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Active removal of biochar by earthworms (*Lumbricus terrestris*)☆

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ABSTRACT

The role earthworms play in the cycling of biochar in the soil column is poorly understood. While some studies show that earthworms favor a biochar-rich environment, they are ambiguous as to whether earthworms actively remove it and/or possibly consume it, or whether biochar indirectly provides a more favorable habitat. More importantly, associating the properties of different biochars to habitat preference by earthworms has received almost no attention. Courts were established with eight biochars from different feedstocks with varying in physicochemical properties. Circular piles (1.5 g) of biochar were applied to the surface of mesocosms containing field soil and 25 earthworms. Removal was quantified by digitally photographing the biochar samples and visually estimating disappearance over time. Most biochars were actively removed by the earthworms. The most preferred biochar was an aged biochar (>70 years) harvested from charcoal mounds, whereas the least preferred was a fast-pyrolysis biochar made from hardwood sawdust. There was an inverse relationship between the removal of biochar by earthworms and total carbon and a proportional increase with the contents of ash, Ca, Mn, and Si, although the correlations were not strong and may not explain earthworm preference. Other physicochemical properties of the biochars, such as the % volatile C, % H, porosity, and cumulative surface area, were associated with increased aerobic bacteria and fluorescent pseudomonads in soil, but not associated with biochar removal by earthworms. More research is needed to determine if tailoring specific biochars for surface removal by earthworms could be achieved by supplementing biochar with Ca, Mn, and Si and thus lead to a non-disruptive system for delivery of biochar into lower soil horizons of perennial crops. More importantly, this procedure may be useful in screening biochars for attractiveness to earthworms.
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Introduction

Biochar has shown much promise as a soil amendment for increasing soil fertility and water-holding capacity in nutrient-depleted soils and for remediating contaminated soils (Lehmann, 2007). Reports have also demonstrated the potential for biochar to reduce the damaging effect of allelopathy and root disease (Elmer and Pignatello, 2011) and to protect plants from foliar pathogens (Graber et al., 2010; Elad et al., 2010). A property associated with biochar is its ability to increase microbial communities in the rhizosphere, such as arbuscular mycorrhizae, fluorescent pseudomonads, *Flavobacterium* spp. and chitin and cellulose degraders (Elmer and Pignatello, 2011; Harel et al., 2012; Kolton et al., 2011). Elad et al. (2010) suggested that biochar promotes microflora in the rhizosphere that induce resistance to plant disease. Given that these biochar responses may be associated with the rhizosphere (Elad et al., 2010; Elmer and Pignatello, 2011), a prerequisite for using biochar in agriculture may require its incorporation into the soil at depths where it has optimum contact with living roots. However, with perennial plants, getting biochar to the root system without damaging the plants presents a challenge.

The use of earthworms as vehicles for delivery of biochar deeper into the soil column may circumvent this problem. Success has been achieved using earthworms to act as delivery agents for biological control organisms that can pass through the gut of the earthworms without appreciable reduction in viability (Doube et al., 1995; Menge, 1999). Furthermore, several studies have documented that charred plant material was observed in earthworm castings within the soil column indicating the ability of earthworms to vertically transport biochar to lower horizons (Eckmeier et al., 2007; Topoliantz and Ponge, 2003, 2005).

Many studies have examined earthworm density and behavior as indicators for biochar toxicity (Gomez-Eyles et al., 2011; Sizmur et al., 2011; Weyers and Spokas, 2011). The review by Weyers and

