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Review Article

Impact of Biochar on Earthworm Populations: A Review

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Despite the overwhelming importance of earthworm activity in the soil system, there are a limited number of studies that have examined the impact resulting from biochar addition to soil. Biochar is part of the black carbon continuum of chemo-thermal converted biomass. This review summarizes existing data pertaining to earthworms where biochar and other black carbon substances, including slash-and-burn charcoals and wood ash, have been applied. After analyzing existing studies on black carbon, we identified that these additions have a range from short-term negative impacts to long-term null effects on earthworm population density and total biomass. Documented cases of mortality were found with certain biochar-soil combinations; the cause is not fully understood, but hypothesized to be related to pH, whether the black carbon is premoistened, affects feeding behaviors, or other unknown factors. With wood ashes, negative impacts were overcome with addition of other carbon substrates. Given that field data is limited, soils amended with biochar did not appear to cause significant long-term impacts. However, this may indicate that the magnitude of short-term negative impacts on earthworm populations can be reduced with time.

1. Introduction

The importance of earthworms in soil genesis (i.e., bioturbation) has long been recognized and dates back to the 1800's with some of the initial work by Charles Darwin [1]. In his seminal publication, Darwin [2] noted that earthworm burrowing and casting activity together were the primary force in mixing soil layers and burying surface debris. Through this bioturbation, earthworms increase soil porosity affecting soil aeration as well as water infiltration. Earthworm casts are also important protective and dispersal vehicles for soil microbes and nutrients. Taken altogether, earthworms have been recognized as ecosystem engineers, or organisms that can have a profound influence on the structure and functioning of soils [3]. By way of function, earthworms have profound direct and indirect impacts on the availability of nutrients, particularly through increased decomposition of plant residues and turnover of soil organic matter. Thus, what positively or negatively affects soil biota [4] may indirectly affect soil function and plant growth.

The functioning of intensively managed soil systems has increasingly become dependent on external inputs to maintain high levels of productivity. Management practices which degrade soil organic matter, including heavy tillage, degrade a soil's inherent quality and reduce fertility [5, 6]. For soil quality improvement, recommendations call for organic inputs of animal manures, green manures and cover crops to replace lost carbon, and reduction of tillage to prevent soil loss and/or rapid C turnover [7, 8]. Longstanding evidence points to the positive increases in earthworm populations when amending soils with organic inputs. Along these same lines, increased plant productivity [9] is frequently cited, but with high abundance of large surface-continuous macropores associated with deep burrowing species [10] increased loss of nutrients through infiltration could occur [11].

In a new drive to improve soil fertility and increase C sequestration, recommendations to amend soil with biochar, which is black carbon, are surfacing around the globe. However, recent evidence has indicated that some biochars may have negative effects on the soil biota, in particular earthworms [12]. Potential mortality aside, earthworms may interact with biochar amendments to increase macro- and micronutrient availability, in positive (e.g., increased plant productivity; [13]) or potentially detrimental ways (e.g., increased leaching of heavy metals; [14]). The availability of information to determine what likely will happen to earthworm populations, nutrient cycling, and overall soil function with land application of biochar is limited. The purpose of this review is to evaluate the existing data on earthworm effects from biochar application. We will define biochar and delineate the direct and indirect impacts of biochar and like substances on earthworms, including Enchytraeidae, and their associated soil functions. We will identify knowledge gaps and provide recommendations for future research directions.

2. What Is Biochar?

The terms and definitions applied to black carbon and "biochar" are dynamic [15]. In its current application, biochar is the solid residual remaining after biomass pyrolysis, which is produced as a vehicle of atmospheric carbon sequestration [16, 17]. Biochar spans the entire continuum of black carbon residual thermochemical conversion products [18]. The International Biochar Initiative extends this definition to describe the enhanced black earths, or *Terra preta* soils, formed by historical inputs of pyrolyzed agricultural waste or other organic material turned into a soil enhancing amendment and currently shown "to improve soil functions and to reduce emissions" of greenhouse gases [19]. However, it is important to realize that the unique aspect of biochar is rooted in the carbon sequestration potential.

Research relevant to biochar encompasses studies on black carbon which includes black earths, wildfire charcoals, chars, and wood ash. For instance, numerous studies have examined the potential impacts of biochar amendments on soil fertility [20–23] and greenhouse gas production [24–29]. Evaluation of biochar stability [18, 30] and economic/life cycle analyses [31–35] has also been performed. These studies and others evaluating potential implications of biochar which are not pertinent to earthworms are reviewed elsewhere [4, 36–39]. In evaluating these biochar studies caution does have to be applied as the method of production, that is, temperature and oxygen conditions, in addition to the feedstock will affect the chemical and physical properties of the biochar produced [18] and likely their impact on the soil environment. Thus, allowing for biochar customization for a particular soil improvement need [40].

