers (Soil Measurement System, Tucson, USA) on the both parts of the vessel with the porous tip at the 7.5–12.5 cm depth. The pH of the soil was measured with a portable pH meter with a stainless steel pH probe (Model 150; IQ Scientific Instruments, San Diego, USA) from the both parts of the vessel. Similarly to the avoidance test with earthworms, the soil was replenished with deionized water according to the weight loss after one week. The measurements were accidentally finished after only 11 days from the beginning, so the differences in soil water potential and pH at the end of 14-day period could not be fully replicated.

2.4. Statistical analyses

Earthworm avoidance data was treated as Tally data and analyzed by assuming it to follow the Bernoulli distribution with the probability of 0.5 for the individual earthworms avoiding the biochar-amended soil (binomial test). The statistical significance of

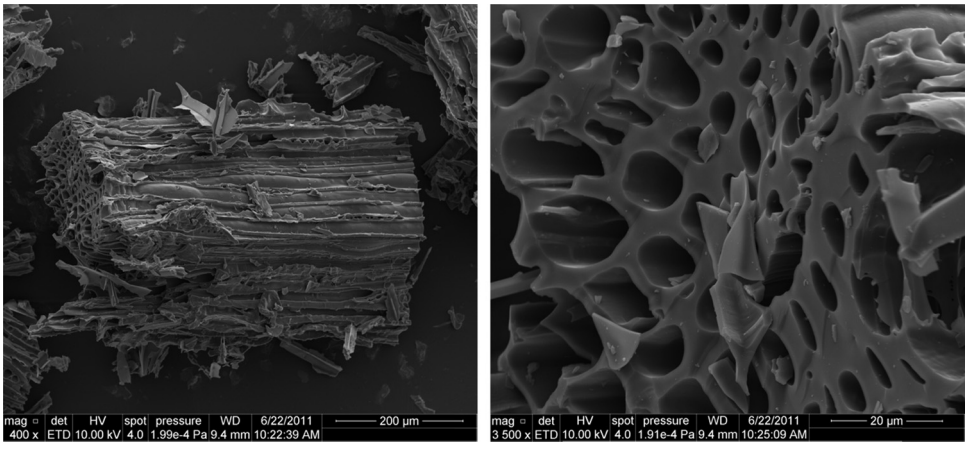

Fig. 2. SEM images of the spruce chip biochar taken at 400× magnification (on left) and at 3500× magnification (on right).

Table 1
Physicochemical properties of the spruce chip biochar, means with standard deviation (SD).

Property[a]	Total	SD	Unit
BET SSA	265		$m^2\ g^{-1}$
pH_{H_2O}	8.1	0.08	
C/N	251		$g\ g^{-1}$
H/C_{org}	0.34		$mol\ mol^{-1}$
Ash	26.6	0.55	$g\ kg^{-1}$
VM	121.6	11	$g\ kg^{-1}$
$CaCO_3$ equivalence	9.0	1.41	$g\ kg^{-1}$
Carbonate–C	1.2	0.08	$g\ kg^{-1}$
C_{org}	881.3		$g\ kg^{-1}$
Al	0.09	0.006	$g\ kg^{-1}$
Ca	4.66	0.018	$g\ kg^{-1}$
Fe	0.34	0.014	$g\ kg^{-1}$
K	4.52	0.498	$g\ kg^{-1}$
Mg	0.92	0.005	$g\ kg^{-1}$
Mn	0.33	0.041	$g\ kg^{-1}$
Na	0.21	0.076	$g\ kg^{-1}$
P	1.83	0.017	$g\ kg^{-1}$
S	0.22	0.006	$g\ kg^{-1}$
C	882.5	13	$g\ kg^{-1}$
N	3.52	0.31	$g\ kg^{-1}$
Cd	0.01	0.002	$mg\ kg^{-1}$
Co	0.25	0.003	$mg\ kg^{-1}$
Cu	11.46	4.609	$mg\ kg^{-1}$
Ni	3.56	0.393	$mg\ kg^{-1}$
Pb	2.51	0.871	$mg\ kg^{-1}$
Sr	25.57	0.145	$mg\ kg^{-1}$
Zn	64.79	0.632	$mg\ kg^{-1}$
Total PAH	10.06	0.77	$mg\ kg^{-1}$

[a] $n=2$ for BET SSA, pH and $CaCO_3$ equivalence, $n=6$ for VM; and $n=3$ for other analyses.

the calculated X_{avoid} was determined with the Fisher's least significant difference test.