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Spokas (2011) concluded that most studies were designed to assess biochar toxicity or avoidance, and that the majority observed either toxicity to earthworms or no effect on earthworm activity. However, a few studies showed that biochar mixed with soil attracted earthworms. Topoliantz and Ponge (2003, 2005) suggested that char from an open fire increased earthworm activity in soil by binding toxins that served to suppress microbial growth. This was observed when the Amazonia earthworm, *Pontoscolex corethrurus*, did not ingest biochar alone, but did so when it was combined with mineral soil, thus pointing to a positive feedback that improved the soil habitat by increasing carbon content. Van Zwieten et al. (2010) observed that earthworm activity in acid soils was favored by biochar amendment, but the effect was minimal in neutral to alkaline soils. Li et al. (2011) discovered that soil moisture alone can determine whether earthworms avoid biochar-amended soil. Chan et al. (2008) found that earthworms favored soil that was amended with biochar from poultry litter pyrolyzed at 450 °C, but not at 550 °C. Hale et al. (2013) observed that *Aporrectodea caliginosa* and *Eisenia fetida*, preferred biochar-amended soil over soil alone. Although these studies show that earthworms favor biochar-amended soils, they are ambiguous as to whether earthworms actively consume it, or whether biochar indirectly provides a more favorable habitat by, for example, increasing pH. Weyers and Spokas (2011) advised that future studies report feedstock, elemental and ash content of the biochar, the soils used, and pH to assist in associating properties with earthworm activity.

Since earthworms obtain the majority of their nutrition from microbes that colonize decaying plant residues (Edwards, 1998), attraction to biochar might also be indicative of how biochar affects resident microbial populations. The review by Lehmann et al. (2011) on the impact of biochar on soil organisms highlights the large knowledge gaps associating physicochemical properties of biochar with their effects on soil microorganisms, fauna, and plant roots.

Here, we demonstrate that earthworms actively removed some biochars. Our objectives in this study were, (1) to rank the ability of earthworms to remove different biochars and to affect soil bacteria, and (2) to associate these abilities with different physicochemical properties of biochar to determine what properties are most closely associated with earthworm behavior.

Material and methods

Origin and properties of different biochars

Eight biochars were obtained from a variety of feedstocks and prepared at different pyrolysis temperatures. Of these, four (Agrichar, CQuest, Pure Black, Soil Reef) were commercially available at the time, three were experimentally prepared, and one was harvested from charcoal mounds in Union, CT formed around 1938 or shortly thereafter. Biochars were passed through 0.5 cm mesh, air-dried at 45 °C, and stored in plastic bags at 20 °C. Each biochar was analyzed for C, H, ash, volatile C, surface area, pH, and elemental content (Braida et al., 2003 (Table 1). Ash content was determined as the residue after combustion at 900 °C. Volatile carbon was determined by anoxic thermal analysis (Hazen Research, Inc., Golden, CO, USA). Surface area was determined using the Brunaur–Emmett–Teller fit of the N_2 adsorption isotherm at 77°K. Micropore surface area and microporosity were determined from the CO_2 isotherm at 273°K based on Grand Canonical Monte Carlo Density Functional Theory analysis (Braida et al., 2003). The pH was measured in a char-to-pure-water suspension of 1:20 (w/v) after gentle mixing for 48 h. The elements Ca, Fe, Mg, Mn, Ni, P, S, Si, and Zn in each biochar were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Five-gram samples were digested (0.5 g) in 50-ml polypropylene digestion tubes with 5 ml of concentrated nitric acid at 115 °C for 45 min using a hot block (DigiPREP System; SCP Science, Champlain, NY). Elements were quantified on a iCAP 6500; (Thermo Fisher Scientific, Waltham, MA).

Earthworm studies

Experiments were conducted with earthworms and the eight different biochars. Drainage holes (2 cm diam.) were cut into four 76-L plastic tubs (0.58 × 0.40 × 0.40 m) lined with 100 μm nylon cloth. Bins were filled with 18 kg of air-dried topsoil (Agway, Inc., Westfield, MA) providing approximately 20 cm depth. According to the manufacturer, there was no charcoal added to the soil. The pH of the soil was 6.7. The soil was moistened to field capacity and set in incubators at 20 °C. Soil temperature monitored at a depth of 10 cm averaged 19 ± 3 °C. Earthworms (*Lumbricus terrestris*) were purchased from a commercial fishing supply company (N.A.S. Inc., Marblehead, OH) and kept at 10 °C for no more than 2 weeks before being washed in tap water and used in the following studies. Twenty-five healthy adult earthworms of the same size were placed on the surface of the soil whereupon they immediately formed burrows. We chose *L. terrestris* because it makes deep burrows. Four to five grams of wheat bran residue that had been ground in a Wiley mill and passed through a 1 mm sieve was sprinkled on the surface and moistened with tap water using a spray bottle. Twenty-four hours later, the wheat bran residue was gone, which signified that the worms were active and feeding.