3. Lab and Field Studies on Biochar

The majority of studies on biochar, and related materials, conducted over the last few decades have been laboratory assays. The converted feedstocks evaluated ranged from crop residues to manures, to hardwood and softwood materials. The conversion products can be placed into three categories: charcoals resulting from slash-and-burn; synthetic biochar produced for industrial purposes; and wood ash. Though less clearly related to biochar, wood ash, which has a lower carbon content than biochar, is analogous to biochar amendments because of similarity in the liming impact, soil fertility, and soil moisture content alterations [41–43]. Various direct impacts on earthworm behavior, growth, survivorship, population dynamics, and cell damage have been observed. These impacts along with characteristics of the material tested, study location, soil type and pH, and earthworm species are summarized in Table 1. As few field studies were available, we present new data on field populations of earthworms potentially impacted by application of synthetic biochars (Figure 1; see Section 3.4.1).

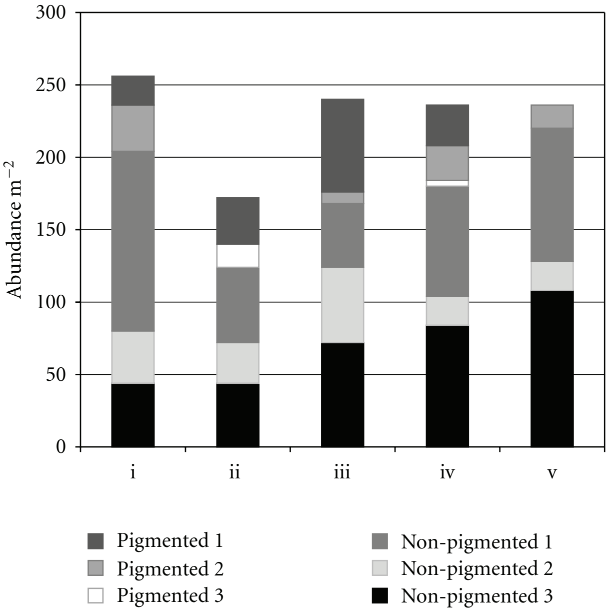

Figure 1: Abundance of earthworms by pigmentation and size class, in biochar plots sampled at Rosemount, MN; treatments are (i) control (no amendment), (ii) composted manure; (iii) fast pyrolysis hardwood biochar, (iv) fast pyrolysis hardwood biochar + manure, (v) fast pyrolysis macadamia nut biochar. See text for description of size class.

3.1. Slash-and-Burn Char. Slash-and-burn practices are often used to prepare forested land or fallow land with existing crop residues for subsequent crop production. Charcoal additions along with slash-and-burn practices paved the way for the formation of carbon-dense fertile black soils [44] (see Section 3.4). Under this premise, Topoliantz and Ponge [45, 46] undertook the evaluation of an earthworm's reaction to charcoal obtained from a slash-and-burn field in laboratory analyses. In these two studies, a geophagous tropical peregrine earthworm, *Pontoscolex corethrurus*, was presented with pure soil (Oxisol), pure charcoal, or 60% sieved (<2 mm) wood-derived charcoal-soil mixtures, and growth rates, ingestion, burrowing, and casting activity were evaluated. The exact pyrolysis conditions producing the charcoal are not known. However, neither study indicated any pronounced effect on earthworm survivorship or growth

Table 1: Summary of laboratory (L) and field (F) studies using biochar and wood ash and reporting direct impacts on earthworms.

