Impact of biochar on earthworm populations: A review.

Sharon L. Weyers[1*] and Kurt A. Spokas[2]

[1]USDA Agricultural Research Service, North Central Soil Conservation Research Lab, 803 Iowa Avenue, Morris MN 56267, USA

[2]USDA Agricultural Research Service, Soil and Water Management Unit, University of Minnesota, 1991 Upper Buford Circle, Saint Paul, MN, 55108, USA

*Corresponding Author: Sharon.Weyers@ars.usda.gov

Abstract

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 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 pre-moistened, affects on 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

22 surface-continuous macropores associated with deep burrowing species [10] increased loss of

23 nutrients through infiltration could occur [11].

24   In a new drive to improve soil fertility and increase C sequestration, recommendations to

25 amend soil with biochar, which is black carbon, are surfacing around the globe. However,

26 recent evidence has indicated some biochars may have negative effects on the soil biota, in

27 particular earthworms [12]. Potential mortality aside, earthworms may interact with biochar

28 amendments to increase macro and micro-nutrient availability, in positive (e.g. increased plant

29 productivity; [13]) or potentially detrimental ways (e.g. increased leaching of heavy metals;

30 [14]). The availability of information to determine what likely will happen to earthworm

31 populations, nutrient cycling and overall soil function with land application of biochar is limited.

32 The purpose of this review is to evaluate the existing data on earthworm effects from biochar

33 application. We will define biochar and delineate the direct and indirect impacts of biochar and

34 like substances on earthworms, including Enchytraeidae, and their associated soil functions. We

35 will identify knowledge gaps and provide recommendations for future research directions.

2. What is Biochar?

37   The terms and definitions applied to black carbon and "biochar" are dynamic [15]. In its

38 current application, biochar is the solid residual remaining after biomass pyrolysis, which is

39 produced as a vehicle of atmospheric carbon sequestration [16, 17]. Biochar spans the entire

40 continuum of black carbon residual thermo-chemical conversion products [18]. The

41 International Biochar Initiative extends this definition to describe the enhanced black earths, or

42 *Terra Preta* soils, formed by historical inputs of pyrolyzed agricultural waste or other organic

43 material turned into a soil enhancing amendment and currently shown "to improve soil functions

44 and to reduce emissions" of greenhouse gases [19]. However, it is important to realize that the

45 unique aspect of biochar is rooted in the carbon sequestration potential.

46 Research relevant to biochar encompasses studies on black carbon which includes black

47 earths, wildfire charcoals, chars, and wood ash. For instance, numerous studies have examined

48 the potential impacts of biochar amendments on soil fertility [20 - 23] and greenhouse gas

49 production [24 - 29]. Evaluation of biochar stability [18, 30] and economic/life cycle analyses

50 [31 - 35] have also been performed. These studies and others evaluating potential implications

51 of biochar which are not pertinent to earthworms are reviewed elsewhere [4, 36-39]. In

52 evaluating these biochar studies caution does have to be applied as the method of production, i.e.

53 temperature and oxygen conditions, as well as the feedstock will affect the chemical and physical

54 properties of the biochar produced [18] and likely their impact on the soil environment. Thus,

55 allowing for biochar customization for a particular soil improvement need [40].

56 **3. Lab and Field Studies on Biochar**

57 The majority of studies on biochar, and related materials, conducted over the last few

58 decades have been laboratory assays. The converted feedstocks evaluated ranged from crop

59 residues to manures, to hardwood and softwood materials. The conversion products can be

60 placed into three categories: charcoals resulting from slash-and-burn; synthetic biochar produced

61 for industrial purposes; and wood ash. Though less clearly related to biochar, wood ash, which

62 has a lower carbon content than biochar, is analogous to biochar amendments because of

63 similarity in the liming impact, soil fertility, and soil moisture content alterations [41 - 43].

