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Earthworm avoidance of biochar can be mitigated by wetting

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ABSTRACT

Biochar has a great potential for enhancing soil fertility and carbon sequestration while enabling beneficial waste disposition. Because of the potential for widespread application, it is essential to proactively assess and mitigate any unintended consequences associated with soil biochar amendment. We conducted soil avoidance tests, growth and reproduction tests, and oxidative stress assays with the earthworm *Eisenia foetida* to assess the potential toxicity of soil amended with biochar produced from apple wood chips. Earthworms avoided soils containing 100 and 200 g/kg dry biochar at statistically significant levels ($p < 0.05$), and after 28-day incubation, these earthworms lost more weight than those in control (unamended) soil. However, biochar did not affect the reproduction of earthworms. We investigated whether the observed avoidance was due to nutrition deficiency, desiccation, or the presence of toxic polynuclear aromatic hydrocarbons (PAHs) formed during biochar production by pyrolysis. Nutrition deficiency was excluded by the lack of earthworm avoidance to soil amended with nutrient-deficient sand instead of biochar. Although traces of PAH were detected in the tested biochar (e.g., 25.9 μg/kg fluorene, 3290 μg/kg naphthalene, and 102 μg/kg phenanthrene), the lack of lipid peroxidation and no increase in superoxide dismutase activity in biochar-exposed earthworms suggests that presence of toxic compounds was not a likely reason for avoidance. Furthermore, wetting the biochar to its field capacity resulted in statistically undetectable avoidance relative to control soil, indicating that insufficient moisture could be a key factor affecting earthworm behavior in soil amended with dry biochar. To avoid desiccation of invertebrates and enable their beneficial ecosystem services, we recommend wetting biochar either before or immediately after soil application.

1. Introduction

Biochar, a form of charcoal produced by pyrolysis of carbon-rich biomass, draws tremendous interest worldwide due to its potential to enhance soil fertility, facilitates soil water management, sequester CO_2, and manage organic waste. Application of biochar to soil has been shown to increase soil water and nutrients retention (e.g., by trapping them in micropores (Lehmann and Joseph, 2009)), improve soil fertility, and alter soil structure (e.g., enhanced porosity and soil aeration) (Edwards and Bohlen, 1996; Glaser et al., 2002; Lehmann et al., 2003). The relatively stable nature of biochar and its subsequent long soil residence time make biochar soil amendment a promising approach to enhance plant growth and reduce CO_2 emissions (Lehmann et al., 2006). Biochar is also effective in removing organic contaminants from water (Chen et al., 2008; Zhu et al., 2005) and has been demonstrated to be six times more effective in absorbing heavy metals compared to activated carbon (Cao et al., 2009). Therefore, it is very likely that biochar will be broadly used in the near future, underscoring the need to proactively assess and mitigate any unintended consequences. In particular, the literature has not yet addressed the potential impact of biochar amendment on terrestrial organisms and the organisms' associated response.

Earthworms perform many essential and beneficial functions in soil ecosystems, including decomposition, nutrient mineralization, and soil structure improvement (Edwards and Bohlen, 1996), and their ability to perform these functions can be inhibited upon exposure to harmful substances. Many ecotoxicological studies have used earthworms as model system to assess potentially toxic materials in soils (Amorim et al., 2005; da Luz et al., 2004; Erstfeld and Snow-Ashbrook, 1999; He et al., 2007; Lanno et al., 2004; Lukkari and Haimi, 2005; Schaefer, 2004; Zhou et al., 2007). Earthworms are useful model organisms because many aspects of their response to environmental perturbations can be assessed and

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connected to environmental outcomes, including their avoidance behavior, growth rate, enzyme activity level, mortality, and reproduction patterns (Yeardley et al., 1996).

The acute earthworm avoidance test was first developed in 1996 (Yeardley et al., 1996). The International Standards Organization (ISO) has established earthworm avoidance test guidelines for rapid screening and evaluation of soil function and influence of contaminants and chemicals on earthworm behavior (ISO, 2008). Environment Canada has also published a standard earthworm avoidance method to test toxicity of contaminated soil (Environment Canada, 2004). Avoidance tests have higher sensitivity to contaminants and require less experimental time than other earthworm toxicity tests (Schaefer, 2004; Yeardley et al., 1996), making them ideal for a rapid screen of emerging substances or materials that may be deliberately or incidentally applied to soils.