Study	Earthworm species	Study type	Charcoal/biochar description and application rate	Location	Soil/pH	Earthworm impact
Topoliantz and Ponge [45]	*Pontoscolex corethrurus*	L	Char from slash-and-burn field 60% char mixture	French Guiana	Oxisol pH 4.6 inc 6.9	Normal growth rates; less casting and burrowing activity in char:soil mixtures; 1 of 10 earthworms died
Topoliantz and Ponge [46]	*Pontoscolex corethrurus*	L	Charred wood from slash-and-burn 60% char mixture	French Guiana	Oxisol PH 4.2–4.6 inc 6.9	Nonsignificant growth increases; direct charcoal consumption observed; surface cast production greater with soil : char mixture
Topoliantz et al. [74]	*Pontoscolex corethrurus*	L	"local homemade charcoal" no further description One half of a 67 L m^{-2} mixture of organic amendment mounded and covered with soil	French Guiana: (3°39′N; 54°2′W)	Oxisol pH 4.4 inc 4.9	Decrease in abundance of juveniles not significant; decrease in number of cocoons significant (charcoal + saw dust only)
Chan et al. [49]	Earthworms, spp. not stated	L	Poultry litter Slow pyrolysis 450 (pH 9.9) and 550 + steam activation (pH 13) 0, 10, 25 and 50 t ha^{-1}	Australia	Alfisol pH 4.8–5.0 inc 6.0–7.8	Earthworms showed no preference/avoidance for soil over soil : char mixtures (specific data on char concentrations not provided); preference better for lower pH char
Cui et al. [47]	*Eisenia fetida*	L	Crop ash from burned rice residue 1, 3, 5, and 10% mixtures	China	Sediment pH = 6.9	No mortality, but genotoxicity (damage to earthworm DNA) occurred at ash concentrations of 10%
Van Zwieten et al. [22]	*Eisenia fetida*	L	Wood chip biochar Slow pyrolysis, 550°C 50 : 50 and 30 : 70 Paper pulp sludge to 10 t ha^{-1} 2% Ferrosol 1.5% calcarosol	Australia	Ferrosol pH 4.2 inc'd to 5.9 Calcarosol pH 7.6 did not change	Biochar : Ferrosol mixture preferred; no preference for biochar : Calcarosol mixture; biochar with 70% wood chips slightly more preferred over biochar with 50% wood chips

Table 1: Continued.

Study	Earthworm species	Study type	Charcoal/biochar description and application rate	Location	Soil/pH	Earthworm impact
Liesch et al. [12]	*Eisenia fetida*	L	Poultry litter and pine chip biochars 400°C 30 min 0, 22.5, 45, 67.5, and 90 Mg ha^{-1}	US	Simulated soil pH initially 7.0	Poultry litter biochar mortality and weight loss increased with application rate; Pine chip biochar no significant effect on mortality
Li et al. [50]	*Eisenia fetida*	L	Apple wood chips Batch reactor slow pyrolysis 525°C 90–180 t ha^{-1} 1, 10, 20% mixtures	US	Simulated soil pH initially ~7.0	Avoidance of biochar amended soil—eliminated by wetting biochar; weight loss increasing with application rate
Gomez-Eyles et al. [55]	*Eisenia fetida*	L	Deciduous, hardwood-derived biochar; 600°C 10% char mixture	UK	Contaminated soil, type not statedpH 7.63	Weight loss observed with biochar; reduced contaminant accumulation in body tissue
Husk and Major [75]	*Earthworms spp. not stated*	F	Hardwood waste material Fast pyrolysis 5.6 Mg ha^{-1}	Canada	Soil type not stated; pH variable between 6.4–7.4	Generally higher abundance in biochar plots but not statistically compared
Current study	European lumbricids	F	Wood-derived biochar (+/− manure) by fast pyrolysis Macadamia nut— derived biochar by slow pyrolysis 22.5 Mg ha^{-1}	US	Waukagan silt loam; pH 6.3–6.6	No impact on field populations
Haimi et al. [56]	*Cognettia sphagnetorum*	F	Wood ash 1000 and 5000 kg ha^{-1}	Finland	Forest soil (podzolized sandy soil)	Decreased abundance following 2500 and 5000 kg ha^{-1} Insignificant decrease at 1000 kg ha^{-1}

TABLE 1: Continued.

Study	Earthworm species	Study type	Charcoal/biochar description and application rate	Location	Soil/pH	Earthworm impact
Liiri et al. [58]	*Cognettia sphagnetorum*	L	Wood ash 5000 kg ha^{-1}	Finland	Pine forest humus pH 4.7–5.8 inc. 6.8–7.8	Decreased biomass but only when wood ash mixed into treated humus
Liiri et al. [59]	*Cognettia sphagnetorum*	L	Wood ash 5000 kg ha^{-1}	Finland	Pine forest humus pH 4.5	Decreased biomass
Cox et al. [60]	Earthworms; spp not stated	F	Coal ash110 t ha^{-1}	US	Naff silt loam	No significant difference in total biomass or abundance
Nieminen [61]	*Cognettia sphagnetorum*	L	Wood ash	Finland	Norway Spruce forest humus pH 4.6	Decreases with solely wood ash No significant effect with the combination of sucrose + wood ash
Lundkvist [89]	*Cognettia sphagnetorum*	L/F	Wood ash	Sweden	Forest soil	No significant differences
Huhta et al. [90]	*Cognettia sphagnetorum*	F	Wood ash	Finland	Forest soil	Decreased biomass following ash addition; Controls lacked earthworms; few earthworms found where ash applied
Lundkvist [62]	*Cognettia sphagnetorum;* Earthworms species not stated	F	Wood ash Wood ash +/− NH_4NO_3	Sweden	Forest soil	No population effects; Increased Cd in body tissue; Increase in earthworm population after 2 yrs
Nieminen [91]	*Cognettia sphagnetorum*	L	Wood Ash (0.5 Mg ha^{-1})	Finland	Mineral soil pH 6	Wood ash reduced enchytraeid size, but no significant effect on total biomass
Nieminen and Haimi [92]	*Cognettia sphagnetorum*	L	Wood ash (birch ash)	Finland	Norway Spruce forest humus pH 4.6	Initially decreased body size; lower reproductive rates