In the field experiment, the effects of biochar and fertilizer effects on the total density and biomass of earthworms were first tested for the two sampling depths (0–15 and 15–28 cm) separately and for the combined data (0–28 cm) with two-way analyses of covariance (ANCOVA), using the highly variable initial soil C content as a covariate and comparing the adjusted least-square means after Bonferroni correction. The biochar level, fertilizer application and their interaction were used as fixed effects and replication as a random effect. Next, a Generalized Linear Mixed Model with the sampling depth as a correlated factor was used for detecting the effects of sampling depth on the main effects and their interactions. Statistical tests were carried out with the software package SPSS v.20.0 (SPSS Corp., Chicago, USA) using $p<0.05$ as the threshold level of significance.

3. Results

3.1. Biochar properties

The biochar particle size distribution (by weight) was 26% 0–0.25 mm, 22% 0.25–1 mm, 40% 1–5 mm, 10% 5–10 mm and 2% >10 mm. The biochar contained considerable amounts of total Ca, K, P and Mg (Table 1). In contrast, the liming equivalent of the biochar (9 g $CaCO_3$ kg^{-1}) and the carbonate–C content of the biochar (1.2 g kg^{-1} equivalent to 10 g $CaCO_3$ kg^{-1}) were both low. The high content of C_{org} (881.3 g kg^{-1}) and low atomic (0.34) H/C_{org} ratio of the biochar (Table 1) provided evidence of a relatively high degree of carbonization during the pyrolysis. The biochar had comparatively high porosity, both on nm and μm scale, as indicated by the high BET SSA (265 $m^2\ g^{-1}$; Table 1) and the high amount of open micropores on the SEM images (Fig. 2), respectively.

The content of heavy metals (Cd, Cu, Ni and Pb) in biochar was comparable with the naturally occurring levels in Finnish arable soils (Mäkelä-Kurtto et al., 2007). The content of Zn (65 mg kg^{-1}) was somewhat higher than the mean Zn content in soils (55 mg kg^{-1}; Mäkelä-Kurtto et al., 2007), but much lower than the 1500 mg kg^{-1} allowed for sewage sludge used as a soil amendment in Finland (Council of State of Finland, 1994). The total PAH content of the biochar (Table 1) was lower than the legislative limits for soil in Finland (30 mg kg^{-1}; Carlon, 2007) and the limit set by European Biochar Certificate (2013, 12 mg kg^{-1}).

3.2. Avoidance test

In the avoidance test, *A. caliginosa* actively burrowed in both biochar-treated and control parts of the vessels and after both test periods all experimental animals were recovered. After 2 days incubation, there was no significant difference in the distribution of the individuals between the treatments ($p=0.382$) whereas after 14 days, the earthworms tended to avoid biochar application ($p=0.033$) (Fig. 3a). However, the difference in the earthworm preference/avoidance of biochar application (X_{avoid}) was not statistically significant at either of the incubation times ($p=0.381$ and $p=0.174$ for 2 and 14 days incubation, respectively; Fig. 3b).

Soil water potential declined from −2.6 to −4.3 kPa in the unamended soil and from −2.4 to −4.8 kPa in the biochar-amended soil over 5 days. After the soil moisture content was replenished on day 7, the water potentials of the biochar-treated soil continued to be −0.2 kPa lower than those in the control. The pH of the control soil was initially lower than that of the biochar treatment (pH 5.2 vs. 5.6, respectively, $n=2$), but the differences by day 11 were negligible (pH 5.7 and pH 5.6 for control and biochar treatments, respectively).

3.3. Field experiment

Biochar application resulted in significantly higher increase in soil K and organic C levels and greater decrease in soil NO_3^-–N level after the first growing season compared with the non-biochar plots (Table 2). The earthworm community of the experimental field was dominated by the genus *Aporrectodea* (86.5% of the 444 individuals collected), with 25% being mature *Aporrectodea caliginosa*, 59.5% juveniles, mostly *A. caliginosa* and 2% *A. rosea* Sav. One *Allolobophora chlorotica* Sav. and one *Lumbricus terrestris* L. were identified (both comprising 0.2% of total). Unidentified earthworms accounted for 13.1% of the total number, and were typically small individuals (weight less than 0.3 g).

The earthworm density and biomass were higher in the topsoil (0–15 cm), with a mean of 129 individuals m^{-2} (range 43–267) and 60 g fresh weight (fwt) m^{-2} (range 14–129), compared to 19 individuals m^{-2} (range 5–80) and 10 g fwt m^{-2} (range 1–37) in the lower (15–28 cm) soil layer (the depth effect was significant for both density and biomass, Table 3). The interactions between the sampling depth and treatments were, however, not significant (Table 3).