In this study, we chose the earthworm *Eisenia foetida* which is widely used as a model soil organism in research and government guidelines (Environment Canada, 2004; Li et al., 2010; Van Zwieten et al., 2010), thus facilitating comparisons with previous studies. We conducted acute soil avoidance tests and viability assays (e.g., reproduction and weight loss) to assess the potential toxicity of biochar produced by apple wood chips to the earthworms. We also measured lipid oxidation and superoxide dismutase (SOD) activity in earthworm tissue to investigate whether exposure to biochar induces oxidative stress.

2. Materials and methods

2.1. Chemicals and reagents

Tris–HCl (molecular grade), EDTA (disodium) solution (2.5% (w/v)), sucrose (99+%), sodium lauryl sulfate, sodium acetate (99+%), acetic acid (99.8%), thiobarbituric acid (99+%), trichloroacetic acid (99+%), 1,1,3,3-tetraethoxypropane (97%), nitro blue tetrazolium, methionine (99+%), $CaCO_3$ and riboflavin (98%) were purchased from Fisher Scientific (Pittsburgh, PA).

2.2. Organism

E. foetida was purchased from The Worm Farm (Durham, CA). Worms were maintained according to the method described previously (Li et al., 2010). Sexually mature earthworms (i.e., clitellated), in a range of 0.3–0.6 g, were selected for all the experiments.

2.3. Biochar preparation and characterization

We prepared biochar in a custom-built demonstration-scale batch reactor at Rice University from apple wood sawdust purchased from Allied Kenco, Houston, Texas. The feedstock was composed of wood fragments which were 5–10 mm × 0.5–1 mm × 0.5–1 mm when added to the biochar reactor; the particle size decreased by 25–50% following pyrolysis. The Rice reactor used in this experiment produced approximately 2 kg biochar per 4 h run. Biomass was sealed within a 20 L reactor vessel constructed with 306 stainless steel and heated in a propane-fired furnace. Exhaust gases were passively vented to a series of heat exchangers at ambient temperature to remove condensable liquids and bio-oils and prevent their condensation into the biochar. Non-condensable gases were combusted in a secondary (venturi-style) burner to heat the reactor. The initial heating rate was approximately 5 °C/min to a temperature of ~400 °C. Thereafter, the heating rate slowed to 1 °C per minute, to a maximum temperature of approximately 525 °C. Total heating time was approximately 250 min (slow pyrolysis, no gasification). No active purging of the atmosphere was involved. The reactor was allowed to cool to ambient temperature (overnight) before removing the biochar. When removed from the reactor, the biochar was odorless and visually homogenous with no ash formed. The biochar was not rinsed before use.

The surface area of this biochar was $8.93 \pm 0.95\ m^2/g$, based on the Brunauer–Emmett–Teller method using a Quantachrome Autosorb-3B Surface Analyzer (Quantachrome Instruments, Boynton Beach, FL, USA). Polycyclic aromatic hydrocarbon (PAH) content in biochar was analyzed by a commercial lab (Pace Analytical Services Inc., Lenexa, KS) using EPA method 8270.

2.4. Artificial soil preparation and amendment

Artificial soil (AS) was prepared as described by Environment Canada (Environment Canada, 2004). It consisted of 10% *Sphagnum* peat moss (previously sieved through 2 mm mesh), 20% kaolin clay and 70% quartz sand. The pH of dry soil was adjusted with $CaCO_3$ to the optimal range (6.5–7.5).

We chose biochar amendment levels of 10, 100, and 200 g biochar/kg soil. The background level of charcoal in US agricultural soils ranges from about 1 to 15 g/kg (Skjemstad et al., 2002), with larger values typically occurring in prairie soils with a history of wildfire (Czimczik and Masiello, 2007; Rodionov et al., 2010). An application rate of 100 g/kg, or 10% biochar by mass, corresponds to an application rate of 90 ton/ha at a tillage depth of 10 cm or 180 ton/ha at a tillage depth of 20 cm. This is at the high end of application rates currently employed (8–116 ton/ha) (Gaskin et al., 2010; Major et al., 2010a, 2010b). An application of 200 g/kg (180 ton/ha at a tillage depth of 10 cm, or 360 ton/ha tilled to 20 cm) is at the outer range of maximum plausible application levels (Lehmann et al., 2006). Phenanthrene, a representative PAH, was added separately at 0.1 g phenanthrene/kg soil as positive control in the avoidance test. Application method of phenanthrene to soil was described previously (Li et al., 2010).