rates (Table 1). The presence of charcoal did affect earthworm activity, as discussed in Section 4. One noted impact of the charcoal application was the increase in soil pH from moderately acidic to nearly neutral pH values (Table 1). The neutralizing of soil pH was provided as a reason why earthworms were not deterred from burrowing into the charcoal-soil mixtures [46].

The prospect of using char from slash-and-burn management practices in rice crops in China for reducing environmental contaminants was evaluated [47]. Rice crop residue was charred in the laboratory to mimic field slash-and-burn residue and mixed into sediment of 4.7% C and a pH of 6.9. Addition of the rice char raised the C content of the sediment to 11% but resulting pH was not reported. Sorption of organic pollutants to the char was determined by evaluating the genotoxicity to the earthworm *Eisenia fetida*. The charred rice crop residue, containing some black carbon, at low mixture rates (1%, 3% and 5%) reduced genotoxic damage of the organic pollutants, however at a mixture rate of 10% the rice-char itself caused genotoxic damage to the earthworm [47].

Even though the chars used in these studies [46, 47] are both considered slash-and-burn residue, the materials are quite different as indicated by the high C content of the wood-derived char-soil mixture (39% C) versus the low C of the rice-derived char-soil mixture (11% C). Therefore, one could expect different responses due to the inherent differences in black carbon chemistries. The low C content of the rice char also indicates that this material was likely more ash, and probably had high residual mineral content as well. Application rate was another substantial difference between the studies. Regardless, even though genotoxic damage occurred in one case, short-term survivorship was not affected by either slash-and-burn product. In comparing these studies, the type of char rather than any soil or resultant pH effect probably contributed most to the observed outcome. This illustrates the current limitation in further comparisons, due to the lack of adequate characterization and documentation of the black carbon additions.

3.2. Synthetic Pyrolysis Char. Slow or fast pyrolysis in small batch reactors has allowed small scale synthetic production of biochars from feedstocks including hardwood, softwood, poultry litter, and tree nut shells at temperatures ranging as low as 400°C to as high as 600°C (Table 1). Laboratory and field testing of these biochars at rates of 5 to 180 $\mathrm{Mg\,ha^{-1}}$ have been conducted. In laboratory trials, standard preference/avoidance assays have been used whereby earthworms, typically *E. fetida*, are offered a choice between a soil containing no char and a soil containing biochar at increasing concentrations. Two of the three studies using this approach showed no preference to slightly greater preference for the biochar-soil mixtures over the nonamended soil [48, 49]. In the third study [50] earthworms significantly avoided both a 10% and 20% apple wood chip biochar-soil mixture, until the biochar was prewetted (see below).

Toxicity of wood-derived and poultry litter-derived biochars were directly tested in 28-day or longer-term incubations. In a 28-day assay, Liesch et al. [12] examined the impact of two biochars (pine chip and poultry litter) on the mortality and growth of earthworms (*E. fetida*) in a simulated soil (70% sand, 20% kaolin, and 10% sphagnum peat). The authors attributed mortality and reduced growth rates at the two highest biochar amendment rates, 68 and 90 $\mathrm{Mg\,ha^{-1}}$, to alterations in soil pH. They also noted a quick mortality (within the first five days) with poultry litter biochar amended soils. The authors speculated that this could be due to the rapid pH alteration or ammonia concentration [12]. It is well established that earthworms are sensitive to pH [51, 52]. However, other causes of quick mortality in earthworm studies have been observed. For instance, Schmidt et al. [53] observed initial mortality (within the first 7-d) of earthworms during studies with dried maize residue, which they attributed to potential physical damage arising from the dry material sticking to the earthworm's body. More recently, Li et al. [50] discovered that once biochar was premoistened, the initial avoidance of the biochar by earthworms was overcome. Similarly, once the corn stover residue was premoistened, initial mortality disappeared [53]. Since the moisture status of biochars could be different due to different chemical and physical properties [23, 39, 54] as well as storage conditions, the alteration of earthworm behavior by dry biochar additions is a probable cause of short-term negative impacts observed in earthworm-biochar incubations, as noted by Li et al. [50].