Irrespective of the fertilizer treatments, the higher mean earthworm density and biomass was observed in the biochar-amended soil. This trend was, however, not statistically significant ($p>0.05$) at any of the sampling depths (0–15, 15–28 cm or combined 0–28 cm; Table 3, Fig. 4). Similarly, the fertilizer treatment had no statistically significant effect on earthworm density or biomass.

4. Discussion

4.1. Avoidance test

The lack of effect of 16 g kg^{-1} of biochar on the habitat choice of *A. caliginosa* after two days, but significant avoidance of the biochar at 14 days, may be attributable to the increased water retention caused by the biochar application. Li et al. (2011) reported no significant effect of 10 g kg^{-1} of biochar on *Eisenia fetida*. in an avoidance test lasting for 48 h, but significant avoidance of 100 g kg^{-1} of biochar was reported unless the biochar was wetted to its field capacity (2.2 mL water per g biochar) before application (Li et al., 2011). As nutrient deficiency and the presence of PAHs in the biochar were ruled out in supplementary tests, Li et al. (2011) concluded that desiccation caused by the biochar was the main reason for the avoidance shown by the earthworms. Busch et al. (2011) reported *Eisenia* spp. preference toward additions of 10 and 30 t ha^{-1} of activated peanut hull biochar in loamy sand (pH 5.5), when the soil on both treatment sides was wetted according to their water holding capacities.

Earthworms are more sensitive to the changes in the matric potential of the soil than to the soil moisture content (Kretzschmar and Bruchou, 1991; Doube and Styan, 1996) as the former better reflects the availability of water to them. In our study, however, the biochar effects on soil matric potential were rather small: the decrease in soil matric potential was only slightly larger in the biochar-amended soil than in the control during the 11-day follow-up. In wet soils (matric potentials higher than −5 kPa), the sensitivity of earthworm response to increased water suction varies between earthworm species and soils (Kretzschmar and Bruchou, 1991; Doube and Styan, 1996; Holmstrup, 2001). Neither Kretzschmar and Bruchou (1991) or Doube and Styan (1996)

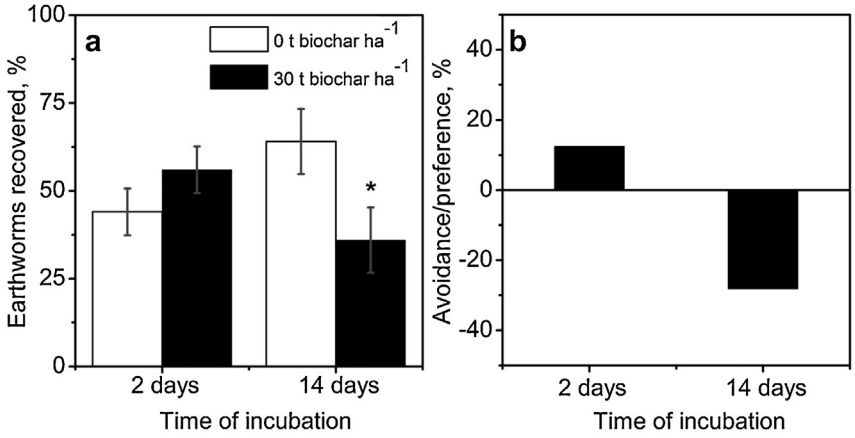

Fig. 3. (a) Percentage of *A. caliginosa* individuals recovered in biochar-amended (30 t biochar ha^{-1}) vs. unamended soil compartments in the avoidance test and (b) *A. caliginosa* preference/avoidance (X_{avoid}) of biochar-amended soil after 2 days and 14 days incubation. * indicates a significant difference of biochar treatments at $p < 0.05$ level. Error bars represent the standard error of the mean ($n = 8$).

reported any significant differences in the response of *A. longa, A. trapezoides* Dugès or *A. rosea* between −2 and −5 kPa matric potentials in sandy soils, but Holmstrup (2001) found that the growth of juvenile *A. caliginosa* was significantly higher at −2 kPa than at −6 kPa in Danish loamy sand (7% clay). It is therefore possible that in our low clay content (2%) soil, the same gravimetric soil moisture content used for both the biochar and control treatments led to increased water retention sufficient to cause the significant avoidance of biochar treatment after the 14-day incubation.