64 Various direct impacts on earthworm behavior, growth, survivorship, population dynamics, and

65 cell damage have been observed. These impacts along with characteristics of the material tested,

66 study location, soil type and pH, earthworm species, are summarized in Table 1. As few field

67 studies were available, we present new data on field populations of earthworms potentially

68 impacted by application of synthetic biochars.

69 *3.1. Slash and Burn Char*

70   Slash-and-burn practices are often used to prepare forested land or fallow land with

71 existing crop residues for subsequent crop production. Charcoal additions along with slash-and-

72 burn practices paved the way for the formation of carbon dense fertile black soils [44](see

73 Section 3.4). Under this premise, Topoliantz and Ponge [45, 46] undertook the evaluation of an

74 earthworm's reaction to charcoal obtained from a slash-and-burn field in laboratory analyses. In

75 these two studies, a geophagous tropical peregrine earthworm, *Pontoscolex corethrurus,* was

76 presented with pure soil (Oxisol), pure charcoal, or 60% sieved (<2mm) wood-derived charcoal -

77 soil mixtures, and growth rates and ingestion, burrowing, and casting activity were evaluated.

78 The exact pyrolysis conditions producing the charcoal are not known. However, neither study

79 indicated any pronounced effect on earthworm survivorship or growth rates (Table 1). The

80 presence of charcoal did affect earthworm activity, as discussed in Section 4. One noted impact

81 of the charcoal application was the increase in soil pH from moderately acidic to nearly neutral

82 pH values (Table 1). The neutralizing of soil pH was provided as a reason earthworms were not

83 deterred from burrowing into the charcoal-soil mixtures [46].

84   The prospect of using char from slash-and-burn management practices in rice crops in

85 China for reducing environmental contaminants was evaluated by [47]. Rice crop residue was

86 charred in the laboratory to mimic field slash-and-burn residue and mixed into sediment of 4.7%

87 C and a pH of 6.9. Addition of the rice-char raised the C content of the sediment to 11% but

5

88 resulting pH was not reported. Sorption of organic pollutants to the char was determined by

89 evaluating the genotoxicity to the earthworm *Eisenia fetida*. The charred rice crop residue,

90 containing some black carbon, at low mixture rates (1%, 3% and 5%) reduced genotoxic damage

91 of the organic pollutants, however at a mixture rate of 10% the rice-char itself caused genotoxic

92 damage to the earthworm [47].

93   Even though the char used in these studies [46, 47] are both considered slash-and-burn

94 residue, the materials are quite different as indicated by the high C content of the wood-derived

95 char-soil mixture (39% C) versus the low C of the rice-derived char-soil mixture (11% C).

96 Therefore, one could expect different responses due to the inherent differences in black carbon

97 chemistries. The low C content of the rice-char also indicates this material was likely more ash,

98 and probably had high residual mineral content as well. Application rate was another substantial

99 difference between the studies. Regardless, even though genotoxic damage occurred in one case,

100 short-term survivorship was not affected by either slash-and-burn product. In comparing these

101 studies, the type of char rather than any soil or resultant pH effect probably contributed most to

102 the observed outcome. This illustrates the current limitation in further comparisons, due to the

103 lack of adequate characterization and documentation of the black carbon additions.

104 *3.2. Synthetic Pyrolysis Char*

105   Slow or fast pyrolysis in small batch reactors has allowed small scale synthetic

106 production of biochars from feedstocks including hardwood, softwood, poultry litter and tree nut

107 shells, at temperatures ranging as low as 400°C to as high as 600°C (Table 1). Laboratory and

108 field testing of these biochars at rates of 5 to 180 Mg ha^{-1} have been conducted. In laboratory

109 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 non-amended 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 pre-wetted (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 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 worm's body. More recently, Li et al. [50] discovered that once biochar was pre-moistened the initial avoidance of the biochar by earthworms was overcome. Similarly, once the corn stover residue was pre-moistened, 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 worm behavior by dry biochar additions is a probable cause of short-term negative impacts observed in worm-biochar incubations, as noted by Li et al. [50].