Li et al. [50] evaluated biochar toxicity in a 28-day lab incubation and found that the biochar at 10% and 20% mixtures significantly increased weight loss over controls, but did not affect reproduction. Polyaromatic hydrocarbons (PAHs) were detected in the biochar at concentrations below environmental concern, but no evidence of oxidative stress, indicating uptake of these potentially toxic compounds, occurred [50]. Gomez-Eyles et al. [55] also conducted 28-day and 56-day toxicity studies but the scope of the study was designed to evaluate the deciduous hardwood-derived biochar as a bioremediation tool. The biochar was mixed into a contaminated soil collected from a gas works site at 10%. Although no earthworms died, they did lose weight, and after 56-d did uptake PAHs and heavy metals from the contaminated soil; however, in the presence of biochar they were found to have a reduced accumulation of contaminants in their body tissue [55]. Notably, however, Gomez-Eyles et al. [55] attributed the observed weight loss primarily to the presence of biochar. However, it was uncertain if reduced feeding activity of contaminated soil in the presence of biochar (see Section 4) was responsible for the reduced body accumulation of contaminants.

3.3. Wood Ash. There have been mixed observations of earthworm dynamics following wood ash additions, but many studies reported reductions in population numbers (Table 1). Haimi et al. [56] noted a virtual immediate decrease (within 20-d) in earthworm numbers when wood ash was added to soil, but difference in the microarthropod population took 4 months to develop, with total numbers of microarthropds being decreased at the highest two amendment levels (2500 and 5000 kg ha^{-1}). However, the general conclusion was that wood ash above 2500 kg ha^{-1} decreased earthworm population densities. This is interesting since the overall impact on microbial populations (microbial biomass C or fungal ergosterol) was insignificant among the wood ash additions from this same study [57]. The total biomass of enchytraeid worms was also reduced by wood ash application when it was mixed with the soil (5000 kg ha^{-1}) [58]. In further studies, the negative impact on *Cognettia sphagnetorum* (Enchytraeidae) populations was confirmed when wood ash was added solely to acidic forest soils [59]. Cox et al. [60] observed that there was no significant difference on total mass or abundance of earthworms in coal ash amended soils, despite the alkaline nature of the amendment.

However, these decreases in enchytraeid populations from wood ash additions can be overcome. If the wood ash was left on the soil surface and not incorporated, no significant impact on enchytraeid numbers was observed [58]. Nieminen [61] observed that the negative impacts of wood ash additions on the enchytraeid populations could be overcome through labile carbon additions. Population numbers were also noted to increase 2 yrs after an ash application occurred [62].

3.4. Historical Impacts and Other Field Studies. Earthworm populations are prevalent in many soil systems where charcoal from natural fires or controlled burns occurs. Populations of native *Diplocardia* spp. (Megascolecidae) occur in the subtropical southern half the United States [63, 64]. These megascolecids, including *Diplocardia mississippiensis*, influence nutrient cycling in fire-controlled pinelands [65, 66]. Populations of European lumbricids along with native megascolecids are found in fire-affected Southern California chapparal soils [67, 68], where they also are important to nutrient availability [69]. In tropical regions, populations of the peregrine earthworm species, *P. corethrurus*, are capable of translocating charcoal residues from slash-and-burn land clearings deeper into the soil profile [70]. This activity by *P. corethrurus* indicates its potentially vital role in stabilizing organic matter and historical development of *Terra preta* soils [46, 71]. Because of their vital role, some researchers have proposed that earthworms, particularly in the tropics, can be used as ecosystem engineering tools to maintain and/or improve soil fertility and ecosystem function [72] particularly in conjunction with charcoal additions [73].

Limited studies were available reporting earthworm populations in agricultural systems receiving biochar-like substances or biochar; these studies included [74, 75]. Topoliantz et al. [74] observed a difference in earthworm abundance for combined charcoal + other organic amendments, compared to a natural fallow field. However, in this study they observed no statistically significant differences at harvest time in the distribution of cocoons and adults. The authors did not compare the results to an equivalently tilled field, nor did they evaluate the impact of a charcoal-only amendment, which complicates the comparisons. For the total numbers, all tilled treatments reduced the numbers of earthworms, which is known to occur in other studies on the impact of tillage [76]. Husk and Major [75] provide a nonpeer reviewed report on earthworm populations sampled by handsorting and mustard application over a two-year nonreplicated study on field application of a wood-derived biochar. Their first sampling was taken two months after biochar application at a rate of 5.6 Mg ha^{-1}. Earthworm populations in six out of eight sample dates were generally greater in the biochar plot than the control plot, however, standard error bars from within-plot replicates generally overlapped, suggesting lack of significant statistical differences between biochar and control plots.