The mechanisms by which increased water retention may have caused the biochar avoidance effect of earthworms were not studied in detail in the present study. The factors explaining the avoidance may include e.g. the desiccation of earthworms (Li et al., 2011) and the increased soil strength (Chan and Barchia, 2007). The latter may have been caused by the addition of small-sized biochar into the coarse-textured soil slightly decreasing the soil porosity and filling some of the large pores. This could have increased the particle contacts, the amount of smaller pores, and the degree of water saturation in soil. If both water suction and the degree of water saturation in soil increase, the effective stress between soil particles determining the soil strength also increases (Fredlund et al., 1995; Baumgartl and Koeck, 2004). Nevertheless, as neither the desiccation of earthworms nor the increased soil strength were measured in our study, and the effects of biochar on matric tension

Table 2
Soil chemical properties at the experimental field before the experiment (spring 2011) and after the first growing season (autumn 2011) at 0–20 cm depth. Means of treatments used for field sampling of earthworms are shown ($n = 4$). For statistical comparisons, see Tammeorg et al. (2014).

	Treatment	EC (μS m^{-1})	pH	Acid ammonium acetate extractable (g m^{-3} soil)					2 M KCl extractable (g m^{-3} soil)			Total (g kg^{-1})	
				Ca	P	K	Mg	S	NH_4^+–N	NO_3^-–N	N_{min}	N	C_{org}
Spring	B_0F_0	78	6.4	1200	19.8	61.5	109.0	5.2	6.4	6.2	12.6	2.61	35.2
	$B_{30}F_0$	73	6.3	1050	20.5	66.3	92.0	5.1	6.1	5.5	11.6	2.41	31.8
	B_0F_I	85	6.4	1175	19.3	64.8	108.5	5.8	8.5	5.9	14.4	2.66	37.1
	$B_{30}F_I$	80	6.3	1093	20.3	66.8	94.8	5.2	6.9	5.7	12.6	2.07	29.0
Autumn	B_0F_0	65	6.3	1300	18.8	67.5	125.0	6.9	6.4	4.4	10.8	2.34	34.6
	$B_{30}F_0$	53	6.4	1050	20.0	87.8	97.3	6.7	5.6	3.0	8.6	2.15	35.3
	B_0F_I	73	6.3	1225	18.3	70.5	116.3	6.0	5.6	7.4	13.1	2.40	35.2
	$B_{30}F_I$	53	6.3	1078	21.3	93.3	97.3	6.1	5.8	5.1	10.8	2.15	34.9

Abbreviations: B = biochar application rate, t ha^{-1}; EC = Electrical conductivity; F = Fertilizer treatment: F_0 = unfertilized control, F_I = inorganic fertilizer; N_{min} = mineral nitrogen (NH_4^+–N + NO_3^-–N); C_{org} = Soil organic C.

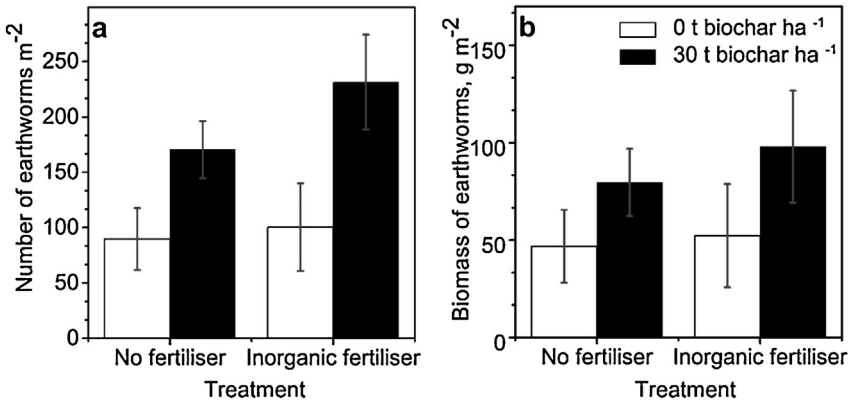

Fig. 4. (a) Density and the (b) Biomass of earthworms in the field trial at the depth of 0–28 cm. Values shown are Bonferroni-adjusted least-square means of 4 replicates after correcting for initial C content of the soil by ANCOVA. The differences between treatments were not statistically significant at $p < 0.1$ level. The error bars represent the standard error.

Table 3
Results from ANCOVA for the effects of biochar (B), fertilizer application (F) and their interactions on the density and biomass of earthworms sampled from the field at depths 0–15, 15–28 cm and the whole profile 0–28 cm. Additionally, the effect of sampling depth (D) and its interaction with experimental factors are presented as an output from Generalized Linear Mixed Models analysis with the sampling depths as correlated factors.