133   Li et al. [50] evaluated biochar toxicity in a 28-day lab incubation, and found that the

134   biochar at 10% and 20% mixtures significantly increased weight loss over controls, but did not

135   affect reproduction. Polyaromatic hydrocarbons (PAHs) were detected in the biochar at

136   concentrations below environmental concern, but no evidence of oxidative stress, indicating

137   uptake of these potentially toxic compounds occurred [50]. Gomez-Eyles et al. [55] also

138   conducted 28-day and 56-day toxicity studies but the scope of the study was designed to evaluate

139   the deciduous hardwood-derived biochar as a bioremediation tool. The biochar was mixed into a

140   contaminated soil collected from a gas works site at 10%. Although no earthworms died, they

141   did lose weight, and after 56-d did uptake PAHs and heavy metals from the contaminated soil;

142   however, in the presence of biochar they were found to have a reduced accumulation of

143   contaminants in their body tissue [55]. Notably, however, Gomez-Eyles et al. [55] attributed the

144   observed weight loss primarily to the presence of biochar. However, it was uncertain if reduced

145   feeding activity of contaminated soil in the presence of biochar (see Section 4) was responsible

146   for the reduced body accumulation of contaminants.

147   *3.3. Wood ash*

148   There have been mixed observations of earthworm dynamics following wood ash

149   additions, but many studies reported reductions in population numbers (Table 1). Haimi et al.

150   [56] noted a virtual immediate decrease (within 20-d) in earthworm numbers when wood ash was

151   added to soil, but difference in the microarthropod population took 4 months to develop, with

152   total numbers of microarthropds being decreased at the highest two amendment levels (2500 and

153   5000 kg ha^{-1}). However, the general conclusion was that wood ash above 2500 kg ha^{-1}

154   decreased earthworm population densities. This is interesting, since the overall impact on

155 microbial populations (microbial biomass C or fungal ergosterol) was insignificant among the

156 wood ash additions from this same study [57]. The total biomass of enchytraeid worms was also

157 reduced by wood ash application when it was mixed with the soil (5000 kg ha^{-1}) [58]. In further

158 studies, the negative impact on *Cognettia sphagnetorum* (Enchytraeidae) populations was

159 confirmed when wood ash was added solely to acidic forest soils [59]. Cox et al. [60] observed

160 that there was no significant difference on total mass or abundance of earthworms in coal ash

161 amended soils, despite the alkaline nature of the amendment.

162 However, these decreases in enchytraeid populations from wood ash additions can be

163 overcome. If the wood ash was left on the soil surface and not incorporated, no significant

164 impact on enchytraeid numbers was observed [58]. Nieminen [61] observed that the negative

165 impacts of wood ash additions on the enchytraeid populations could be overcome through labile

166 carbon additions. Population numbers were also noted to increase 2 yrs after an ash application

167 occurred [90].

168 *3.4. Historical impacts and other field studies*

169 Earthworm populations are prevalent in many soil systems where charcoal from natural

170 fires or controlled burns occurs. Populations of native *Diplocardia* spp. (Megascolecidae) occur

171 in the sub-tropical southern half the United States [62, 63]. These megascolecids, including

172 *Diplocardia mississippiensis*, influence nutrient cycling in fire-controlled pinelands [64, 65].

173 Populations of European lumbricids along with native megascolecids are found in fire affected

174 southern California chapparal soils [66, 67], where they also are important to nutrient availability

175 [68]. In tropical regions, populations of the peregrine earthworm species, *P. corethrurus,* are

176 capable of translocating charcoal residues from slash-and-burn land clearings deeper into the soil

177 profile [69]. This activity by *P. corethrurus* indicates its potentially vital role in stabilizing

178 organic matter and historical development of *Terra Preta* soils [46, 70]. Because of their vital

179 role, some researchers have proposed earthworms, particularly in the tropics, can be used as

180 ecosystem engineering tools to maintain and/or improve soil fertility and ecosystem function

181 [71] particularly in conjunction with charcoal additions [72].