3.4.1. Rosemount Biochar Field Plots. Earthworm abundance was evaluated in a subset of experimental biochar plots at the University of Minnesota Research and Outreach Center in Rosemount, MN USA (44°N, 93°W). These plots are part of the USDA-ARS multilocation biochar and pyrolysis research effort (Spokas, unpublished). Eight treatments were established using a completely randomized design with three replications: (i) control (no amendment), (ii) composted manure, (iii) fast pyrolysis hardwood biochar, (iv) fast pyrolysis hardwood biochar + manure, (v) fast pyrolysis macadamia nut biochar, (vi) slow pyrolysis wood pellet biochar, (vii) slow pyrolysis wood waste biochar, and (viii) a slow pyrolysis hardwood biochar. Each individual plot measures 4.88 m on a side ($16' \times 16'$) with a 3-m ($10'$) buffer zone between plots. The biochar was applied at a rate of 22.5 Mg ha^{-1} and incorporated by rotary tillage (15 cm depth) in the fall of 2008.

Earthworm assessments were made in the spring of 2011 after two full years of continuous no-till corn. Sampling within a circular 0.25 m^2 area in each of five treatments was aided by an electrical field sampling device [77]. Briefly, three step-increases in field strength with corresponding alterations in the electrical field orientation by an octet arrangement of electrical poles were conducted over a 20 minute sampling period. A two- or three-pole electrical field was held for approximately two minutes with the increase in the field strength made after a complete circuit was accomplished. Earthworms were removed once fully exposed at the soil surface and placed in a bucket for quantification. Earthworms were classed as pigmented or nonpigmented then sorted into three size categories and counted. Size categories were roughly equivalent to hatchlings, juveniles and near-clitellate adults within pigmentation class, and actual lengths do not specifically overlap. One plot was manually excavated within the circle influenced by the electrical sampling device and hand-sorted; a total of 82% of the earthworms were retrieved by the electrical device in this plot, additional confirmation checks were not performed. Only one near-clitellate adult earthworm, *Aporrectodea* sp. possibly *A. rosea*, was sampled in the fast pyrolysis + manure treatment (treatment iv), no clitellated or other near-clitellate earthworms were observed. Due to the field logistic issues and the sampling time required per plot, no replicates were achieved. However, based on this limited data there were no drastic impacts on total earthworm abundance as a function of the different biochar types after two years in field production (Figure 1). The reduced abundance in the composted manure treatment was of unknown cause, but probably related to spatial heterogeneity of earthworm populations, since all plots were fertilized equivalently (accounting for initial manure-N in year 1 only).

The field studies indicate that biochar, charcoal, or occurrence of fire does not significantly affect long-term field populations of earthworms. In the studies evaluated however, short-term impacts, those which may occur within the first several days to weeks after burning or application, are unknown, with one noted exception: successful harvesting of *Diplocardia spp*. for the fishing industry (bait) is known to take place primarily in recently (within days) control-burned forest areas in the Appalachicola National Forest, Florida, USA [64]. Topoliantz and Ponge [45, 46] have already shown that *P. corethrurus* was unaffected by biochar in short-term studies, but field application rates were difficult to discern. As with Husk and Major [75] a low application rate of biochar, particularly one derived from wood, might not have had a substantial effect in the field. However, probable short-term effects with higher biochar application rates could not be substantiated by the Rosemount field trial because population assessment occurred two years after application.

4. Biochar Effects on Earthworm Activity

Some details on earthworm activity, including burrowing, feeding and casting, were available in the studies evaluated. Effects on earthworm mating activity, assessed via cocoon production, were noted earlier. Observations on earthworm casts and gut materials indicate that charcoal fragments are ingested by earthworms [45, 46, 71, 78]. Topoliantz and Ponge [45] utilized 2-D microcosms to study *P. corethrurus* activity in soil and charcoal amended soil. Two soils were placed in a plexiglass frame, which enabled viewing of earthworm burrowing and casting activity. Ten replicates were run and they observed a few significant differences. The first was a drastic difference in the burrowing activity, with 14.6 cm^3 of burrows created in the soil only side and a total burrow volume of 1.7 cm^3 on the soil + charcoal side [45]. This data suggests that *P. corethrurus* did not prefer the environment in the soil + charcoal side. Furthermore, there were significant differences in the volume of casts, with 5.5 cm^3 in the soil alone and 0.3 cm^3 in the charcoal + soil side. Even though the cast density was lower, the earthworm still ingested and created some casts with charcoal. Furthermore, and perhaps most important, there was an absence of feeding burrows observed in soil + charcoal side, with all feeding burrows present solely in the control soil. This observation, coupled with the differences in the cast production, would suggest that this particular charcoal was not being utilized by the earthworms as a food source [45] and suggested that the earthworms were pushing the charcoal bits aside rather than ingesting them.