2.5. Soil avoidance bioassays

We conducted soil avoidance bioassays following a modified method developed by Environment Canada (Environment Canada, 2004). This test was conducted in stainless-steel avoidance wheels (Fig. 1) with six pie-shaped compartments connected to a circular, center chamber that served as the test arena. Negative control soil (unamended artificial soil) was placed into alternating compartments (3 compartments/unit, 250 g of soil/compartment), while test soil was transferred to the remaining compartments. Test soil

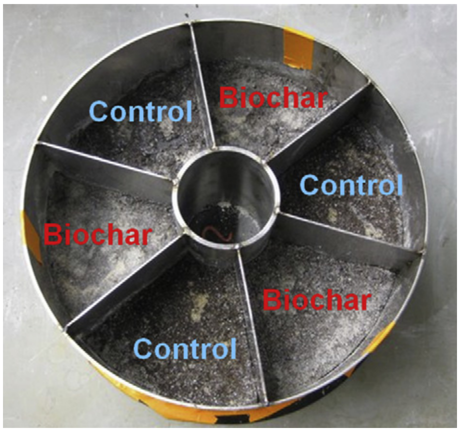

Fig. 1. Avoidance wheel. The avoidance wheel contains six compartments separated by removable partitions. Worms are added to the center area, which contains open passages to the six compartments. Holes on the radial partitions allow earthworms to move freely between compartments.

and control soil were separated by removable aluminum partitions. Soil in each compartment was hydrated with Milli-Q water to 85% of water-holding capacity according to the method published by Coleman et al. (2010). Partitions were removed afterward. Ten worms were selected for each avoidance wheel. Worms were introduced to the center of the avoidance wheel individually. After the addition of ten worms to an avoidance wheel, a lid was placed to prevent escape and time was recorded ($t = 0$). At least five replicates were conducted for each concentration of biochar. The wheels were placed in a dark area at 22 °C for 48 h. At the end of 48 h time period, the lid was removed and the partitions were inserted back to the chambers to prevent further worm movement between the compartments. The numbers of worms in each compartment were recorded. If a worm was cut by the partition and separated in two compartments, 0.5 was recorded for both of the compartments. The percentage of worms (10 in total) in control and test soils was then calculated separately.

2.6. Growth and reproduction tests

To measure the effects of prolonged exposure of biochar to earthworms on their growth and reproduction, we conducted a 28-day toxicity test according to Environment Canada protocols (Environment Canada, 2004). After depuration on filter paper hydrated with deionized (DI) water for 24 h, earthworms were transferred to 500-ml glass jars filled with soil (10 worms/200 g soil) containing different concentrations of biochar (0, 10, 100, and 200 g/kg) for 28 days. Soils in all treatments were hydrated with Milli-Q water to 85% of water-holding capacity. Worm weight was measured and recorded at day 0 and day 28 after depuration. Cocoon numbers in each treatment were counted and recorded to assess worm reproduction. All treatments were prepared in triplicate.

2.7. Lipid peroxidation and superoxide dismutase activity measurement

We exposed worms to the highest level of biochar-amended soil (200 g/kg) for 1, 2, 7, and 14 days. We chose this biochar concentration because it represented an upper limit of what is likely to be applied to agricultural soils. At each time point, 5 worms were picked out randomly and sacrificed for oxidative stress tests. Worm tissue was homogenized on ice in nine parts (w/v) of 50 mM Tris–HCl buffer containing 1 mM EDTA and 0.25 M sucrose (pH 7.6) with a motor-homogenizer (Brinkmann Instruments, Inc., Westbury, NY). The homogenate was centrifuged at 8000 rpm for 20 min and the supernatant was saved for future tests.