Depth	Factor	df*	Earthworm density		Earthworm biomass	
			F	p	F	p
0–15 cm	B	1	3.563	0.099	2.159	0.194
	F	1	3.883	0.106	1.476	0.279
	B × F	1	0.980	0.368	0.580	0.481
15–28 cm	B	1	0.415	0.538	0.034	0.858
	F	1	0.524	0.502	0.891	0.389
	B × F	1	0.049	0.833	1.386	0.292
0–28 cm	B	1	4.381	0.077	1.532	0.269
	F	1	2.435	0.179	0.599	0.474
	B × F	1	0.694	0.443	0.101	0.763
	D	1	46.728	**<0.001**	37.253	**<0.001**
	D × B	2	0.809	0.505	1.268	0.354
	D × F	2	0.877	0.499	0.715	0.546
	D × B × F	2	0.155	0.863	0.559	0.610

* df = degrees of freedom. Significant p values ($p < 0.05$) are in bold font.

were small and determined only at a single depth, the contributions of these mechanisms in earthworm avoidance remain to be studied in future experiments.

Other factors besides water retention could contribute to the avoidance of biochar by earthworms (Li et al., 2011), including soil pH (Van Zwieten et al., 2010; Busch et al., 2011), heavy metal content, or other contaminants (Busch et al., 2011). Van Zwieten et al. (2010) reported significant earthworm preference for the soil amended with biochar from paper mill waste when the application raised the pH of a Ferrosol (pH 4.2) but not when it was used on a Calcarosol (pH 7.7). The biochar used in present study contained very low amount of carbonates and had respectively low liming equivalence (9 g $CaCO_3$ kg^{-1}) and did not notably affect the soil pH. It is thus unlikely that pH could have affected the earthworm preferences in the present study. Similarly, the low contents of heavy metals and PAH in our biochar suggest that the earthworm avoidance was probably not caused by these components. It remains possible that other undetermined factors were effective in the experiment, and more work is needed to explore the factors affecting biochar avoidance or preference of earthworms.

4.2. Field experiment

The earthworm community of our field site, with its dominance by endogeic *A. caliginosa*, was typical for frequently ploughed fields, and the overall earthworm density was close to the typical values reported for arable soils (Fraser et al., 1996; Nieminen et al., 2011). The significantly higher earthworm abundance in the 0–15 cm soil layer probably relates to the greater content of organic matter in the topsoil compared with the subsoil, but also to the favorable physical conditions that prevailed in this layer for the dominant endogeic earthworms.

Biochar and fertilizer treatments had no statistically significant effects on the density and biomass of earthworms. The trend toward higher values over the control (for instance, $p = 0.077$ for density in 0–28 cm layer) by adding 30 t biochar ha^{-1} suggests, however, a possible influence. A positive effect of biochar on the earthworm abundance has been reported by Husk and Major (2010) in an unreplicated experiment in which 3.9 t ha^{-1} of fine (<2 mm) hardwood biochar was added to a temperate clay loam in Canada. Weyers and Spokas (2011), in another unreplicated study, reported that no clear trends on earthworm abundance were observed two years after application of 22.5 t ha^{-1} of woody biochar to a silt loam in Minnesota, USA.

In addition to its effects on soil physicochemical properties, biochar may affect earthworm density through its effects on the microbial biomass and microbial metabolites (Lehmann et al., 2011). Particles of fine (<2 mm sieved) charcoal found in earthworm guts (Topoliantz and Ponge, 2003, 2005) may increase gut pH and thus assist the assimilation of other resources (Weyers and Spokas, 2011), as well as enhance gut microbial communities favoring the production of digestive enzymes (Topoliantz and Ponge, 2003). In our study, the biochar contained larger particles (12% larger than 5 mm; 52% larger than 1 mm), that probably reduced its potential for ingestion. The particle size of biochar however decreases with time in soil. For this reason, long-term studies are needed for detecting the effects of different biochars on earthworm abundance both under arable and pastoral cropping systems and for revealing the mechanisms involved.

5. Conclusions

This study is to our knowledge the first where the biochar effects on earthworms were investigated both in a laboratory avoidance test and in a replicated field experiment that allowed statistical evaluation of treatment effects. In the avoidance test, biochar did not affect the habitat choice of earthworms within two days, but after two weeks, the earthworms tended to avoid biochar slightly but significantly. As the liming equivalence and contents of heavy metals and PAH in the biochar were low, we attribute the avoidance mainly to the slight decline in soil water potential. In the field experiment, four and half months after biochar application, the highest earthworm densities and biomasses were measured in biochar-amended soils. Nevertheless, the differences to the control were not statistically significant. The duration of the field experiment was not sufficient for the detection of differences in the population growth rates due to reproduction or immigration, so a follow-up study would be useful for obtaining a more definitive picture of the treatment effects.

Conflicts of interest

The authors declare that they have no conflicts of interest in the research.