Eight plastic rings (5.5 cm diam.) were placed equidistantly from each other, but at least 3.5 cm from the edge of the bin. Onto the surface of the soil within the circle defined by each ring was placed an evenly-distributed layer of 0.5 g of a biochar, and the ring was then gently removed. This amount just covered the surface of the soil. The soil surface was then misted with deionized water until the biochars were thoroughly moistened. Seven days later, another 4–5 g of ground wheat bran and alfalfa were uniformly sprinkled onto the surface to ensure the earthworms had sufficient nutrition, and the soil surface was remoistened. Care was taken to avoid sprinkling the bran and alfalfa on the biochar samples. At day 0 and every 24 h thereafter for the next 9 days, the four bins were digitally photographed. The photos were examined by two individuals. The percent of biochar remaining in each circle was estimated by visually comparing the photos for each day with the photos for Day 0. The two estimates were averaged and the percent disappearance was calculated as a single integrated value of biochar disappearance (IBD):

$$\mathrm{IBD} = \sum_{i=1}^{9} \frac{Y_i + Y_{i-1}}{2}(t_i - t_{i-1})$$

where Y_i = the percentage of biochar present at time, t_i, relative to day 0. The biochars were randomly arranged in each or the four bins. After 2 months, the entire experiment was repeated with new earthworms and fresh soil.

Microbial assays

We made an assumption that earthworms might move the biochar into the soil in order to garden microbes for consumption. Each biochar was mixed with a soil suspension, incubated, and assayed for its effect on bacterial densities. Ten g of air-dried soil were mixed with 500 ml sterile normal saline and agitated for 30 min on a stir plate. Fifty ml were removed from the suspension

Table 1
Physicochemical properties of biochars under study.

	CQuest	AgriChar	Soil Reef	Pure Black	CT Char	BC1	BC2	BC3
Feedstock	Hardwood sawdust	Hardwood litter	Hardwood chips	Mixed hardwoods	Mixed Hardwoods	Animal cage litter	Yard waste	Mixed hardwoods
Method of production	Fast pyrolysis, 450 °C	Slow pyrolysis, 600 °C	Slow pyrolysis, 600 °C	Slow pyrolysis; Temp. unknown	Slow pyrolysis, Temp. unknown	Slow pyrolysis, 430 °C	Slow pyrolysis 430 °C	Slow pyrolysis, 430 °C
Atomic H/C	0.57	0.12	0.30	0.39	0.62	0.76	0.66	0.60
Ash, % dry weight[a]	10.9	1.4	7.9	3.1	16.2	11.5	9.3	5.5
Volatile C, % of volatile plus fixed C[b]	31.0	6.5	14.4	20.7	31.4	32.0	40.0	28.4
N_2-B.E.T surface area ($m^2\,g^{-1}$)[c]	∼0.1	427	338	46.6	9.71	ND	0.32	10.97
CO_2 GCMC cumulative surface area, 0–1.4 nm ($m^2\,g^{-1}$)[d]	157	614	578	392	238	223	255	349
CO_2 cumulative porosity (0–1.4 nm) ($cm^3\,g^{-1}$)[d]	0.05	0.18	0.17	0.11	0.07	0.06	0.07	0.10
pH[e]	6.3	7.9	7.9	7.55	5.3–5.4	7.4	7.7–7.8	7.3
C, % dry weight[a]	70.5	91.7	77.4	80.9	50.0	63.3	60.4	73.7
H, % dry weight[a]	3.3	0.9	2.0	2.6	2.6	4.0	3.3	3.7
N, % dry weight	ND[f]	ND	ND	ND	>0.5	4.71	1.51	>0.5
P (μg/g)	2.735	32.4	1.188	5.53	3.12	12.90	22.65	33.4
K (μg/g)	60.18	60.13	14.705	56.02	16.735	44.65	52.39	59.125
Ca (μg/g)	49.80	62.67	21.84	93.81	85.78	62.76	62.70	63.67
Mg (μg/g)	6.57	28.07	3.178	12.05	12.17	15.02	21.54	28.07
S (μg/g)	2.15	3.59	0.71	3.22	2.76	2.67	3.13	3.49
Fe (μg/g)	10.78	67.37	4.38	9.07	59.31	36.38	51.87	66.37
Cu (μg/g)	0.26	0.08	0.02	0.06	0.13	0.11	0.09	0.08
Mn (μg/g)	1.74	1.63	2.81	0.47	3.47	1.96	1.79	1.62
Ni (μg/g)	0.06	0.14	0.04	0.01	0.05	0.07	0.115	0.14
Si (μg/g)	0.36	7.02	0.59	1.02	6.67	3.78	5.40	7.1
Zn (μg/g)	0.11	0.40	0.32	1.66	0.42	0.55	0.47	0.40