Protein concentration of the worm homogenate was determined following the Bradford method (Bradford, 1976) using bovine serum albumin as standard. Oxidative stress damage in the form of lipid peroxidation was assessed by malondialdehyde (MDA) formation using the method described by Hannam et al. (2010) with modifications. Briefly, worm tissue homogenate (200 μl) was added to a 5-ml glass test tube containing 200 μl sodium lauryl sulfate (SDS) solution (8.1% w/v sodium lauryl sulfate), 1.5 ml of 0.2 M NaAc–HAc buffer solution (pH 3.6), 750 μl TBA solution (1% w/v thiobarbituric acid), 750 μl TCA solution (10% w/v trichloroacetic acid), and 1 ml of Milli-Q water. The cocktail was incubated in water bath at 90 °C for 60 min, cooled down to room temperature, and centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant was measured using an Ultrospec 2100 *pro* spectrophotometer (GE Healthcare, Piscataway, NJ) at 530 nm. A blank was prepared by substituting 200 μl of worm homogenate with 200 μl of Milli-Q water. The MDA concentration was determined against the standard curve with 1,1,3,3-tetraethoxypropane. Results were expressed as nmol MDA per mg protein.

The activity of SOD was measured by using the nitroblue tetrazolium chloride (NBT) method described by Song et al. (2009). One unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition of the NBT photoreduction (measured as 50% of the absorbance of the SOD-free control), and the result was expressed as U per mg protein.

2.8. Statistical analysis

Whether differences between two sets of treatments were statistically significant was determined using Student's *t*-tests at the 95% confidence level.

3. Results and discussion

3.1. Earthworms response to the presence of biochar in soil

We assessed the effects of biochar on the earthworms' avoidance behavior, growth rate, and reproduction. Earthworms did not avoid soil containing 10 g/kg biochar (Fig. 2), a level within the upper limits of natural soil biochar concentrations. However, a statistically significant earthworm avoidance effect was observed with soils amended with dry biochar at higher concentrations (100 and 200 g/kg). Phenanthrene, which is a commonly studied PAH and can be formed during biomass pyrolysis, was used as a positive control. Phenanthrene elicited a more significant avoidance effect at concentrations as low as 0.1 g/kg, three orders of magnitude lower than our highest soil biochar concentrations (Fig. 2).

In a separate test, earthworm weight loss was assessed after 28-day incubation in soil amended with different concentrations of dry biochar (10, 100, and 200 g/kg). Because earthworms were not fed during the exposure period, they experienced weight loss even in the control soil. The weight loss for earthworms collected from soils containing 100 and 200 g/kg biochar was 37.1 ± 1.7% and 40.3 ± 2.5%, respectively, which is significantly higher ($p < 0.05$) than the weight loss for worms in the control soil (32.1 ± 1.0%) (Fig. 3A). Soil containing 10 g/kg dry biochar did not significantly affect weight loss. No significant effect on earthworm reproduction was observed for all tested biochar concentrations (Fig. 3B).

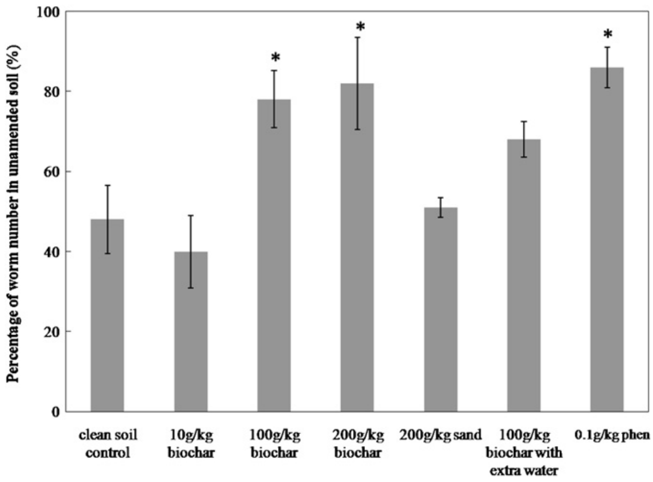

Fig. 2. Percentage of worms recovered in amended versus unamended soil compartments after 48-h avoidance tests. Worms showed a significant avoidance effect in soils amended with 100 g/kg and 200 g/kg biochar ($p < 0.05$). Phenanthrene (phen), the positive control, also showed a significant avoidance effect at 0.1 g/kg * indicates a significant difference from controls at the 95% confidence level. Error bars represent ± one standard error ($n = 5$).