Acknowledgments

The authors thank Sampo Tukiainen (Preseco Oy) for providing the experimental biochar, Mikko Hakojärvi for his help with the TDR measurements and Johannes Mäkinen for his help during earthworm sampling from the field. The authors acknowledge Ilya Belevich for the scanning electron microscopy imaging, Miia Collander and Johanna Muurinen for technical assistance with the biochar and soil analysis, Festus Anasonye for PAH analyses of the biochar and Arja Tervahauta for the analysis of the H content of the biochar. The English language of the manuscript was revised by Dr F.L. Stoddard. The insightful comments from the editor and two anonymous reviewers significantly improved the quality of the manuscript. This study was financed by Jenny and Antti Wihuri Foundation and the Ministry of Agriculture and Forestry of Finland.

References

ASTM D3175-02, 2002. Standard Test Method for Volatile Matter in the Analysis Sample of Coal and Coke. American Society for Testing and Materials, Conshohocken, PA.

Baumgartl, T., Koeck, B., 2004. Modeling volume change and mechanical properties with hydraulic models. Soil Sci. Soc. Am. J. 68, 57–65.

Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D., Brun, J.J., 2013. A review of earthworm impact on soil function and ecosystem services. Eur. J. Soil Sci. 64, 161–182.

Brockhoff, S.R., Christians, N.E., Killorn, R.J., Horton, R., Davis, D.D., 2010. Physical and mineral-nutrition properties of sand-based turfgrass root zones amended with biochar. Agron. J. 102, 1627–1631.

Busch, D., Kammann, C., Grünhage, L., Muller, C., 2011. Simple biotoxicity tests for evaluation of carbonaceous soil additives: establishment and reproducibility of four test procedures. J. Environ. Qual. 40, 1–10.

Butt, K.R., Nieminen, M.A., Sirén, T., Ketoja, E., Nuutinen, V., 2005. Population and behavioural level responses of arable soil earthworms to boardmill sludge application. Biol. Fertil. Soils 42, 163–167.

Carlon, C. (Ed.), 2007. Derivation Methods of Soil Screening Values in Europe. A Review and Evaluation of National Procedures towards Harmonization. EUR 22805-EN. European Commission, Joint Research Centre, Ispra, Italy, p. 306.

Chan, K.Y., Barchia, I., 2007. Soil compaction controls the abundance, biomass and distribution of earthworms in a single dairy farm in south-eastern Australia. Soil Tillage Res. 94, 75–82.

Chaoui, H.I., Zibilske, L.M., Ohno, T., 2003. Effects of earthworm casts and compost on soil microbial activity and plant nutrient availability. Soil Biol. Biochem. 35, 295–302.

Cheng, C.-H., Lehmann, J., Thies, J.E., Burton, S.D., 2008. Stability of black carbon in soils across a climatic gradient. J. Geophys. Res. 113, 1–10, http://dx.doi.org/10.1029/2007JG000642.

Council of State of Finland, http://www.finlex.fi/fi/laki/alkup/1994/19940282 Accessed 27 January 2014 1994. Decision of the Council of State (Vnp) No 282/1994.

Doube, B.M., Styan, C., 1996. The response of *Aporrectodea rosea* and *Aporrectodea trapezoides* (Oligochaeta: Lubricidae) to moisture gradients in three soil types in the laboratory. Biol. Fertil. Soils 23, 166–172.

Eckmeier, E., Gerlach, R., Skjemstad, J.O., Ehrmann, O., Schmidt, M.W.I., 2007. Minor changes in soil organic carbon and charcoal concentrations detected in a temperate deciduous forest a year after an experimental slash-and-burn. Biogeosciences 4, 377–383.

Elonen, P., 1971. Particle size analysis of soil. Acta Agral. Fenn. 122, 1–122.

European Biochar Certificate, 2013. Guidelines for a Sustainable Production of Biochar. Version 4.8 of 13 December 2013, http://www.european-biochar.org/biochar/media/doc/1364855734492.pdf. Accessed 27 January 2014.

FMI, 2012. Monthly Climatological Statistics of Finland. Finnish Meteorological Institute, http://ilmatieteenlaitos.fi/kuukausitilastot. Accessed 22.06.12.

Fraser, P.M., Williams, P.H., Haynes, R.J., 1996. Earthworm species, population size and biomass under different cropping systems across the Canterbury Plains. N. Z. Appl. Soil Ecol. 3, 49–57.

Fredlund, D.G., Vanapalli, S.K., Xing, A., Pufahl, D.E., 1995. Predicting the shear strength for unsaturated soils using the soil water characteristic curve. In: Alonso, E.E., Delage, P. (Eds.), Proceedings of the 1st International Conference on Unsaturated Soils. Balkema, Rotterdam, The Netherlands, pp. 63–69.

Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—a review. Biol. Fertil. Soils 35, 219–230.

Gomez-Eyles, J.L., Sizmur, T., Collins, C.D., Hodson, M.E., 2011. Effects of biochar and the earthworm *Eisenia fetida* on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements. Environ. Pollut. 159, 616–622.

Güereña, D., Lehmann, J., Hanley, K., Enders, A., Hyland, C., Riha, S., 2013. Nitrogen dynamics following field application of biochar in a temperate North American maize-based production system. Plant Soil. 365, 239–254, http://dx.doi.org/10.1007/s11104-012-1383-4.

Hale, S.E., Lehmann, J., Rutherford, D., Zimmerman, A.R., Bachmann, R.T., Shitumbanuma, V., O'Toole, A., Sundqvist, K.L., Arp, H.P.H., Cornelissen, G., 2012. Quantifying the total and bioavailable polycyclic aromatic hydrocarbons and dioxins in biochars. Environ. Sci. Technol. 46, 2830–2838.

Hilber, I., Blum, F., Leifeld, J., Schmidt, H.P., Bucheli, T.D., 2012. Quantitative determination of PAHs in biochar: a prerequisite to ensure its quality and safe application. J. Agric. Food Chem. 60, 3042–3050.

Holmstrup, M., 2001. Sensitivity of life history parameters in the earthworm *Aporrectodea caliginosa* to small changes in soil water potential. Soil Biol. Biochem. 33, 1217–1223.

Hund-Rinke, K., Wiechering, H., 2001. Earthworm avoidance test for soil assessments. An alternative for acute and reproduction tests. J. Soils Sediments 1, 15–20.

Husk, B., Major, J., 2010. Commercial Scale Agricultural Biochar Field Trial in Quebec, Canada over Two Years: Effects of Biochar on Soil Fertility, Biology and Crop Productivity and Quality. BlueLeaf, http://www.blue-leaf.ca/main-en/files/BlueLeaf%20Biochar%20Field%20Trial%2008-09%20Report-1.pdf. Accessed 27.01.14).

Jones, D.L., Rousk, J., Edwards-Jones, G., DeLuca, T.H., Murphy, D.V., 2012. Biochar-mediated changes in soil quality and plant growth in a three year field trial. Soil Biol. Biochem. 45, 113–124.

Kretzschmar, A., Bruchou, C., 1991. Weight response to the soil water potential of the earthworm *Aporrectodea longa*. Biol. Fertil. Soils 12, 209–212.

Lavelle, P., 1988. Earthworm activities and the soil system. Biol. Fertil. Soils 6, 237–251.

Lavelle, P., Pashanasi, B., Charpentier, F., Gilot, C., Rossi, J.P., Derouard, L., Andre, J., Ponge, J.-F., Bernier, N., 1998. Large-scale effects of earthworms on soil organic matter and nutrient dynamics. In: Edwards, C.A. (Ed.), Earthworm Ecology. CRC Press, Boca Raton, FA, pp. 103–122.

Lehmann, J., Skjemstad, J., Sohi, S., Carter, J., Barson, M., Falloon, P., Coleman, K., Woodbury, P., Krull, E., 2008. Australian climate–carbon cycle feedback reduced by soil black carbon. Nat. Geosci. 1, 832–835.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – a review. Soil Biol. Biochem. 43, 1812–1836.

Li, D., Hockaday, W.C., Masiello, C.A., Alvarez, P.J.J., 2011. Earthworm avoidance of biochar can be mitigated by wetting. Soil Biol. Biochem. 43, 1732–1737.

Liang, B., Lehmann, J., Sohi, S.P., Thies, J.E., O'Neill, B., Trujillo, L., Gaunt, J., Solomon, D., Grossman, J., Neves, E.G., Luizão, F.J., 2010. Black carbon affects the cycling of non-black carbon in soil. Org. Geochem. 41, 206–213.

Liesch, A.M., Weyers, S.L., Gaskin, J.W., Das, K.C., 2010. Impact of two different biochars on earthworm growth and survival. Ann. Environ. Sci. 4, 1–9.

Major, J., Rondon, M., Molina, D., Riha, S., Lehmann, J., 2010. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna Oxisol. Plant Soil 333, 117–128.

Major, J., Rondon, M., Molina, D., Riha, S.J., Lehmann, J., 2012. Nutrient leaching in a Colombian savanna Oxisol amended with biochar. J. Environ. Qual. 41, 1076–1086, http://dx.doi.org/10.2134/jeq2011.0128.