182   Limited studies were available reporting earthworm populations in agricultural systems

183 receiving biochar-like substances or biochar; these studies included [73] and [74]. Topoliantz et

184 al. [73] observed a difference in worm abundance for combined charcoal + other organic

185 amendments, compared to a natural fallow field. However, in this study they observed no

186 statistically significant differences at harvest time in the distribution of cocoons and adults. The

187 authors did not compare the results to an equivalently tilled field, nor did they evaluate the

188 impact of a charcoal only amendment, which complicates the comparisons. For the total

189 numbers, all tilled treatments reduced the numbers of worms, which is known to occur in other

190 studies on the impact of tillage [75]. Husk and Major [74] provide a non-peer reviewed report on

191 earthworm populations sampled by handsorting and mustard application over a two-year non-

192 replicated study on field application of a wood-derived biochar. Their first sampling was taken

193 two months after biochar application at a rate of 5.6 Mg ha^{-1}. Earthworm populations in six out

194 of eight sample dates were generally greater in the biochar plot than the control plot, however,

195 standard error bars from within-plot replicates generally overlapped, suggesting lack of

196 significant statistical differences between biochar and control plots.

197   *3.4.1. Rosemount Biochar Field plots*

198   Earthworm abundance was evaluated in a sub-set of experimental biochar plots at the

199   University of Minnesota Research and Outreach Center in Rosemount, MN USA (44°N, 93°W).

200   These plots are part of the USDA-ARS multi-location biochar and pyrolysis research effort

201   (Spokas, unpublished). Eight treatments were established using a completely randomized design

202   with three replications: (i) control (no amendment), (ii) composted manure; (iii) fast pyrolysis

203   hardwood biochar, (iv) fast pyrolysis hardwood biochar + manure, (v) fast pyrolysis macadamia

204   nut biochar, (vi) slow pyrolysis wood pellet biochar, (vii) wood waste slow pyrolysis biochar,

205   and (viii) a slow pyrolysis hardwood biochar. Each individual plot measures 4.88 m on a side

206   (16' x 16' with a 3-m (10') buffer zone between plots. The biochar was applied at a rate of 22.5

207   Mg ha^{-1} and incorporated by rotary tillage (15 cm depth) in the fall of 2008.

208   Earthworm assessments were made in the spring of 2011 after two full years of

209   continuous no-till corn. Sampling within a circular 0.25 m^2 area in each of five treatments was

210   aided by an electrical field sampling device [76]. Briefly, three step increases in field strength

211   with corresponding alterations in the electrical field orientation by an octet arrangement of

212   electrical poles were conducted over a 20 minute sampling period. A two- or three-pole

213   electrical field was held for approximately two minutes with the increase in the field strength

214   made after a complete circuit was accomplished. Worms were removed once fully exposed at

215   the soil surface and placed in a bucket for quantification. Earthworms were classed as pigmented

216   or non-pigmented then sorted into three size categories and counted. Size categories were

217   roughly equivalent to hatchlings, juveniles and near-clitellate adults within pigmentation class,

218   and actual lengths don't specifically overlap. One plot was manually excavated within the circle

219 influenced by the electrical sampling device and hand sorted; a total of 82% of the worms were

220 retrieved by the electrical device in this plot, additional confirmation checks were not performed.

221 Only one near-clitellate adult earthworm, *Aporrectodea* sp. possibly *A. rosea*, was sampled in the

222 fast pyrolysis + manure treatment (treatment iv), no clitelliated or other near-clitellate

223 earthworms were observed. Due to the field logistic issues and the sampling time required per

224 plot, no replicates were achieved. However, based on this limited data there were no drastic

225 impacts on total earthworm abundance as a function of the different biochar types after two years

226 in field production. The reduced abundance in the composted manure treatment was of unknown

227 cause, but probably related to spatial heterogeneity of worm populations, since all plots were

228 fertilized equivalently (accounting for initial manure-N in year 1 only).