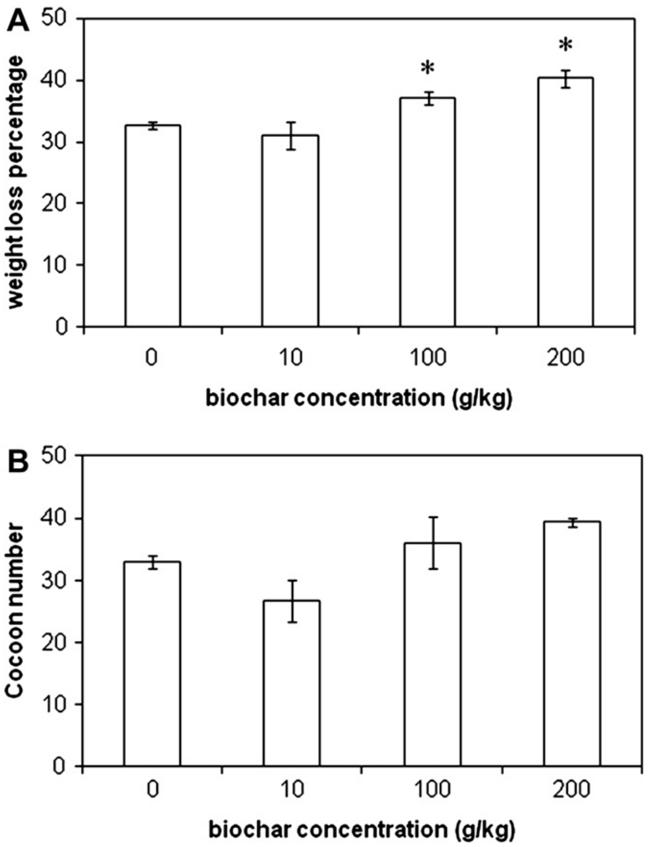

Fig. 3. Worm weight loss (A) and cocoon production (B) after 28-d incubation in unamended soil or soils amended with dry biochar. Significantly higher weight loss was observed in both treatments with 100 and 200 g/kg dry biochar-amended soils. Biochar did not have a significant effect on the earthworms' cocoon production. * indicates significant difference from controls at the 95% confidence level. Error bars represent ± one standard error ($n = 3$).

3.2. Three hypotheses for avoidance response

We posited three hypotheses that could explain the observed avoidance response: 1) the response was driven by nutrient scarcity, since biochar is non-nutritive and its addition in large amounts to soils would dilute the total amount of nutrients available in the soil; 2) biochar had a high water-holding capacity and dry biochar may desiccate earthworms; and 3) contaminants such as PAHs, which could be formed during biochar production by pyrolysis (Barbosa et al., 2006), were toxic and avoided by the earthworms.

To test the first hypothesis, we conducted an avoidance test with soil amended with 200 g/kg sand instead of biochar. Sand contains no earthworm nutrients and similarly to this biochar it would dilute those present in the artificial soil. Fig. 2 shows that earthworms did not differentiate between the control soil and the reduced-nutrient soil containing extra sand, indicating that nutrient deficiency did not drive the avoidance behavior. Nutrient deficiency due to nutrient sorption by biochar was unlikely given the experimental design. The only possible source of nutrients in the tested artificial soil was one of the soil ingredients, peat moss (about 2 mm diameter), which cannot penetrate into the much smaller pores of biochar (on the order of tens of microns (Nguyen et al., 2010)). Furthermore, the short duration of exposure (2 days) in the absence of water flow makes significant leaching of nutrients from peat moss into the biochar highly improbable.

To investigate the effects of biochar's water-holding capacity, we repeated the 100 g/kg experiment with biochar wetted to field capacity. The field capacity of this biochar was more than four times greater than that of the artificial soil (2.2 versus 0.47 ml water per g char/soil). Extra water (beside the standard amount of water added to reach 85% of the artificial soil's water-holding capacity) was added to every 250 g of amended soil (100 g/kg biochar) to saturate the biochar to its field capacity. With this extra moisture, the avoidance effect was no longer statistically significant relative to the unamended control ($p > 0.05$) (Fig. 2), indicating that moisture was likely a key factor affecting earthworm behavior in biochar-amended soil. Accordingly, desiccation may also explain the significantly higher weight loss in earthworms exposed to dry biochar for 28 d (Fig. 3A). However, additional (as yet undetermined) factors besides desiccation may have also contributed to the observed behavior since decreased avoidance upon wetting was significant relative to unamended controls but not relative to dry biochar ($p > 0.05$) (Fig. 2).