Even though charcoal has been found in earthworm gut material, ingestion does not necessarily indicate utilization as an energy source. Ingestion of a basic pH charcoal would modify earthworm internal gut pH, which could assist in the assimilation of other resources. Notably, this has been the presumed function of earthworm calciferous glands [79]. The application rate of nearly 60% charcoal could be the reason Topoliantz and Ponge [45] observed less feeding activity in the char-soil mixture whereas lower more applicable field rates might not have had the same impact. However, other laboratory studies that used lower application rates did not indicate that charcoal was a food source for earthworms, but in fact, inhibited feeding activity and induced earthworm weight loss (Table 1) [12, 50, 55]. Microbes are hypothesized to colonize charcoal [80] and may be protected within the charcoal pores. Therefore, less food might be available to the earthworms [45]. This phenomenon could also explain the lack of feeding burrows observed within the charcoal amended soil in the Topoliantz and Ponge [45] study. These observations could be true for particular soil-biochar combinations but might not be universally the case, particularly in light of the differing responses observed for different biochars and soil combinations [12, 48]. Regardless of any nutritive value, the ingestion of charcoal particles by earthworms and resulting bioturbation and transport of these particles into the soil profile is an important force in the maintenance and improvement of soil function, as discussed earlier.

5. Biochars, Soils, and Earthworm Interactions

Biochar and soil type have an influence on the response of earthworms following biochar additions (Table 1). Data from Van Zweiten et al. [48] indicates that earthworm preference is a function of both biochar and soil type. They observed the preferences of *E. fetida* in combination with two different biochars (two different mixture ratios of paper mill sludge and waste wood chips) in two different soil types [a ferrosol (productive red Australian agricultural soil) and a calcarosol (calcareous/calcite rich soil; lower productivity) [81]]. *E. fetida* preferred the biochar amended ferrosol soil compared to the unamended ferrosol soil, whereas no significant difference in earthworm preference was observed for biochar amended calcarosol soil. One aspect that stands out in this study is that the biochar addition to the ferrosol increased soil pH from 4.2 to 5.9, but addition to the calcarosol did not change pH from 7.6 (Table 1). Also notable, the biochar which had a greater proportion of waste wood to paper sludge (70 : 30 mix) was also preferred by the earthworms. In our own studies [12], survivorship of *E. fetida* on pine chip-derived biochar was higher than poultry litter-derived biochar.

Noguera et al. [13] assessed the effect of two different biochars with *P. corethrurus* on growth dynamics of rice plants in two different soils in a laboratory study. One was a eucalyptus-derived biochar made at a temperature of 350°C and applied at a rate of 2.5% to a nutrient rich Inceptisol, the second was a household-use charcoal tested at a rate of 4.5% in a nutrient poor oxisol with and without added fertility. Effects on earthworm survivorship, growth, or behavior were not reported. In mixtures of worms + biochar more plant growth was observed in the nutrient rich soil than with biochar or earthworms alone, however, an earthworm-only effect but no biochar or worm + biochar influence was found in the nutrient poor soil [13]. In a second study with only the eucalyptus-derived biochar, Noguera et al. [82], determined that there was a variable response in growth due to differences among rice cultivars when biochar and earthworms were added to the nutrient rich Inceptisol.

Beesley and Dickenson [14] applied a biochar made from hardwoods at 400°C in steel ring furnaces at 30% (volume basis) to an urban soil with a sand : silt : clay content of 83 : 16 : 1 and a 7.9 pH. They added fifteen juvenile *Lumbricus terrestris* to the mesocosms, but direct effects on the earthworms were not reported. The biochar caused a significant increase in pore water concentrations of As, Cu, and Pb within the year of testing. However, when earthworms were present, the concentrations of As and Cu in the leachate collected from biochar amended soils were decreased. The authors attributed this decrease to the effect earthworms had on reducing the concentration of dissolved organic C (DOC) as well as the amount of pore water moving through the soil chambers. Beesley and Dickenson [14] hypothesized that an increased pH (6.6 of the soil to 9.9) caused by the addition of biochar might have positively influenced the earthworms and their subsequent effects on DOC. However, they did not assess the potential for *L. terrestris* to construct and line burrows with organic matter shown to reduce leaching of organic pesticides [83]. These statements made by Beesley and Dickenson [14] support observations made earlier in this review that biochar application to soil will impact earthworm activity (see Section 4).