229   The field studies indicate that biochar, charcoal, or occurrence of fire does not

230 significantly affect long-term field populations of earthworms. In the studies evaluated however,

231 short-term impacts, those which may occur within the first several days to weeks after burning or

232 application, are unknown, with one noted exception: successful harvesting of *Diplocardia sp.* for

233 the fishing industry (bait) is known to take place primarily in recently (within days) control-

234 burned forest areas in the Appalachicola National Forest, Florida, USA [63]. Topoliantz and

235 Ponge [45, 46] have already shown *P. corethrurus* was unaffected by biochar in short-term

236 studies, but field application rates were difficult to discern. As with Husk and Major [74] a low

237 application rate of biochar, particularly one derived from wood, might not have had a substantial

238 effect in the field. However, probable short-term effects with higher biochar application rates

239 could not be substantiated by the Rosemount field trial because population assessment occurred

240 two years post-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 charcoal fragments are ingested by earthworms [45, 46, 70, 77]. Topoliantz and Ponge [45] utilized 2-D microcosms to study worm 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 worm 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 the worm 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 worm 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 worms as a food source [45], and suggested that the worms were pushing the charcoal bits aside rather than ingesting it.

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 [78]. The

263 application rate of nearly 60% charcoal could be the reason Topoliantz and Ponge [45] observed

264 less feeding activity in the char-soil mixture whereas lower more applicable field rates might not

265 have the same impact. However, other laboratory studies that used lower application rates did not

266 indicate that charcoal was a food source for worms, but in fact, inhibited feeding activity and

267 induced earthworm weight loss (Table 1) [12, 50, 55]. Microbes are hypothesized to colonize

268 charcoal [79], and may be protected within the charcoal pores. Therefore, less food might be

269 available to the worms [45]. This phenomenon could also explain the lack of feeding burrows

270 observed within the charcoal amended soil in the Topoliantz and Ponge [45] study. These

271 observations could be true for particular soil-biochar combinations but might not be universally

272 the case, particularly in light of the differing responses observed for different biochars and soil

273 combinations [12, 48]. Regardless of any nutritive value, the ingestion of charcoal particles by

274 earthworms and resulting bioturbation and transport of these particles into the soil profile is an

275 important force in the maintenance and improvement of soil function as discussed earlier.

276 **5. Biochars, soils and earthworm interactions**

277   Biochar and soil type have an influence on the response of earthworms following biochar

278 additions (Table 1). Data from Van Zweiten et al. [48], indicates that earthworm preference is a

279 function of both biochar and soil type. They observed the preferences of worms (*Eisenia fetida*)

280 in combination with two different biochars (two different mixture ratios of paper mill sludge and

281 waste wood chips) in two different soil types [a ferrosol (productive red Australian agricultural

282 soil) and a calcarosol (calcareous/calcite rich soil; lower productivity) [80]]. *E. fetida* preferred

283 the biochar amended ferrosol soil compared to the unamended ferrosol soil, whereas no

284 significant difference in worm preference was observed for biochar amended calcarosol soil.

285   One aspect that stands out in this study is that the biochar addition to the ferrosol increased soil

286   pH from 4.2 to 5.9, but addition to the calcarosol did not change pH from 7.6 (Table 1). Also

287   notable, the biochar which had a greater proportion of waste wood to paper sludge (70:30 mix)

288   was also preferred by the earthworms. In our own studies [12], survivorship of *E. fetida* on pine

289   chip-derived biochar was higher than poultry litter-derived biochar.