Potential toxins in biochar, such as PAHs produced during pyrolysis, may be assimilated by the earthworms. Out of the 16 PAHs regulated by the EPA, only three were detected within the biochar prior to its addition to the soil: fluorene (25.9 μg/kg),

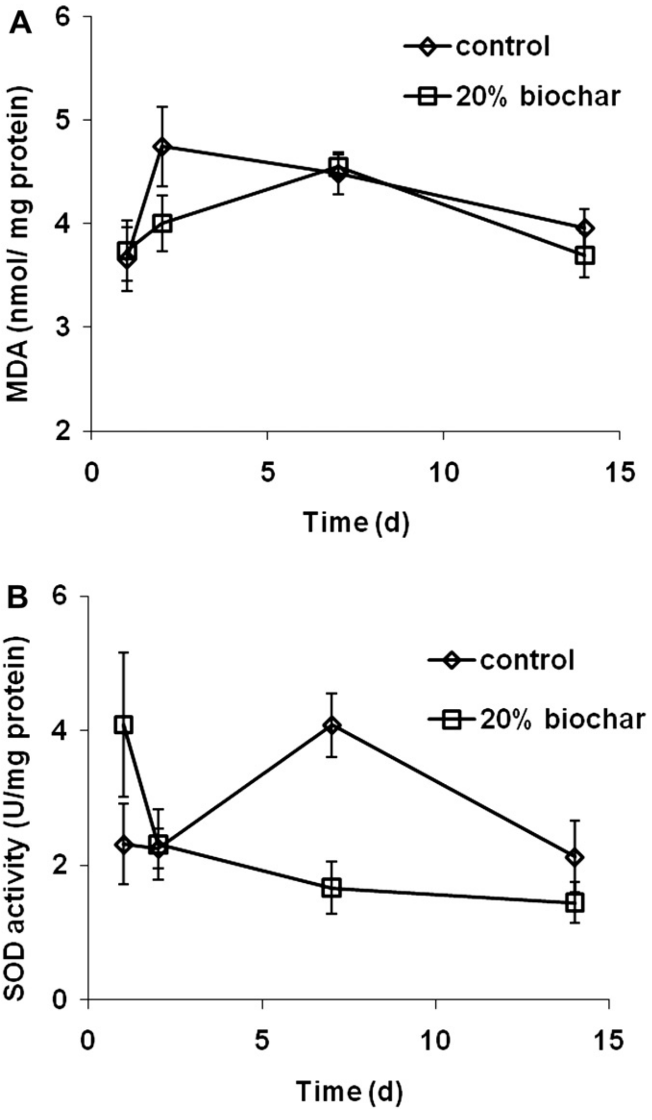

Fig. 4. Malondialdehyde (MDA) (A) and superoxide dismutase (SOD) (B) measurements in worm tissue in control soil and soil amended with 200 g/kg biochar during 28-day exposure. Error bars represent ± one standard error ($n = 5$).

naphthalene (3290 μg/kg), and phenanthrene (102 μg/kg). These values are below cleanup action levels for PAH-contaminated soil (e.g., TRRP, 2010), which are 2300 mg/kg for fluorene, 120 mg/kg for naphthalene, and 1700 mg/kg for phenanthrene. Thus, there is no amendment level at which it would be possible to exceed legally acceptable PAH limits with this biochar. Nevertheless, PAH could induce the intracellular production of reactive oxygen species (ROS) (Stegeman and Lech, 1991), which in turn cause oxidative stress to cellular components (Lopez et al., 2006) and induce a detectable biochemical response in the organisms. Specifically, the common oxidative stress biomarker MDA, which is an oxidation product of ROS interaction with polyunsaturated fatty acids (Song et al., 2009), could be produced. To assess the possibility that worms experienced oxidative stress, we monitored SOD activity and lipid peroxidation (per MDA formation) in earthworm cells during exposure to control soil and soil amended with 200 g/kg biochar. No discernable differences or increase in either SOD or MDA levels was observed during the 14-day test for earthworms incubated in control versus biochar-amended soils (Fig. 4). Therefore, exposure to this biochar did not cause lipid peroxidation or induce antioxidant defense in the earthworms, indicating that the avoidance response was not likely the result of chemical (e.g., PAH) toxicity.