[a] Galbraith Laboratories, Inc., Knoxville, TN, USA. Ash content was residue after combustion at 900 °C.
[b] By anaerobic thermal analysis; Hazen Research, Inc., Golden, CO, USA.
[c] Based on Brunaur–Emmett–Teller fit of the N_2 adsorption isotherm at 77 K.
[d] Based on Grand Canonical Monte Carlo Density Functional Theory analysis of the CO_2 isotherm at 273 K.
[e] Char-to-pure water ratio 1:20 (w/v), after gentle mixing for 48 h.
[f] ND: not determined.

while it stirred and placed into 125 ml Erlenmeyer flasks containing 10 g of each dried biochar. The flasks were agitated on an orbital shaker (125 rpm) for 25–28 h at 25 °C. Tenfold dilutions in sterile normal saline were prepared and 0.1 ml was spread onto plates of 10% Tryptic soy agar (TSA) (Difco, Inc., Detroit, MI) (dilutions 10^{-4}–10^{-5} g soil/ml) and King's B agar (Dhingra and Sinclair 1985) (dilutions 10^{-3}–10^{-4} g soil/ml). There were three plates per dilution. Plates were incubated in the dark at 25 °C. The experiment was repeated three weeks later using fresh soil. Colonies of heterotrophic bacteria were counted daily on the TSA plates for 3 days and summed. Total counts between 30 and 300 colonies/plates were used for estimating bacterial densities. Fluorescent pseudomonads were counted under black light after 2 days. Bacteria counts were log-transformed before being subjected to analysis of variance.

Statistical methods

To assess interactions among treatments and the repetitions of the experiment, the treatment × experiment interaction term was generated. Both repetitions were combined when interaction terms were not statistically significant. Treatment means were separated using Tukey's HSD test at $P = 0.05$. Correlations among biochar properties, earthworm removal, and microbial densities were tested using Pearson's correlation matrix for continuous data and significance relationships were tested using the General Linear Model at $P = 0.05$. To assess complications from autocorrelation, Durbin Watson statistics were calculated for all significant relationships. All were found to be between 2.4 and 3.2 indicating no autocorrelation among variables. All computations were performed using Systat 13 (Software, Inc., Chicago, IL).

Results

Each biochar was analyzed for % ash content, volatiles C, surface area (N_2-B.E.T.), microporosity, micropore surface area (CO_2 GCMC), and pH, along with elemental analyses for C, H, N, P, K, Ca, Mg, Fe, S, Cu, Mn, Ni, Si, and Zn. While many properties did not differ greatly, we did observe a wide range of values for % ash, volatile C, N_2-B.E.T surface area ($m^2\,g^{-1}$), microporosity, micropore surface area, and elemental content (Table 1).

L. terrestris consistently preferred CT Char biochar and avoided CQuest biochar (Fig. 1). The IBD value for CT Char was statistically greater than all the others. CQuest, which remained relatively untouched during the experiments, had a mean IBD value that was statistically different from that of BC1, BC2 and Pure Black, but was not statistical different from the commercial biochars, Soil Reef and Agrichar nor the experimental biochar BC3. Most of the other experimental and commercial biochars did not differ greatly from one another. At the conclusion of each experiment, the earthworms were exhumed and counted to assess any mortality. We observed on average the loss of one earthworm per bin (4% mortality). Thus, none of the biochars were overtly toxic under our conditions. We made no attempt to determine the final deposition of the biochar.