Miller, R.O., 1998. High-temperature oxidation: dry ashing. In: Kalra, Y.P. (Ed.), Handbook of Reference Methods for Plant Analysis. Soil and Plant Analysis Council. CRC Press, Boca Raton, FA, pp. 53–56.

Mäkelä-Kurtto, R., Eurola, M., Laitonen, A., 2007. Monitoring Programme of Finnish Arable Land: Agua Regia Extractable Trace Elements in Cultivated Soils in 1998. Agrifood Research Reports, No. 104. MTT Agrifood Research, Jokioinen, Finland.

Nieminen, M., Ketoja, E., Mikola, J., Terhivuo, J., Sirén, T., Nuutinen, V., 2011. Local land use effects and regional environmental limits on earthworm communities in Finnish arable landscapes. Ecol. Appl. 21, 3162–3177.

Noguera, D., Rondon, M., Laossi, K.-R., Hoyos, V., Lavelle, P., de Carvalho, M.H.C., Barot, S., 2010. Contrasted effect of biochar and earthworms on rice growth and resource allocation in different soils. Soil Biol. Biochem. 42, 1017–1027.

Penttilä, A., Slade, E.M., Simojoki, A., Riutta, T., Minkkinen, K., Roslin, T., 2013. Quantifying beetle-mediated effects on gas fluxes from dung pats. PLoS One 8, 1–7, http://dx.doi.org/10.1371/journal.pone.0071454.

Pribyl, D.W., 2010. A critical review of the conventional SOC to SOM conversion factor. Geoderma 156, 75–83.

Rowell, D.L., 1994. Soil Science. Methods & applications. Longman Scientific & Technical, Harlow, UK, p. 34.

Soil Survey Division Staff, 1993. Soil Survey Manual. Agric. Handbook No. 18, USDA-NRCS. U.S. Gov. Print. Office, Washington, DC.

Tammeorg, P., Simojoki, A., Mäkelä, P., Stoddard, F.L., Alakukku, L., Helenius, J., 2014. Short-term effects of biochar on soil properties and wheat yield formation with meat bone meal and inorganic fertiliser on a boreal loamy sand. Agr. Ecosyst. Environ. 191, 108–116.

Timm, T., 1999. A Guide to Estonian Annelida. (Naturalist's Handbooks 1). Estonian Academy Publishers, Tartu-Tallinn, Estonia, p. 208.

Topoliantz, S., Ponge, J.F., 2003. Burrowing activity of the geophagous earthworm Pontoscolex corethrurus (Oligochaeta: Glossoscolecidae) in the presence of charcoal. Appl. Soil Ecol. 23, 267–271.

Topoliantz, S., Ponge, J.F., 2005. Charcoal consumption and casting activity by Pontoscolex corethrurus (Glossoscolecidae). Appl. Soil Ecol. 28, 217–224.

Vaccari, P.F., Baronti, S., Lugatoa, E., Genesio, L., Castaldi, S., Fornasier, F., 2011. Biochar as a strategy to sequester carbon and increase yield in durum wheat. Eur. J. Agron. 34, 231–238.

Van Zwieten, L., Kimber, S., Morris, S., Chan, Y.K., Downie, A., Rust, J., 2010. Effect of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. Plant Soil 327, 235–246.

Vuorinen, J., Mäkitie, O., 1955. The Method of Soil Testing in Use in Finland, vol. 63. Agrogeological Publishing, Helsinki, pp. 1–44.

Weyers, S.L., Spokas, K.A., 2011. Impact of biochar on earthworm populations: a review. Appl. Environ. Soil Sci. 2011, 1–12, http://dx.doi.org/10.1155/2011/541592.

Woolf, D., Amonette, J.E., Street-Perrott, F.A., Lehmann, J., Joseph, S., 2010. Sustainable biochar to mitigate global climate change. Nat. commun. 1, 1–9.

WRB, 2007. World Reference Base for Soil Resources 2006, First Update 2007. World Soil Resources Reports No. 103. FAO, Rome, Italy.

Xu, G., Wei, L.L., Sun, J.N., Shao, H.B., Chang, S.X., 2013. What is more important for enhancing nutrient bioavailability with biochar application into a sandy soil: direct or indirect mechanism? Ecol. Eng. 52, 119–124.

Yeardley, R.B., Lazorchak, J.M., Gast, L.C., 1996. The potential of an earthworm avoidance test for evaluation of hazardous waste sites. Environ. Toxicol. Chem. 15, 1532–1537.

Zhang, A., Liu, Y., Pan, G., Hussain, Q., Li, L., Zheng, J., Zhang, X., 2012. Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China Plain. Plant Soil 351, 263–275.