290   Noguera et al. [13] assessed the effect of two different biochars with *P. correthrurus* on

291   growth dynamics of rice plants in two different soils in a laboratory study. One was a

292   eucalyptus-derived biochar made at a temperature of 350°C, and applied at a rate of 2.5% to a

293   nutrient rich Inceptisol, the second was a household-use charcoal tested at a rate of 4.5% in a nutrient

294   poor Oxisol with and without added fertility. Effects on earthworm survivorship, growth, or

295   behavior were not reported. In mixtures of worms + biochar more plant growth was observed in

296   the nutrient rich soil than with biochar or earthworms alone, however, an earthworm-only effect

297   but no biochar or worm+biochar influence was found in the nutrient poor soil [13]. In a second

298   study with only the eucalyptus-derived biochar, Noguera et al. [81], determined that there was a

299   variable response in growth due to differences among rice cultivars when biochar and

300   earthworms were added to the nutrient rich Inceptisol.

301   Beesley and Dickenson [14] applied a biochar made from hardwoods at 400°C in steel

302   ring furnaces at 30% (volume basis) to an urban soil with a sand:silt:clay content of 83:16:1 and

303   a 7.9 pH. They added fifteen juvenile *Lumbricus terrestris* to the mesocosms but direct effects

304   on the earthworms were not reported. The biochar caused a significant increase in pore water

305   concentrations of As, Cu, and Pb within the year of testing. However, when earthworms were

306   present, the concentrations of As and Cu in the leachate collected from biochar amended soils

307   was decreased. The authors attributed this decrease to the effect earthworms had on reducing the

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308 concentration of dissolved organic C (DOC) as well as the amount of pore water moving through

309 the soil chambers. Beesley and Dickenson [14] hypothesized that an increased pH (6.6 of the

310 soil to 9.9) caused by the addition of biochar might have positively influenced the earthworms

311 and their subsequent effects on DOC. However, they did not assess the potential for *L. terrestris*

312 to construct and line burrows with organic matter shown to reduce leaching of organic pesticides

313 [82]. These statements made by Beesley and Dickenson [14] support observations made earlier

314 in this review that biochar application to soil will impact earthworm activity (see Section 4).

315   Despite the limited number of studies specifically examining different biochar types, the

316 general conclusion is that there are different responses as a function of soil and biochar

317 properties. From the existing studies, it still is not clear what particular mechanisms are

318 responsible for these observations. However, Noguera et al. [13] determined the interaction

319 resulting in increased plant growth observed between biochar and earthworms was additive

320 rather than synergistic.   These data strongly suggest that soil characteristics, biochar

321 characteristics as well as plant characteristics will affect the response observed when biochar is

322 added to soils with earthworm populations.

323 **6. Future Steps**

324   Field populations of earthworms occurring in fire-affected systems indicate adaptation to

325 the presence of natural charcoal is possible. But yet unknown is if the input of natural or

326 synthetic biochar has or had any initial impact on the pre-existing earthworm populations.

327 Overall from this data there is the suggestion that the short-term impacts on worms are either

328 non-significant or negative. As indicated by our field study, earthworm populations in biochar

329 amended plots were similar to the control plots after 2 years of continuous no-till corn

330   production in Minnesota. Other field observations were likely made after the population was

331   able to rebound. This interval would include the two-month interval between application and

332   sampling as in Husk and Major [74]. Field studies using small amounts of biochar (<10 Mg ha^{-1})

333   potentially avoid negative short-term impacts. Field studies are needed which evaluate a greater

334   range of application rates, preferably they would be paired with laboratory preference/avoidance

335   assays to establish appropriate ranges of application rates. Assessment of earthworm populations

336   are needed prior to, immediately at, and over the long term after biochar application to the soil.

337   In the evaluations reviewed, biochar was never clearly used as a food substrate.

338   Although we cannot discount the potential use of biochar for digestive purposes by field

339   populations, laboratory studies certainly showed that earthworm behaviors were altered, and soil

340   ingestion was reduced. A few of these laboratory studies demonstrated that some biochars are

341   likely to be potential toxins [47] but lethal results likely depend on amendment rates [12].