Each biochar was also selected to determine whether or not its attractiveness to earthworms was associated with its ability to increase soil microbes (Table 2). We found no interaction between the biochar and the repeated experiments so all studies were combined. Biochars CT Char, BC2, Soil Reef and Agrichar had no effect on soil total aerobic bacteria. However, CQuest, Pure Black, BC1, and BC3 increased bacterial densities more than ten-fold. Activated carbon had no effect on aerobic bacteria when compared to the control, and was not statistically different from Soil Reef, Agrichar, BC12 and

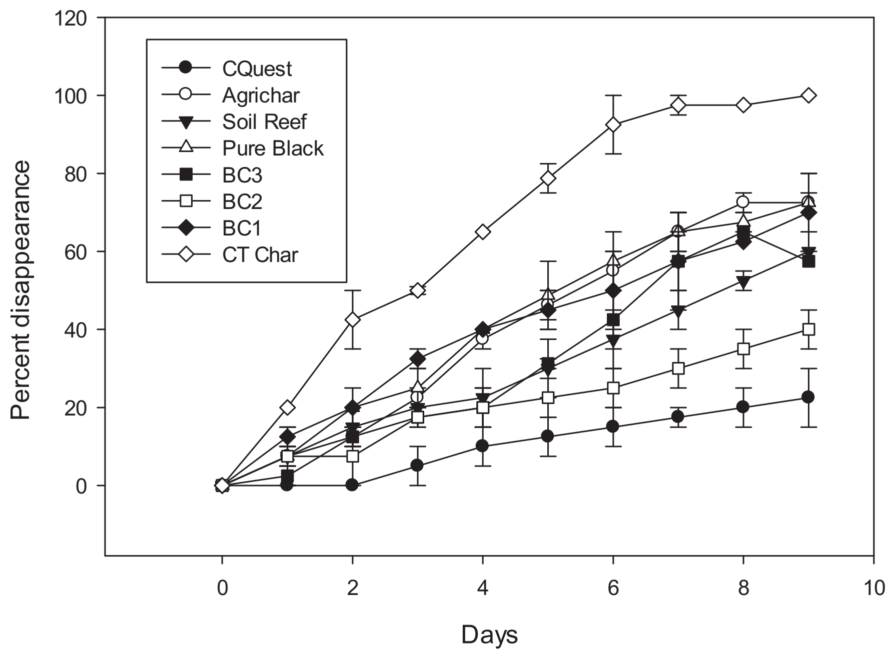

Fig. 1. Biochar removal (% disappearance) by earthworms over time. Each point represents the mean of eight replicates. Error bars represent the standard error of the means.

BC3. Only Pure Black biochar was able to increase the fluorescent pseudomonads in soil significantly.

Weak but significant relationships were observed between earthworm preference and percent carbon of the biochar ($R = -0.47$, $P < 0.01$) (Table 3). Similarly, positive associations exist between earthworm preference and ash content ($R = 0.38$, $P = <0.05$), Ca content ($R = 0.41$, $P < 0.05$), Mn content ($R = 0.42$, $P < 0.05$), and Si content ($R = 0.39$, $P = 0.05$) of the biochar. The parameter K content just missed statistical significance ($R = -0.37$, $P = <0.07$). Interestingly, the ability of biochar to increase soil bacteria was not associated with enhanced removal by earthworms. In fact, many biochar variables that favored increased densities of bacteria were not associated with removal by earthworms; these included % H, atomic H/C, volatile carbon, surface area (N_2-B.E.T), micropore surface area, and microporosity. However, there were two biochar properties (Ca and Mn content) that were associated both with earthworm removal and with increased densities of total aerobic bacteria and fluorescent pseudomonads. Calcium levels in the biochar were positively related to earthworm removal ($R = 0.41$, $P < 0.05$), soil aerobic bacteria ($R = 0.44$, $P < 0.05$) and fluorescent pseudomonads ($R = 0.47$, $P < 0.05$). Similarly, Mn content was positively related to earthworm removal ($R = 0.42$, $P < 0.05$), soil aerobic bacteria ($R = 0.48$, $P < 0.05$) and fluorescent pseudomonads ($R = 0.41$, $P < 0.05$).