Previous studies have shown different response of earthworms to biochar in soil. Chan et al. (2008) found that earthworms preferred soil amended with biochar produced from poultry litter pyrolyzed at 450 °C. However, this effect was not reproducible with biochar from the same feedstock pyrolyzed at a 550 °C (Chan et al., 2008). Field capacity of biochar increases with increasing pyrolysis temperature up to about 500 °C (Kinney et al., in preparation), suggesting that the earthworm behavior observed by Chan et al. (2008) may also be a function of biochar water retention properties. In another study with biochar derived from papermill waste, earthworms exhibited a slight but statistically indiscernible attraction to biochar (Van Zwieten et al., 2010). However, the underlying mechanism(s) for earthworm attraction (or avoidance) have not been discerned. This may require considering how biochar affects the nutritional and water activity properties of the soil, which can potentially affect earthworm behavior. We showed that although soil amendment with dry biochar could desiccate earthworms, this negative effect can be overcome by pre-wetting the biochar. Furthermore, with a much higher field capacity than soil (2.2 versus 0.47 g water-per g biochar/soil for the biochar used here), wet biochar may buffer the soil from large fluctuations in soil moisture (unpublished data showed that biochar slows soil water loss).

4. Conclusions

Earthworms avoided soils amended with high concentrations ($\geq$100 mg/kg) of dry biochar (produced from apple wood chips), and experienced significant weight loss after 28-day exposure. The avoidance response was likely to avert desiccation rather than to avoid potential toxicants (i.e., PAHs formed during biochar production by pyrolysis) or nutrient scarcity. By wetting the biochar to field capacity before exposing the worms, we found that this adverse effect could be completely mitigated in application levels as high as 100 g/kg (90 ton/ha). Therefore, depending on site-specific conditions and irrigation or rainfall patterns, wetting biochar either before or immediately after soil application may be needed to prevent desiccation of earthworms and enable their beneficial effects on plants.

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References

Amorim, M.J., Rombke, J., Soares, A.M., 2005. Avoidance behaviour of *Enchytraeus albidus*: effects of benomyl, carbendazim, phenmedipham and different soil types. Chemosphere 59, 501–510.

Barbosa, J.M., Re-Poppi, N., Santiago-Silva, M., 2006. Polycyclic aromatic hydrocarbons from wood pyrolysis in charcoal production furnaces. Environ. Res. 101, 304–311.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

Cao, X., Ma, L., Gao, B., Harris, W., 2009. Dairy-manure derived biochar effectively sorbs lead and atrazine. Environ. Sci. Technol. 43, 3285–3291.

Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2008. Using poultry litter biochars as soil amendments. Aust. J. Soil Res. 46, 437–444.

Chen, B., Zhou, D., Zhu, L., 2008. Transitional adsorption and partition of nonpolar and polar aromatic contaminants by biochars of pine needles with different pyrolytic temperatures. Environ. Sci. Technol. 42, 5137–5143.

Coleman, J.G., Johnson, D.R., Stanley, J.K., Bednar, A.J., Weiss, C.A., Boyd, R.E., Steevens, J.A., 2010. Assessing the fate and effects of nano aluminum oxide in the terrestrial earthworm, *Eisenia foetida*. Environ. Toxicol. Chem. 29, 1575–1580.

Czimczik, C.I., Masiello, C.A., 2007. Controls on black carbon storage in soils. Global Biogeochem. Cycles 21.

da Luz, T.N., Ribeiro, R., Sousa, J.P., 2004. Avoidance tests with Collembola and earthworms as early screening tools for site-specific assessment of polluted soils. Environ. Toxicol. Chem. 23, 2188–2193.

Edwards, C.A., Bohlen, P.J., 1996. Biology and Ecology of Earthworms. Chapman & Hall.

Environment Canada, 2004. In: Biological Test Method: Tests for Toxicity of Contaminated Soil to Earthworms (*Eisenia andrei*, *Eisenia foetida*, or *Lumbricus terrestris*). Environment Canada, Ottawa, Ontario.

Erstfeld, K.M., Snow-Ashbrook, J., 1999. Effects of chronic low-level PAH contamination of soil invertebrate communities. Chemosphere 39, 2117–2139.

Gaskin, J.W., Speir, R.A., Harris, K., Das, K.C., Lee, R.D., Morris, L.A., Fisher, D.S., 2010. Effect of peanut hull and pine chip biochar on soil nutrients, corn nutrient status, and yield. Agron. J. 102, 623–633.

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.