Despite the limited number of studies specifically examining different biochar types, the general conclusion is that there are different responses as a function of soil and biochar properties. From the existing studies, it still is not clear what particular mechanisms are responsible for these observations. However, Noguera et al. [13] determined that the interaction resulting in increased plant growth observed between biochar and earthworms was additive rather than synergistic. These data strongly suggest that soil characteristics, biochar characteristics, and plant characteristics will affect the response observed when biochar is added to soils with earthworm populations.

6. Future Steps

Field populations of earthworms occurring in fire-affected systems indicate that adaptation to the presence of natural charcoal is possible. But yet unknown is if the input of natural or synthetic biochar has or had any initial impact on the preexisting earthworm populations. Overall from this data, there is the suggestion that the short-term impacts on earthworms are either nonsignificant or negative. As indicated by our field study, earthworm populations in biochar amended plots were similar to the control plots after 2 years of continuous no-till corn production in Minnesota. Other field observations were likely made after the population was able to rebound. This interval would include the two-month interval between application and sampling as in Husk and Major [75]. Field studies using small amounts of biochar (<10 Mg ha^{-1}) potentially avoid negative short-term impacts. Field studies are needed which evaluate a greater range of application rates; preferably they would be paired with laboratory preference/avoidance assays to establish appropriate ranges of application rates. Assessment of earthworm populations are needed prior to, immediately at, and over the long term after biochar application to the soil.

In the evaluations reviewed, biochar was never clearly used as a food substrate. Although we cannot discount the potential use of biochar for digestive purposes by field populations, laboratory studies certainly showed that earthworm behaviors were altered, and soil ingestion was reduced. A few of these laboratory studies demonstrated that some biochars are likely to be potential toxins [47] but lethal results likely depend on amendment rates [12]. Beesley and Dickenson [14] did show that leaching of potential toxins, including Pb, As, and Cu, was increased with biochar application, though the biochar might not have been the direct source of these elements. Regardless, this observation runs counter to the suggested use of biochars to sorb environmental toxins [47, 55]. With earthworms active in the soil, however, reduced concentrations of potential toxins in pore water were found [14]. Thus the natural movement of biochars into the soil through earthworm activity, as shown by Topoliantz and Ponge [45, 46], might assist the use of biochar as a bioremediation tool in contaminated soils. Studies which address earthworm activity, in particular burrowing, ingestion, and casting, which might affect movement of biochar in and around the soil environment, would be useful in determining more specific interactions with soil function. Studies examining activity will also need to account for the ecological strategy the earthworm species present could be categorized into, particularly as these strategies define where and how within the soil profile they feed and burrow and the resulting affects on the soil environment [84]. Another aspect needing to be addressed is potential effects on earthworm migration. The reader is referred to Butt and Grigoropoulou [85] for information on how to properly address analysis of earthworm populations.

The complications of evaluating biochar research stem from the deficiency of many of these studies to report on elemental content, ash content, pH, soils used, feedstock material, and method of production. Though wood-derived biochars used in the studies described here had more null effects, and other biochars from mixes with sludges, manures, or crop residues had negative effects, the data also indicate pH changes in the assessment medium, whether that is field soil or simulated soil, might influence the outcome. The null to positive impacts of wood-based biochars on agronomic yields has also been observed in biochar field studies [86, 87]. There is an identified need to standardize earthworm studies [88], and adequate data must be presented on the biochar properties, the environment in which they are to be used and influence on soil biota, so future meta-analyses can be conducted. More detailed initial and final evaluations of earthworm populations in short as well as long-term studies are necessary to elucidate the immediate and lasting effects of biochar before it becomes a widespread soil amendment.

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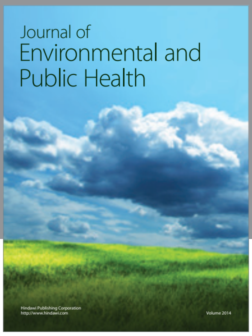

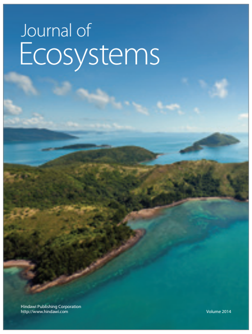

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