Table 2
Earthworm feeding preference of biochars, and their effect on soil bacteria and fluorescent pseudomonads.

Biochar	Integrated estimate of disappearance over time[a]	Total aerobic bacteria (log cfu/g soil)	Fluorescent pseudomonads log cfu/g soil)
CT Char	460 a[b]	7.02 ab	6.53 ab
BC1	303 b	7.70 a	6.83 ab
BC2	274 bc	6.09 b	5.39 a
Pure Black	269 bc	7.62 a	7.11 b
Soil Reef	221 bcd	6.41 ab	6.09 ab
AgriChar	189 cd	6.96 ab	6.24 ab
BC3	184 cd	7.37 a	6.81 ab
CQuest	85 d	7.79 a	6.89 ab
Activated Carbon	ND[y]	6.08 b	5.80 a
Control – No Biochar	ND	5.99 b	5.92 a

[a] Based on visual estimates of disappearance by earthworms integrated over time to produce an integrated biochar disappearance value (IBD); $IBD = \Sigma(Y_i + Y_{(i+1)})/2 \times (t_{(i+1)} - t_i)$, and where Y_i = the percentage of biochar present at time, t_i, relative to Day 0.
[b] Tukey's Test ($P = 0.05$).

Discussion

Numerous studies have used earthworms in avoidance/detoxification studies to assess the toxicity of biochar-amended soils (Weyers and Spokas, 2011). In a few of these studies, earthworms actually preferred biochar-amended soil over non-amended soils (Chan et al., 2008; Hale et al., 2013; Van Zwieten et al., 2010). These observations were often explained by biochar's ability to reduce soil acidity (Chan et al., 2008; Liesch et al., 2010; Topoliantz and Ponge, 2005; Van Zwieten et al., 2010) or as an indirect response of the positive feedback that typically occurs when carbonaceous material is added to soil (Ponge et al., 2006). The current study is novel in that it demonstrates removal or possibly active consumption of biochar particles by earthworms rather than showing just a preference for the habitat in which they reside. The courts were designed to assess if there was preferential removal of different biochars by earthworms. More detailed studies are needed to show that biochars are consumed and passed through the guts of earthworms. Nevertheless, we conclude that the disappearance of the biochar sample over time was an accurate reflection of the attractiveness of the biochar to the earthworms. Starvation factors were ruled out, since all earthworms were furnished with sufficient food substrates and had equal opportunities to interact or not with each biochar in these randomized trials. In addition, recognizing that wetting the biochar can mitigate earthworm avoidance (Li et al., 2011), we ensured the biochars were sufficiently moist at the beginning of the study and during its course by misting the samples whenever food substrates were added. Nevertheless, we cannot rule out the possibility that the results may have been influenced by differences in water repellency and/or drying rate of the biochars.

We demonstrated that earthworms prefer certain biochars more than others. In every trial, the CT Char biochar was consistently removed first by the earthworms and the CQuest biochar was

Table 3
Pearson correlation coefficients (*R*) among biochar properties, active consumption by earthworms and effect of biochar on soil bacteria.

Property	Earthworm feeding	Aerobic bacteria	Fluorescent pseudomonads
C, % dry weight[a]	−0.47**[f]	Ns	Ns
H, % dry weight[a]	Ns	0.57***	0.52***
Atomic H/C	Ns	0.47*	0.42*
Ash, % dry weight[a]	0.38*	Ns	Ns
Volatile carbon, % of volatile plus fixed C[b]	Ns	0.52***	0.47***
N_2-B.E.T surface area ($m^2\,g^{-1}$)[c]	Ns	0.61***	0.63***
CO_2 GCMC cumulative surface area, 0–1.4 nm ($m^2\,g^{-1}$)[d]	Ns	0.62***	0.52***
CO_2 cumulative porosity (0–1.4 nm) ($cm^3\,g^{-1}$)[d]	Ns	0.63***	0.53***
pH [e]	Ns	Ns	Ns
Ca	0.41*	0.44*	0.47*
K	−0.37[g]	0.59**	0.41*
Mn	0.42*	0.48*	0.41*
Si	0.39[h]	Ns	Ns
Earthworm feeding	–	Ns	Ns
Aerobic bacteria		–	0.92***
Fluorescent pseudomonads			–