342   Beesley and Dickenson [14] did show that leaching of potential toxins, including Pb, As, and Cu,

343   was increased with biochar application, though the biochar might not have been the direct source

344   of these elements. Regardless, this observation runs counter to the suggested use of biochars to

345   sorb environmental toxins [47, 55]. With earthworms active in the soil, however, reduced

346   concentrations of potential toxins in pore water were found [14]. Thus the natural movement of

347   biochars into the soil through earthworm activity, as shown by Topoliantz and Ponge [45, 46],

348   might assist the use of biochar as a bioremediation tool in contaminated soils. Studies which

349   address earthworm activity, in particular burrowing, ingestion and casting, which might affect

350   movement of biochar in and around the soil environment, would be useful in determining more

351   specific interactions with soil function. Studies examining activity will also need to account for

352   the ecological strategy the earthworm species present could be categorized into particularly as

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353 these strategies define where and how within the soil profile they feed and burrow and the

354 resulting affects on the soil environment [83]. Another aspect needing to be addressed is

355 potential effects on earthworm migration. The reader is referred to Butt and Grigoropoulou [84]

356 for information on how to properly address analysis of earthworm populations.

357   The complications of evaluating biochar research stem from the deficiency of many of

358 these studies to report on elemental content, ash content, pH, soils used, feedstock material, and

359 method of production. Though wood derived biochars used in the studies described here had

360 more null effects, and other biochars from mixes with sludges, manures, or crop residues had

361 negative effects, the data also indicate pH changes in the assessment medium, whether that is

362 field soil or simulated soil, might influence the outcome. The null to positive impacts of wood

363 based biochars on agronomic yields has also been observed in biochar field studies [85, 86].

364 There is an identified need to standardize earthworm studies [87], and adequate data must be

365 presented on the biochar properties, the environment in which they are to be used and influence

366 on soil biota, so future meta-analyses can be conducted. More detailed initial and final

367 evaluations of earthworm populations in short as well as long-term studies are necessary to

368 elucidate the immediate and lasting effects of biochar before it becomes a widespread soil

369 amendment.

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

Study	Worm Species	Study type	Charcoal/Biochar description and application rate	Location	Soil /pH	Worm 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 worms 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	Non-significant growth increases; direct charcoal consumption observed; surface cast production greater with soil:char mixture
Topoliantz et al. [73]	*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%

Table 1. continued

Study	Worm Species	Study type	Charcoal/Biochar description and application rate	Location	Soil /pH	Worm Impact
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
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 stated pH 7.63	Weight loss observed with biochar; reduced contaminant accumulation in body tissue
Husk and Major [74]	*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

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Table 1. continued

Study	Worm Species	Study type	Charcoal/Biochar description	Location	Soil/pH	Worm Impact
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}
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 ash 110 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 [88]	*Cognettia sphagnetorum*	L/F	Wood ash	Sweden	Forest soil	No significant differences
Huhta et al [89]	*Cognettia sphagnetorum*	F	Wood ash	Finland	Forest soil	Decreased biomass following ash addition; Controls lacked earthworms; few earthworms found where ash applied
Lundkvist [90]	*Cognettia sphagnetorum;* Earthworms species not stated	F	Wood ash Wood ash +/- NH$_4$NO$_3$	Sweden	Forest soil	No population effects; Increased Cd in body tissue; Increase in earthworm population after 2yrs
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

1 Figure legend

2 Figure 1. Abundance of earthworms by pigmentation and size class, in biochar plots sampled at

3 Rosemount, MN; treatments are (i) control (no amendment), (ii) composted manure; (iii) fast

4 pyrolysis hardwood biochar, (iv) fast pyrolysis hardwood biochar + manure, (v) fast pyrolysis

5 macadamia nut biochar.

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