Hannam, M.L., Bamber, S.D., Galloway, T.S., John Moody, A., Jones, M.B., 2010. Effects of the model PAH phenanthrene on immune function and oxidative stress in the haemolymph of the temperate scallop *Pecten maximus*. Chemosphere 78, 779–784.

He, Q., Yan, Z., Wang, Q., Li, F., 2007. The application of earthworm avoidance test in ecological risk assessment of naphthalene-contaminated soils. J. Agro-Environment Sci. 26, 538–543.

ISO, 2008. Soil Quality – Avoidance Test for Determining the Quality of Soils and Effects of Chemicals on Behaviour. Part 1: Test with Earthworms (*Eisenia foetida* and *Eisenia andrei*). International Organization for Standardization.

Lanno, R., Wells, J., Conder, J., Bradham, K., Basta, N., 2004. The bioavailability of chemicals in soil for earthworms. Ecotoxicol. Environ. Saf. 57, 39–47.

Lehmann, J., Joseph, S., 2009. Biochar for Environmental Management. Earthscan, London; Sterling, VA.

Lehmann, J., da Silva, J.P.J., Steiner, C., Nehls, T., Zech, W., Glaser, B., 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant and Soil 249, 343–357.

Lehmann, J., Gaunt, J., Rondon, M., 2006. Bio-char sequestration in terrestrial ecosystems – a review. Mitigation Adaptation Strateg. Glob. Change 11, 403–427.

Li, D., Fortner, J.D., Johnson, D.R., Chen, C., Li, Q., Alvarez, P.J.J., 2010. Bioaccumulation of $^{14}C_{60}$ by the earthworm *Eisenia foetida*. Environ. Sci. Tech. 44, 9170–9175.

Lopez, E., Arce, C., Oset-Gasque, M.J., Canadas, S., Gonzalez, M.P., 2006. Cadmium induces reactive oxygen species generation and lipid peroxidation in cortical neurons in culture. Free Radic. Biol. Med. 40, 940–951.

Lukkari, T., Haimi, J., 2005. Avoidance of Cu- and Zn-contaminated soil by three ecologically different earthworm species. Ecotoxicol. Environ. Saf. 62, 35–41.

Major, J., Lehmann, J., Rondon, M., Goodale, C., 2010a. Fate of soil-applied black carbon: downward migration, leaching and soil respiration. Glob. Change Biol. 16, 1366–1379.

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

Nguyen, B.T., Lehmann, J., Hockaday, W.C., Joseph, S., Masiello, C.A., 2010. Temperature sensitivity of black carbon decomposition and oxidation. Environ. Sci. Tech. 44, 3324–3331.

Rodionov, A., Amelung, W., Peinemann, N., Haumaier, L., Zhang, X.D., Kleber, M., Glaser, B., Urusevskaya, I., Zech, W., 2010. Black carbon in grassland ecosystems of the world. Glob. Biogeochem. Cycles 24.

Schaefer, M., 2004. Assessing 2,4,6-trinitrotoluene (TNT)-contaminated soil using three different earthworm test methods. Ecotoxicol. Environ. Saf. 57, 74–80.

Skjemstad, J.O., Reicosky, D.C., Wilts, A.R., McGowan, M.A., 2002. Charcoal carbon in U.S. agricultural soils. Soil Sci. Soc. Am. J. 66, 1249–1255.

Song, Y., Zhu, L.S., Wang, J., Wang, J.H., Liu, W., Xie, H., 2009. DNA damage and effects on antioxidative enzymes in earthworm (*Eisenia foetida*) induced by atrazine. Soil Biol. Biochem. 41, 905–909.

Stegeman, J.J., Lech, J.J., 1991. Cytochrome P-450 monooxygenase systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. Environ. Health Perspect. 90, 101–109.

TRRP, 2010. TRRP Protective Concentration Levels.

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

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.

Zhou, S.P., Duan, C.Q., Fu, H., Chen, Y.H., Wang, X.H., Yu, Z.F., 2007. Toxicity assessment for chlorpyrifos-contaminated soil with three different earthworm test methods. J. Environ. Sci. (China) 19, 854–858.

Zhu, D., Kwon, S., Pignatello, J.J., 2005. Adsorption of single-ring organic compounds to wood charcoals prepared under different thermochemical conditions. Environ. Sci. Technol. 39, 3990–3998.