[a] Galbraith Laboratories, Inc., Knoxville, TN, USA. Ash content was residue after combustion at 900 °C.
[b] Hazen Research, Inc., Golden, CO, USA.
[c] Based on Brunaur–Emmett–Teller fit of the N_2 adsorption isotherm at 77 K.
[d] Based on Grand Canonical Monte Carlo Density Functional Theory analysis of the CO_2 isotherm at 273 K.
[e] Char-to-pure water ratio 1:20 (w/v), after gentle mixing for 48 h.
[f] Values followed by *, **, or *** are statistically significant at $P < 0.05$, 0.01 or 0.001, respectively, Ns is not significant at $P < 0.05$.
[g] $P = 0.07$.
[h] $P = 0.06$.

consistently avoided. The CT Char is an aged biochar that was produced in charcoal kilns in Connecticut over 70 years ago following the 1938 hurricane that hit the Northeastern United States and made massive amounts of plant biomass available. The CT Char has the lowest C content and among the highest Si, Ca and Mn contents, suggesting it may have some soil mixed in with it. Although we did not study aging as a factor in these biochars, it is known that aging increases the O content of biochar (Liang et al., 2008), thus allowing the surface and pores to be coated more readily by natural organic matter and mineral deposits (Pignatello et al., 2014), but it is unclear whether and how these changes might affect earthworm preference. We observed correlations between the percent carbon (negative), ash content (positive), Ca (positive), and Mn (positive); these correlations were not strong, but passed statistical significance at $P < 0.05$.

It is not clear how carbon content in biochar would affect earthworm activity. The carbon content in biochar can be a function of the pyrolysis temperature (Crombie et al., 2013; Cao and Pignatello (2012)). Temperature was not examined in the current study, but it is interesting that Chan et al. (2008) found that earthworms favored soil amended with biochar produced at 450 °C, but not at 550 °C. Chan et al. (2008) study also found higher %C in biochar produced at high pyrolysis temperature suggesting that %C may be associated with earthworm activity.

The relationship between earthworm preference and biochar Ca was not surprising. The positive impact of Ca and liming on earthworm activity is well known (Edwards, 1998), since earthworm castings are coated with mucilaginous gel containing Ca. Future studies might examine the role of adding lime to biochar to increase its attractiveness to earthworms. The positive correlation between biochar Mn or Si and earthworm preference is intriguing. Previous studies found that biochar-amended soil suppressed disease and increased Mn content in plants, presumably through microbial transformation of Mn oxides by rhizosphere fluorescent pseudomonads (Elmer 2009; Elmer and Pignatello, 2011). Moreover, biochar Si was associated with earthworm removal and has also been implicated in plant disease suppression (Datnoff et al., 2007).

Biochar properties that might explain the difference in preference by the earthworms are not abundantly clear. Since earthworms obtain the majority of their nutrition from the microflora of the soil (Edwards, 1998), we tested whether biochars differed in their effect on soil microbial densities. We found no clear pattern. All biochars except BC2 increased total aerobic bacteria, whereas only Pure Black increased the plant-promoting, fluorescent pseudomonads. Biochar removal correlates weakly with Mn content, and Mn content correlates with aerobic bacteria and fluorescent pseudomonads. It remains to be seen if Mn levels in the feedstock correlate with earthworm preference of the derived biochar.

The actual mechanism for how these parameters influence earthworm activity is not clear. This procedure may be useful in screening biochar for attractiveness to earthworms. Many questions and challenges remain before biochars can be tailored for delivery by earthworms to lower soil horizons. Studies are currently underway to address these questions. Filling in these knowledge gaps may allow for specific biochars to be prescribed for specific uses.

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